Spatiotemporal control of gene expression by a light-switchable transgene system

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We developed a light-switchable transgene system based on a synthetic, genetically encoded light-switchable transactivator. The transactivator binds promoters upon blue-light exposure and rapidly initiates transcription of target transgenes in mammalian cells and in mice. This transgene system provides a robust and convenient way to spatiotemporally control gene expression and can be used to manipulate many biological processes in living systems with minimal perturbation.

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Regulated transgene systems are indispensable tools in biomedical research and biotechnology. During the past decade, chemically regulated gene expression systems[1,2] have been widely used for the temporal control of gene expression. However, as these small molecular inducers diffuse freely and are hard to remove, it is not possible to precisely switch on and off gene expression at an exact location and time. In contrast to chemicals, light is an ideal inducer of gene expression because it is easy to obtain, highly tunable, nontoxic and, most importantly, has high spatiotemporal resolution. A light-switchable gene expression system could be the most promising tool for precisely controlling spatiotemporal gene expression in multicellular organisms. There have been several efforts to control gene expression using light. Caged transactivator or chemical inducers that are activated by UV light have been developed, allowing study of gene function in developing embryos[3–5]. Infrared laser light was used to induce heat shock–mediated expression of transgenes[6]. Recently, synthetic approaches have been developed to regulate gene expression by light illumination using genetically encoded light sensors[7–11].Uptake of these methodologies by biologists has been minimal, however, probably because of technical complexities or limitations. We sought to develop a simple robust transgene system that is directly regulated by a single genetically encoded, photosensitive transactivator.

To create a light-switchable gene promoter system, it is necessary to first design a DNA-binding domain that is activated by light. The well-characterized DNA-binding domain comprising Gal4 residues 1–147, Gal4(147), consists of a DNA-recognition element and a dimerization domain. Removal of the dimerization domain to create Gal4(65), which contains Gal4 residues 1–65, virtually eliminates binding to its consensus cognate DNA sequence, the upstream activating sequence of *Gal* (*UASG*)[12]. Vivid (VVD), the smallest light-oxygen-voltage (LOV) domain–containing protein, forms a rapidly exchanging dimer upon blue-light activation[13–15]. We reasoned that the DNA-binding property of a Gal4(65)-VVD fusion protein would be light-switchable, as light should induce dimerization of the fusion protein, enhance binding to the *UASG* sequence and activate transcription and removing the light should result in gradual dissociation of the dimers, DNA dissociation and inactivation (**Fig. 1a**). Indeed, an electrophoretic mobility shift assay showed that Gal4(65)-VVD dimerized upon 15 W m^{-2} constant blue-light illumination and bound the *UASG* sequence (**Fig. 1b**). Purified Gal4(65)-VVD had similar spectra to VVD[13] in dark or light states (**Supplementary Fig. 1**). These data suggested that the VVD domain in the fusion protein was correctly folded and was bound by flavin adenine dinucleotide (FAD) and that light illumination induced Gal4 dimerization and binding to *UASG*.

To create a system capable of driving light-activated transcription, we fused different transactivation domains to the C terminus of Gal4(65)-VVD (**Fig. 1a** and **Supplementary Fig. 2**). We tested their light-dependent impact on transcriptional activity of a firefly luciferase (*Fluc*) reporter driven by Gal4 binding sites upstream of a TATA box after transient transfection in HEK293 cells, illumination with 0.84 W m^{-2} 460 nm peak light from an LED lamp for 22 h and measurement of expression. Transactivators containing the p65 activation domain (GAVP) or the VP16 activation domain (GAVV) both showed marked light-induced reporter gene transcription, but the GAVP transactivator resulted in much greater gene expression under light exposure conditions (**Fig. 1c** and **Supplementary Fig. 3**). Mutation of Cys108 in VVD to serine blocked light-inducible gene expression as expected[13] (**Fig. 1c** and **Supplementary Fig. 3**). Mutation of Cys71 to valine in VVD is known to enhance the stability of the light-induced VVD dimer[14], and based on the crystal structure of VVD[13] we hypothesized that mutating Gln56 of VVD to lysine would form a salt bridge with Asp68 of the other VVD protein and additionaly stabilize the dimer. Both dimer-enhancing mutations, C71V and N56K, in the VVD domain decreased reporter gene expression in the dark, whereas the N56K,C71V double mutant (optimized GAVP (GAVPO)) additionally decreased the background gene expression to a minimal level (**Fig. 1c** and **Supplementary Note**). We used GAVPO in all subsequent studies, and we referred to the gene promoter system based on GAVPO as the light-on (LightOn) system.

We compared the LightOn system to human cytomegalovirus immediate early promoter (*CMV*)-based induction of reporter

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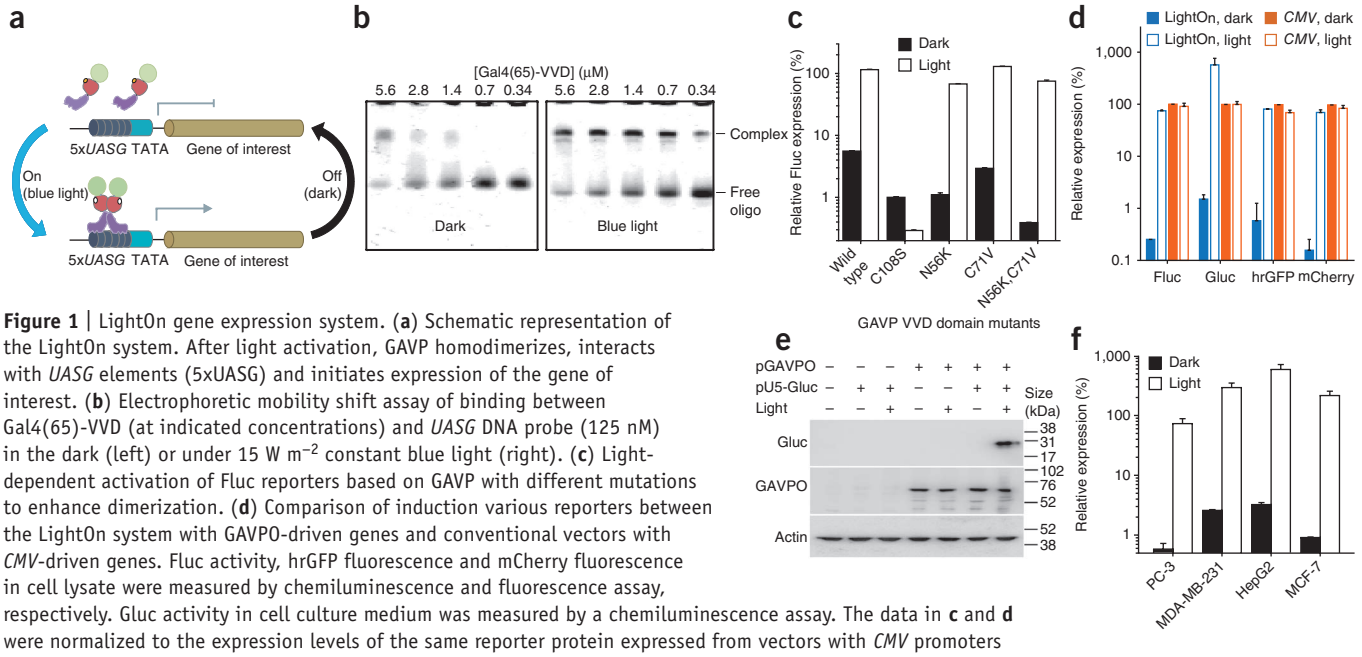

Figure 1 | LightOn gene expression system. (**a**) Schematic representation of the LightOn system. After light activation, GAVP homodimerizes, interacts with *UASG* elements (5xUASG) and initiates expression of the gene of interest. (**b**) Electrophoretic mobility shift assay of binding between Gal4(65)-VVD (at indicated concentrations) and *UASG* DNA probe (125 nM) in the dark (left) or under 15 W m^{-2} constant blue light (right). (**c**) Light-dependent activation of Fluc reporters based on GAVP with different mutations to enhance dimerization. (**d**) Comparison of induction various reporters between the LightOn system with GAVPO-driven genes and conventional vectors with *CMV*-driven genes. Fluc activity, hrGFP fluorescence and mCherry fluorescence in cell lysate were measured by chemiluminescence and fluorescence assay, respectively. Gluc activity in cell culture medium was measured by a chemiluminescence assay. The data in **c** and **d** were normalized to the expression levels of the same reporter protein expressed from vectors with *CMV* promoters in the dark. (**e**) Western blot of the Gluc expression in HEK293 cells transiently transfected with pGAVPO and pU5-Gluc under light or dark conditions. (**f**) Light-switchable Gluc expression from pGAVPO in different cell lines. The data were normalized to the expression of the same reporter protein expressed from the *CMV* promoter under light-on conditions. (**c**,**d**,**f**) Error bars, mean ± s.e.m. (n = 4 samples) from the same experiment. Six (**c**) or ten (**d**–**f**) hours after transfection, cells were illuminated by 0.84 W m^{-2} blue light or remained in the dark for 22 h before measurements.

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vectors driving *Fluc*, *Gaussia princeps* luciferase (Gluc), humanized recombinant GFP (hrGFP) or monomeric (m)Cherry protein expression by transient transfection of vectors encoding all components into HEK293 cells and light exposure as described above (**Fig. 1d** and **Supplementary Figs. 4** and **5**). We confirmed that light-mediated activation required all components by western blots of Gluc (**Fig. 1e**). We routinely observed 200–300-fold induction of *Fluc* and *Gluc* in our experiments and a similarly high on/off ratio of gene expression and induction efficiency of LightOn system in other cells lines (**Fig. 1f**). The blue-light irradiation had little effect on expression of proteins whose transcription was driven by *CMV* promoter (**Fig. 1d**), suggesting that the LightOn system has minimal interference with or toxicity to normal cellular function.

We investigated the time course of light-induced *Gluc* transcription using quantitative real-time PCR. Cellular *Gluc* mRNA amounts increased 13-fold and 81-fold after 0.5 h and 1 h of light induction, with respect to samples not exposed to light (**Fig. 2a**) followed by an increase in secreted Gluc protein ~1 h later (**Fig. 2b**). Removal of light resulted in a slow decay in the amount of *Gluc* mRNA and a plateau in the amount of protein when we did not change the medium (**Fig. 2a**,**b**). *Gluc* expression depended on the duration of illumination, but a 30-min illumination was sufficient for ~25-fold induction of Gluc protein above background expression 10 h after initial illumination (**Supplementary Fig. 6**). Off expression kinetics of *Gluc* mRNA suggested that GAVPO was kept in the activated state with a half life of 2 h (**Supplementary Fig. 7**), which is similar to that of VVD (half life of ~18,000 s)[16].

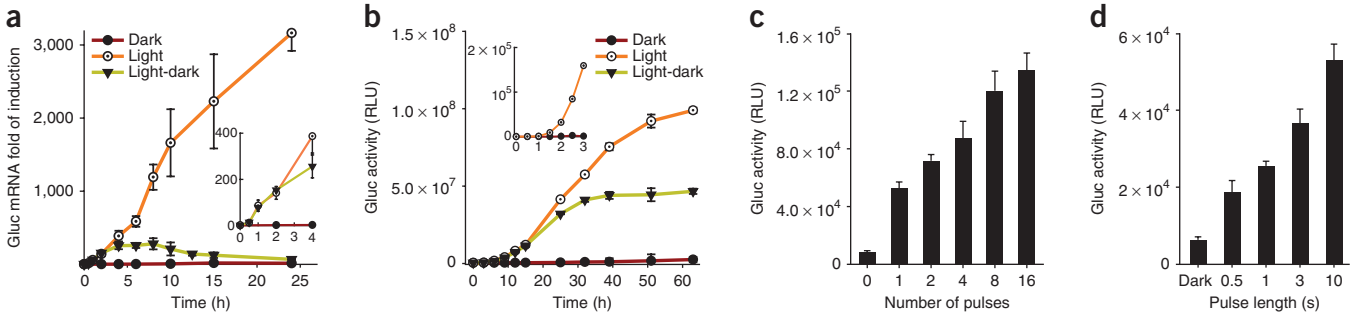

Figure 2 | Time course of light-switchable gene expression using LightOn in HEK293 cells transiently transfected with pGAVPO and pU5-Gluc. (**a**) Cellular *Gluc* mRNA level measured at indicated times in the dark, after illumination under continuous 0.84 W m^{-2} blue light or after illumination under 0.84 W m^{-2} blue light for 2 h and then in the dark (light-dark). (**b**) Expression kinetics of the Gluc reporter in cell culture medium measured at indicated times in the dark, after illumination under continuous 0.84 W m^{-2} blue light or after illumination under blue light for 15 h and then in the dark (light-dark). Insets, kinetics of Gluc mRNA (**a**) or protein activity (**b**) during the 4 h or 3 h after the initial light exposure, respectively. (**c**,**d**) Gluc activity in medium, measured 4 h after the initial light exposure to blue-light pulses (**c**; 10 s pulses, 22 W m^{-2}, 8 min apart) or to a single blue-light pulse of varying duration (22 W m^{-2}). RLU, relative luciferase units. Error bars, mean ± s.e.m. (n = 4 samples) from the same experiment.

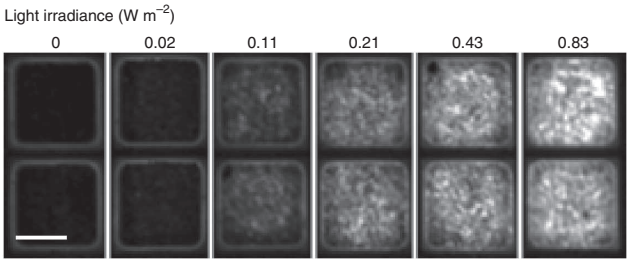

Figure 3 | Graded response of mCherry expression under different blue-light irradiances. Ten hours after transfection of *mCherry* reporter and *GAVPO* vectors, cells were illuminated by blue light of indicated irradiances adjusted by neutral density filters for 22 h before determination. Fluorescence images are shown. Scale bar, 0.5 cm.

This led to continued mRNA synthesis during the first few hours after turning the light off (**Fig. 2a**). The estimated half life of *Gluc* mRNA was 10 h (**Supplementary Fig. 7**), which explained the continuous increase in the amount of Gluc in the medium after light was turned off for another 20 h (**Fig. 2b**). To increase the off rate of the system we modified the 3′ untranslated region of the *Gluc* reporter gene by inserting the conserved AU-rich element (ARE) from the gene encoding GM-CSF, which mediates selective degradation of mRNA[17]. Expression of *Gluc-ARE* stopped much earlier than did expression of the original *Gluc* gene (**Supplementary Fig. 8**). We also investigated the capacity to activate the LightOn system by short pulses of light and observed a strong dose dependence on the number of pulses and the duration of a single pulse, showing that continuous illumination is unnecessary (**Fig. 2c**,**d**).

We next tested the ability of the LightOn system to induce graded protein expression in cells by controlling the irradiance (**Fig. 3** and **Supplementary Figs. 9** and **10**). To spatially control gene expression in cultured cells, we illuminated in a specific pattern HEK293 cells transfected with *mCherry* reporter and *GAVPO*. The mCherry fluorescence image of the cells had the pattern of the original image used as the mask (**Supplementary Fig. 11**).

These data indicate that the LightOn system can be robustly used to quantitatively, spatially and temporally control gene expression in mammalian cells.

Finally, we validated the LightOn system *in vivo*. We transferred *GAVPO* and *mCherry* reporter vector into the livers of mice using a hydrodynamic procedure. Exposure of the mice to blue light from below resulted in the appearance of marked fluorescence from mCherry protein in their livers (**Fig. 4a**). Light-dependent transgene expression was limited to the anterior side and posterior lining of liver that received sufficient blue light irradiance, in contrast to controls transfected with pcDNA3.1 vector containing the *mCherry* gene driven by a *CMV* promoter that resulted in homogenous expression (**Fig. 4a**). Light-induced expression of the *mCherry* gene in the liver was limited to 1 mm or less from the surface (**Fig. 4b**). Spatial control of gene expression in the liver was possible with localized illumination using optical fibers (**Supplementary Fig. 12**).

We then used the LightOn system for Cre recombinase–mediated *LacZ* activation in *Gt(ROSA)26Sor* (*ROSA26*)-*LacZ* mice transfected with pGAVPO and pU5-Cre vectors. We observed *LacZ* expression in the liver after illumination with 90 mW cm^{-2} blue light for 22 h and 48 h in the dark but not in control mice kept only in the dark (**Fig. 4c**). This suggests that light-mediated, tissue-specific expression should be possible. As a very preliminary proof-of-principle demonstration of the potential of our system for regulated gene or cell therapy, we transfected type I diabetic mice with pGAVPO and pU5-insulin vectors and observed that blue-light illumination caused a large drop of blood glucose compared to mice transfected with the vector encoding the nonfunctional GAVPO mutant (**Fig. 4d**).

An ideal regulated gene expression system should have low background expression, low toxicity, low interference with endogenous proteins or genes and the capacity for temporal and spatial control, and should be easy to manipulate. Most existing systems[3,5–11], however, do not simultaneously satisfy all of these above requirements (**Supplementary Table 1**). Approaches involving caged activators[3,5] or heating effects[6] are hard to implement

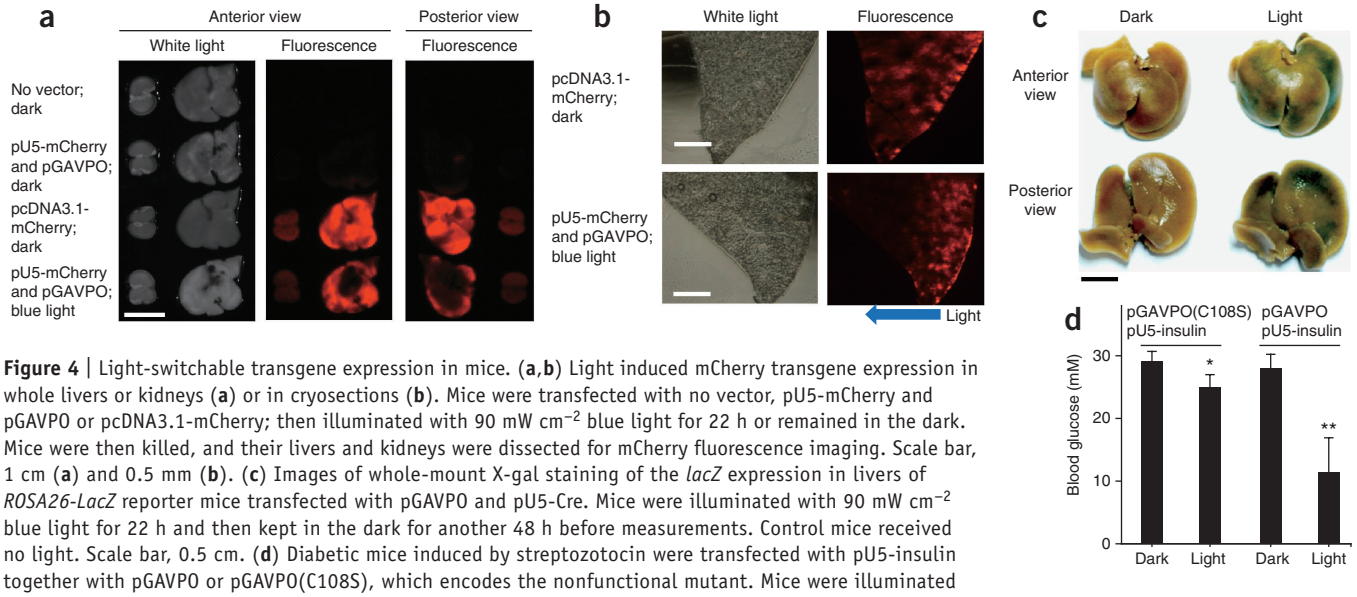

Figure 4 | Light-switchable transgene expression in mice. (**a**,**b**) Light induced mCherry transgene expression in whole livers or kidneys (**a**) or in cryosections (**b**). Mice were transfected with no vector, pU5-mCherry and pGAVPO or pcDNA3.1-mCherry; then illuminated with 90 mW cm^{-2} blue light for 22 h or remained in the dark. Mice were then killed, and their livers and kidneys were dissected for mCherry fluorescence imaging. Scale bar, 1 cm (**a**) and 0.5 mm (**b**). (**c**) Images of whole-mount X-gal staining of the *lacZ* expression in livers of *ROSA26-LacZ* reporter mice transfected with pGAVPO and pU5-Cre. Mice were illuminated with 90 mW cm^{-2} blue light for 22 h and then kept in the dark for another 48 h before measurements. Control mice received no light. Scale bar, 0.5 cm. (**d**) Diabetic mice induced by streptozotocin were transfected with pU5-insulin together with pGAVPO or pGAVPO(C108S), which encodes the nonfunctional mutant. Mice were illuminated with 90 mW cm^{-2} blue light or kept in dark for 8 h. Glucose levels were measured after the mice rested in the dark for another 4 h with sufficient food. Error bars, s.e.m. (n = 8–10) from two independent experiments; statistics by two-tailed t test. *P < 0.04 versus 'dark' control; **P < 0.0002 versus 'dark' control.

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and manipulate, and are associated with potential problems of cell injury or side effects resulting from the UV-light irradiance or heat shock used to activate gene expression. The LightOn system reported here is based on a genetically encoded light sensor that uses FAD as a photon acceptor[13] and offers obvious advantages compared to the above techniques. As FAD naturally exists in cells, it is unnecessary to treat cells with extraneous ligands that are required by phytochrome[7] or caged activators[3,5]. The single-chain 56-kDa genetically encoded light-switchable transactivator in the LightOn system, which operated through homodimerization, additionally reduced the complexity of the multicomponent gene expression methodologies based on two-hybrid technologies previously reported for yeast[7,10] or mammalian cells[9]. Recently, a well-designed synthetic light-regulated circuit has been reported to regulate gene expression in transgenic cells and blood-glucose homeostasis in mice[11]. However, as this technique is based on coupling an exogenously expressed blue light–induced melanopsin receptor to major existing cellular signaling players such as phospholipase, phosphokinase and calcium, it suffers from low on/off ratio of gene expression and mutual interference with endogenous signaling events that may limit its usage. In contrast, the LightOn system is orthogonal to mammalian cellular signaling, which should allow tighter control with minimal perturbation of, or from, existing signaling pathways.

There are many other advantages of the LightOn gene expression system reported here. LightOn has low background and allows high induction with reasonably fast kinetics and reversibility. We showed that continuous illumination was not necessary to activate LightOn, and single brief pulses of light were sufficient. This was possible owing to the high induction level and low background we observed. Because of the unusually stable photoactivated state of VVD[16], the LightOn system is extremely sensitive to light, thus minimizing any potential toxicity of blue-light irradiance on cells. We observed a fourfold increase in Fluc expression when we irradiated cells with blue light five orders of magnitude lower than the sun's irradiance. These characteristics provide the capability for gene activation with good spatial, temporal and quantitative control in an easy-to-use and robust system. LightOn should be a powerful yet convenient tool for life science research, allowing spatial and temporal control of gene expression.

In the past three years, optogenetics has become a booming field by using genetically encoded light-sensitive proteins to control the behavior of living cells and organisms[18,19]. Most of these tools are based on light-gated ion channels, light-switchable enzymes or protein interactions. The LightOn system provides another general way to control biological processes using light-switchable gene expression, thus avoiding the need for case-by-case protein engineering to create light-regulated protein modules. In addition to its use in mammals, the LightOn gene expression system could be used to control gene expression spatiotemporally in other model eukaryotes such as *Danio rerio* and *Drosophila melanogaster*, in which *Gal-UAS* systems are already widely used to control cell type–specific gene expression. We anticipate that the LightOn system will be widely used in many fields of life science research and biotechnology that have great demand for high-resolution spatial and temporal control of gene expression.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemethods/.

Note: Supplementary information is available on the Nature Methods website.

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AUTHOR CONTRIBUTIONS

Y.Y. conceived of the concept; Y.Y., X.W. and X.C. designed the experiments and analysed the data; X.W. preformed the molecular cloning, protein characterization and cell culture experiments; X.C. preformed animal studies; and Y.Y. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

DNA cloning. Full length *VVD* gene was isolated from *Neurospora crassa* (gift of B. Chen, Guangxi Normal University) genomic DNA with both introns and exons. Introns were removed by reverse PCR. Sequences encoding the Gal4(1–65), and VP16 activation domain was amplified from pBIND and pACT (Promega), respectively. Chimeric fusion construct pGAVV consisting of sequences encoding Gal4(1–65), VVD (37–186) and VP16 activation domain was generated using overlapping PCR and inserted into Eco47III and BsrGI sites in pEGFP-N1 vector. To generate chimeric fusion construct pGAVP, sequence encoding VP16 activation domain of pGAVV was replaced with sequence encoding p65 activation domain residues 286–550, which was isolated from HEK293 cDNA by EcoRI and BsrGI. Site-directed mutagenesis, to generate sequences encoding VVD proteins with mutations C71V, N56K and C108S, was performed on sequence encoding the VVD domain according to the MutanBEST protocol (Takara). The reporter vector pU5-Gluc was generated by overlapping poly(A)–5×*UASG*-TATA sequence from pG5luc (Promega) and secreted Gluc sequence from pGLuc-basic (NEB), and subsequent ligation into NruI and BamHI sites of pcDNA3.1/Hygro(+) using CloneEZ PCR Cloning kit (Genescript), thereby replacing *CMV* promoter in pcDNA3.1/Hygro(+) (Invitrogen). The 2×Flag tag was added to C-terminal end of Gluc (added into the gene using BamHI and XbaI) for convenient immunoassay detection. Other reporter vectors including pU5-hrGFP, pU5-Fluc, pU5-mCherry and pU5-Insulin were generated by substituting *Gluc* with genes encoding humanized recombinant (hr)GFP, Fluc, mCherry and a minimal human proinsulin, respectively. Plasmid pU5-Gluc-ARE was constructed by inserting the *ARE* of the sequence encoding GM-CSF into 3′ untranslated region of *Gluc*. A furin consensus cleavage sequence was introduced to the minimal human proinsulin gene, allowing the translational product to be constitutively processed and secreted in liver cells[20]. Genes encoding hrGFP, Fluc, mCherry and insulin were introduced into HindIII and BamHI site of pcDNA3.1/Hygro(+) and *Gluc* was cloned into pcDNA3.1/V5-His-TOPO (Invitrogen) to obtain *CMV* promoter–driven genes in expression vectors. To obtain the pU5-Cre vector, the multiple cloning site of pU5-Gluc was replaced, and *Cre* was introduced into HindIII-Eco47III site of the new vector by substituting *Gluc*. To construct *Escherichia coli* expression vector, sequence encoding Gal4(65)-VVD was amplified from pGAVP and inserted into pET-28a(+) using CloneEZ PCR Cloning kit.

Protein expression and purification. Gal4(65)-VVD was expressed in *E. coli* strain JM109 at 18 °C for 24 h under constant light in the presence of 0.4 mM IPTG, 10 μM $ZnCl_2$ and 5 μM FAD. The cell pellet was collected by centrifugation and sonicated in buffer A containing 20 mM Hepes, 0.5 M NaCl, 10 μM $ZnCl_2$, 20 mM imidazole, 10 mM β-mercaptoethanol and 10% glycerol, pH 7.5. The soluble cell lysate was fractionated by centrifugation. The supernatant was passed over a HisTrap FF column (GE Healthcare) and then washed thoroughly in buffer B containing 20 mM Hepes, 0.5 M NaCl, 50 μM $ZnCl_2$, 300 mM imidazole, 10 mM β-mercaptoethanol and 10% glycerol, pH 7.5. Proteins were desalted in 20 mM Hepes, 0.15 M NaCl, 20 μM $ZnCl_2$ and 10% glycerol, pH 7.5, using a HisTrap desalting column (GE Healthcare). After purification, Gal4(65)-VVD was stored at 4 °C and protected from light for recovery in the dark.

Electrophoretic mobility shift assay. The probes used were as follows: 5′-TCTTCGGAGGGCTGTCACCCGAATATA-3′ and 5′-ACCGGAGGACAGTCCTCCGG-3′[12]. All samples containing VVD-derived proteins were prepared under red LED safe light. The DNA was annealed and diluted in 20 mM Hepes and 50 mM NaCl, pH 7.5 (renaturation buffer), to a final reaction DNA duplex concentration of 125 nM. Protein was diluted in renaturation buffer containing 100 μg ml^{-1} BSA (Jackson ImmunoResearch) by twofold serial dilution from 5.6 μM to 0.34 μM protein in dim red light. Protein and DNA were equilibrated at room temperature (20–25 °C) for 30 min in reaction buffer with an additional 5% (w/v) Ficoll either in the dark or with 15 W m^{-2} constant blue light. After incubation, the dark and light irradiated samples were separately loaded onto different 6% native polyacrylamide gels in 0.5× Tris-borate buffer and were run at 100 V at 4 °C in the dark or with 15 W m^{-2} blue-light irradiance, respectively. After electrophoresis, the gel was stained with GelRed nucleic acid gel stain (Biotium) before fluorescence imaging using the In-Vivo Multispectral System FX (Kodak) with 530 nm excitation and 600 nm emission filters. Images of full-length gels from **Figure 1b** are available in **Supplementary Figure 14**.

Cell culture and blue light irradiation. HEK293, HepG2, MDA-MB-231, MCF7 and PC-3 cells were maintained in high-glucose DMEM (HyClone) supplemented with 10% FBS, penicillin and streptomycin (Invitrogen). Cells were plated in phenol red–free, antibiotic-free high glucose DMEM supplemented with 10% FBS 16 h before transfection. We typically used equal amounts (0.4 μg each) of the light-switchable transactivator and reporter constructs with 2.4 μl Lipofectamine 2000 (Invitrogen) for each well of a 12-well plate according to the manufacturer's protocol. To estimate the transcription efficiency of the LightOn system, equal amounts of *CMV* promoter–driven reporter constructs were used to transfect the cells as a control. Unless indicated, the transfected cells were kept in the dark for 10 h, and then they were illuminated by 0.84 W m^{-2} (average irradiance) blue light from an LED lamp (460 nm peak) from below or remained in the dark for 22 h before characterization. The LED lamps were controlled with a timer to adjust the overall dose of blue light illumination during the specified period (**Supplementary Fig. 13a**). Neutral density filters were used to adjust the light irradiance. To spatially control gene expression in cultured mammalian cells, single layers of HEK293 cells cultured on glass bottom dishes were transiently transfected with an *mCherry* reporter and the *GAVPO* transactivator, and then, the cells were illuminated with a spatial pattern using a photomask printed with a specific image for 24 h. LightOn system is sensitive to ambient light. One minute exposure to 0.16 W m^{-2} white fluorescent lamp light lead to substantial induction of gene expression, whereas there was minimal gene induction when cells were illuminated with 630 nm red LED light. In this study, experiment procedures after cell transfection were carried out under red LED light, and cells were cultured inside dedicated CO_2 incubators.

Animal experiments. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Shanghai and were conducted in accordance with the National Research Council Guide for Care and Use of Laboratory Animals. Unless otherwise mentioned, 10 μg of pGAVPO and 300 μg of pU5 vector carrying target gene were transferred into mice by

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hydrodynamic procedure[21]. For better illumination, abdominal fur of the mice was removed by shaving the mice and 8% sodium sulfide. Mice then rested in cages with glass bottoms and illuminated from below with blue LED lamp (90 mW cm^{-2}) (**Supplementary Fig. 13b**) or in the dark. For localized illumination, mice skin was illuminated by 7 mW 450 nm blue diode laser (Nichia) through optical fiber bundle 600 μm in diameter attached to abdomen surface (**Supplementary Fig. 13c**). To control the motion of the animal and their internal organs, we fixed the mouse on a plastic board using nylon rope. Mice were released from the board and fed for 20 min every 8 h. The *ROSA26-LacZ* mice (obtained from Shanghai Research Center for Model Organisms) were used for *in vivo* light-induced DNA recombination. After transfection with pU5-Cre and pGAVPO vectors and illuminated with blue light for 22 h, mice were kept in the dark for another 48 h. Four-week-old male ICR mice (SLRC Laboratory Animals) or Chinese Kunming (KM) mice (Animal Center of FUDAN University) of ~20 g body weight were used for mCherry reporter expression or type I diabetes animal model studies, respectively. To induce diabetes, the KM mice received intraperitoneal injection of streptozotocin at 150 mg kg^{-1} body weight and then fed with sufficient water and food. Two weeks after streptozotocin treatment, the mice with blood glucose levels around 30 mM were selected and transfected with 20 μg of pU5-insulin together with 10 μg of pGAVPO or 10 μg of pGAVPO(C108S), which encodes the nonfunctional mutant. After transfection, mice were illuminated with blue light without food for 8 h. After, the mice rested in the dark for another 4 h with sufficient food, and their blood glucose levels were determined with ACCU-CHEK Integra Glucose Meter (Roche). Control mice received no light. Experiments were done at the same phase of the circadian rhythm to minimize effects of the circadian clock. Food-intake by each group of mice was similar, therefore the effect of different food intake was minimal.

RNA analysis. Total RNA was isolated from transfected HEK293 cells using a total RNA extraction kit (Tiangen) according to the manufacturer's instructions. RNA was converted to single-stranded cDNA using ImProm-II reverse transcriptase (Promega). Specific primers (**Supplementary Table 2**) were used to amplify target genes. For real-time quantitative reverse transcription (RT)-PCR, 1 μl of the cDNA was used for the assay with 2× TaKaRa SYBR Green Premix (TaKaRa) and specific primers according to the manufacturer's recommendations on a Bio-Rad CFX96 system. The specificity of amplification was verified by melt-curve analysis, and the data were collected using BioRad CFX manager software. Amplification conditions were 1 cycle of 95 °C for 3 min followed by 40 cycles of 95 °C for 10 s and 60 °C for 60 s, with a final melt curve analysis step (heating the PCR mixture from 65 °C to 95 °C by 0.5 °C every 5 s) to confirm specificity of amplification and lack of primer dimers. All samples were normalized to the β-actin values and the results expressed as fold changes of cycle threshold (Ct) value relative to samples before light induction by using the $2^{-\Delta\Delta Ct}$ formula.

Chemiluminescence, fluorescence and absorption assays. A Synergy 2 multi-mode microplate reader (BioTek) was used to measure the chemiluminescence, fluorescence and absorption spectrum of samples. The Fluc assay was carried as previously described with minor modifications[22]. Ten microliters cell lysate and 15 μl assay buffer were transferred to each well of a white 384 well plate (Greiner), and 15 μl 0.2 mM D-luciferin (SynChem) was added before detection. Light emission was recorded as relative light units. Cell culture supernatants were assayed for secreted Gluc activity using a *Gaussia* luciferase assay kit according to the manufacturer's protocol (NEB). For the Fluc assay, data were normalized based on protein concentrations measured using the DC protein assay (BioRad). The fluorescence intensities of the cell lysates containing hrGFP and mCherry were measured using a 485/20 excitation filter and a 528/20 emission filter and a 590/20 excitation filter and a 635/40 emission filter, respectively. Absorption spectrum was acquired from 300 nm to 700 nm with 5 nm increments.

Electrophoresis and western blot. For native gel electrophoresis, equal amounts of lysate protein were loaded onto a 15% native Tris-glycine gel and run at 20 mA for 2 h. For western blots, equal amounts of total lysate protein were separated on a NuPAGE 4–12% gradient Bis-Tris gel (Invitrogen) and then transferred to polyvinylidene fluoride (PVDF) membranes (Invitrogen). An equal volume of culture medium was used instead for secreted Gluc measurements. Membranes were then blocked, and the signal was detected with a BM Chemiluminescence Blotting kit (Roche Diagnostics) according to the manufacturer's instructions. Mouse monoclonal antibody to Flag (clone M2) 1:200 and antibody to β-actin (clone AC-15) 1:1,000 were purchased from Sigma-Aldrich. Horseradish peroxidase–conjugated secondary antibodies 1:10,000 were obtained from Jackson ImmunoResearch. Flag-tagged GAVPO and Gluc were detected with antibodies to Flag. Images of full-length blots from **Figure 1e** are available in **Supplementary Figure 15**.

Imaging. mCherry and hrGFP fluorescence and chemiluminescence images were taken using an Kodak In-Vivo Multispectral System FX (Carestream Health). For chemiluminescence, we typically used an exposure of 1 s to 5 s for actin and 1 min to 5 min for Flag-tagged proteins with 4 × 4 binning. For the electrophoretic mobility shift assay, fluorescence images of gels stained by GelRed nucleic acid gel stain were acquired with 530 nm excitation and 600 nm emission filters. For fluorescence protein assays of native PAGE and cultured cells, images were acquired with 550 nm excitation and 600 nm emission filters for mCherry and 480 nm excitation and 535 nm emission filters for hrGFP. For mCherry fluorescence imaging of mouse internal organs, liver and kidney were dissected from killed mice using standard surgical procedures. Multispectral images were acquired with excitation filters from 500 nm to 620 nm with 10-nm intervals, and emission filter of 670 nm. The mCherry fluorescence was resolved from background fluorescence by CareStream Multispectral program. For live-cell fluorescence microscopy, images were acquired using an S Plan Fluor ELWD 20×, 0.45 numerical aperture (NA) objective and a digital sight camera on an Eclipse Ti inverted microscope system (Nikon), using a GFP filter for hrGFP and a Texas Red filter for mCherry. A Nikon Plan 4×, 0.10 NA objective was used for fluorescence imaging of liver cryosections.

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