SUPPORTING INFORMATION

"A single-component optogenetic system allows stringent switch of gene expression in yeast cells"

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This file includes:

- **Figures S1 to S10**
- **Tables S1 to S3**
- **Supplementary Note**

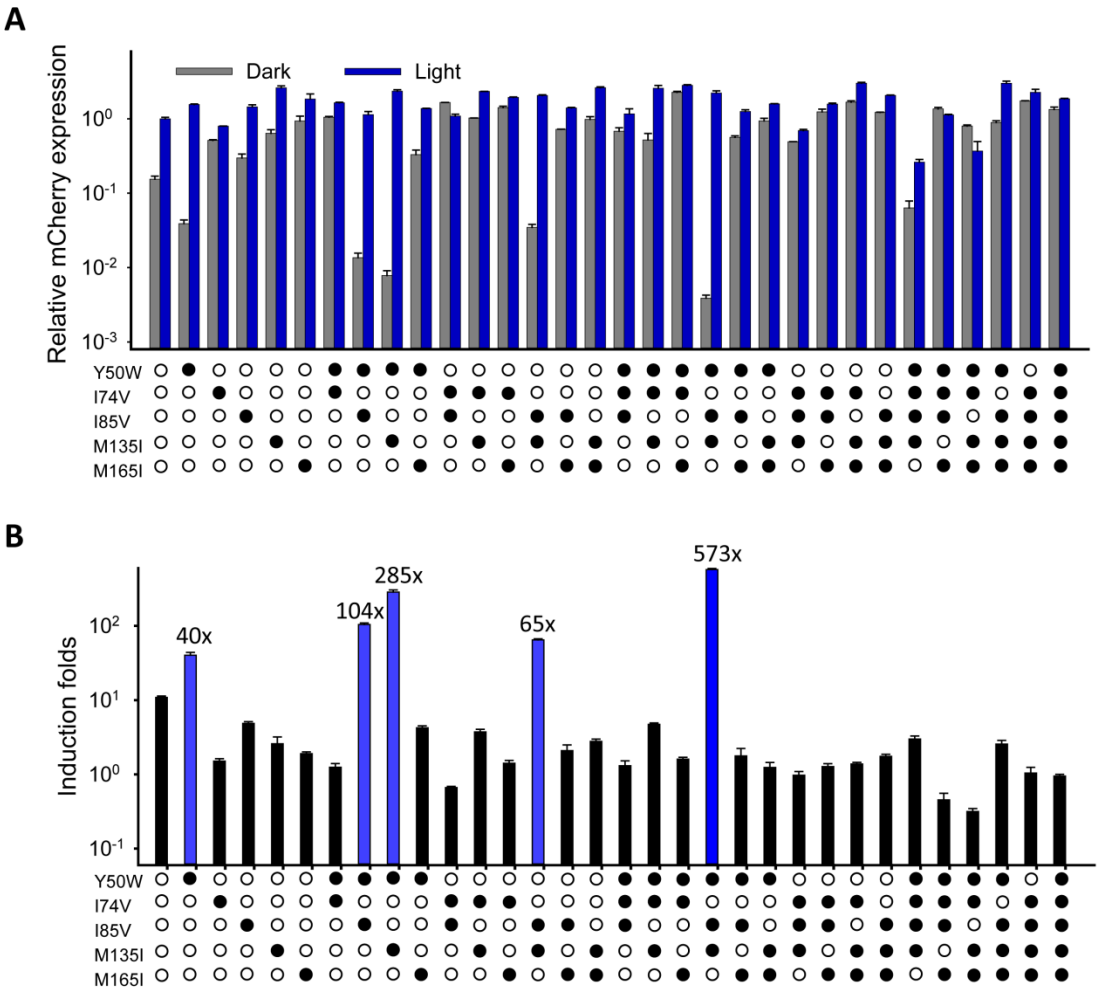

Figure S1 Light-induced gene activation by LVAD mutants. The engineered BY4742 cells transformed with the response plasmid (*8xLexA$_{op}$-Gal1$_{min}$-mCherry*) and the activator plasmid expressing different LVAD mutants were cultured in light or dark conditions for 24 h before mCherry fluorescence was measured (**A**) and induction folds (the ratio of mCherry fluorescence in light and dark conditions) were calculated (**B**). White circle indicated no mutation at this site. Black circle indicated mutation at this site. Data in (**A**) and (**B**) are means of three independent experiments, and error bars indicate the standard deviation.

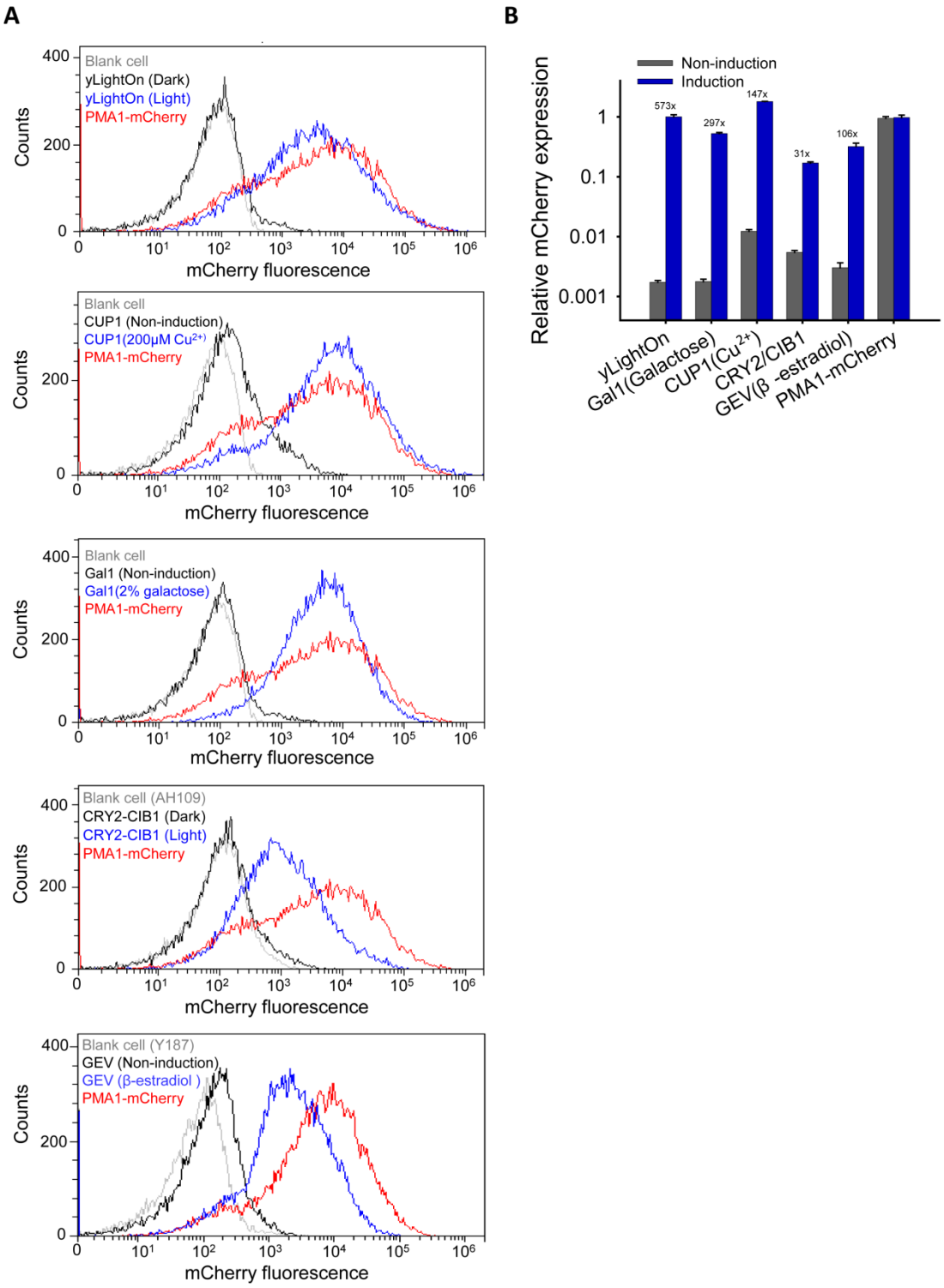

Figure S2 Flow cytometry and statistic results of the light- or chemical-inducible systems. **(A)** FACS analysis of the light- or chemical-inducible systems. The BY4742 cells were transformed with pL8Gal1-mCherry and pADH1(410)-LVADO, or with pCUP1-mCherry, or with pGal1-mCherry. The AH109 cells were transformed with pU5Gal1-mCherry, pGal4BD-CRY2 and pGal4AD-CIB1. The Y187 cells were transformed with pRS316-ACT1-GEV and pRS315-U5Gal1-mCherry. The engineered cells were cultured in non-inducing conditions (for light-inducible systems: dark; for

CUP1, Gal1 and GEV: in the absence of Cu^{2+}, galactose and β-estradiol, respectively) or inducing conditions (for light-inducible systems: blue light; for CUP1 and Gal1: in the presence of 200 μM Cu^{2+}, 2% galactose and 100 nM β-estradiol, respectively). *PMA1*, a strong yeast constitutive promoter, was used as the control. **(B)** Statistics of the FACS results in (**A**). Fluorescence was normalized to yLightOn system in light illumination. Data are means of three independent experiments, and error bars indicate the standard deviation.

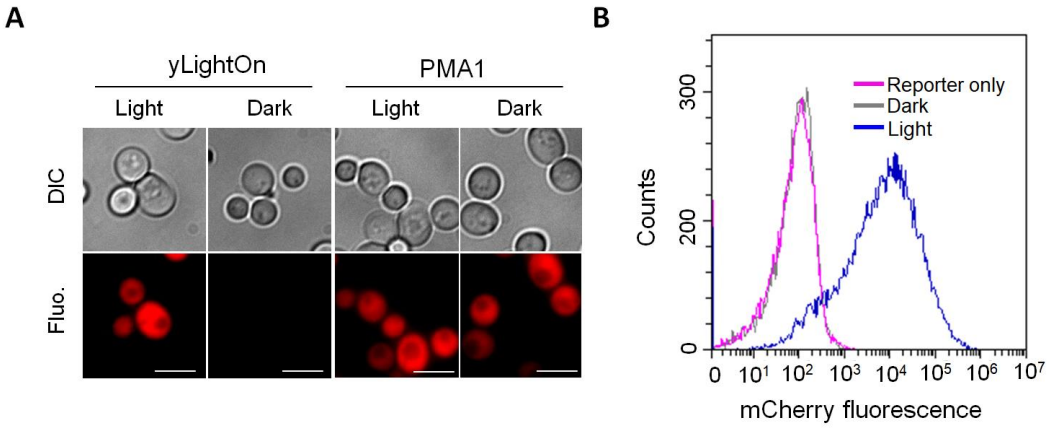

Figure S3 Light-switchable gene expression by yLightOn system. **(A)** The engineered BY4742 cells transformed with yLightOn system using mCherry as the reporter were cultured in light or dark conditions for 24 h before imaging of mCherry fluorescence. *PMA1*, a strong yeast constitutive promoter, was used as the control. Scale bars, 5 μm. (**B**) FACS analysis of light-switchable gene expression by yLightOn system (n=20,000). The BY4742 cells transformed with only the response plasmid were used as the control.

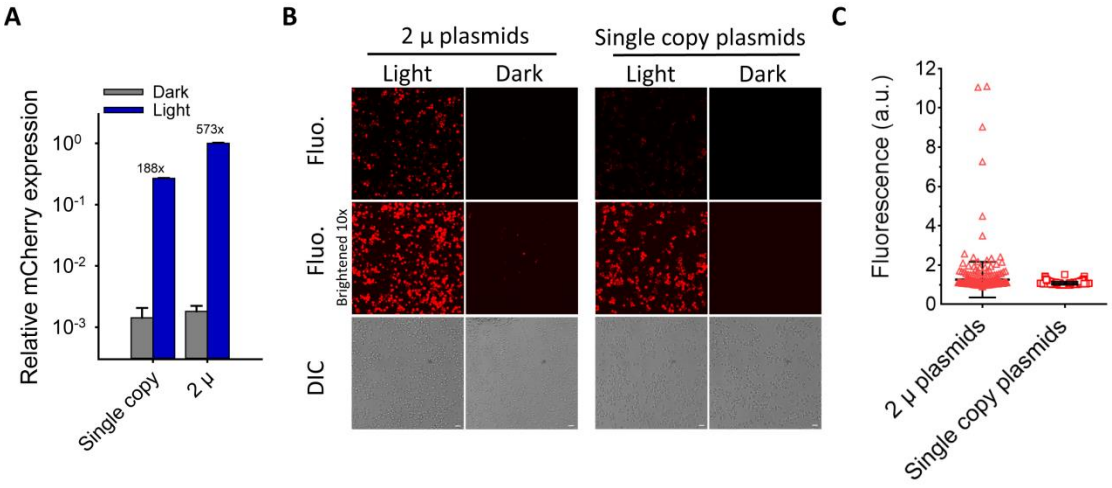

Figure S4 Light-induced mCherry expression by yLightOn system in 2 μ plasmids or single copy plasmids. **(A)** The engineered BY4742 cells transformed with yLightOn system in 2 μ plasmids or single copy plasmids using mCherry as the reporter were cultured in light or dark conditions for 24 h before imaging of mCherry fluorescence. Data are means of three independent experiments, and error bars indicate the standard deviation. **(B)** Fluorescence imaging of mCherry fluorescence. Scale bars, 10 μm. **(C)** Statistics analysis of mCherry expression in dark conditions (n=400 from 3 fields).

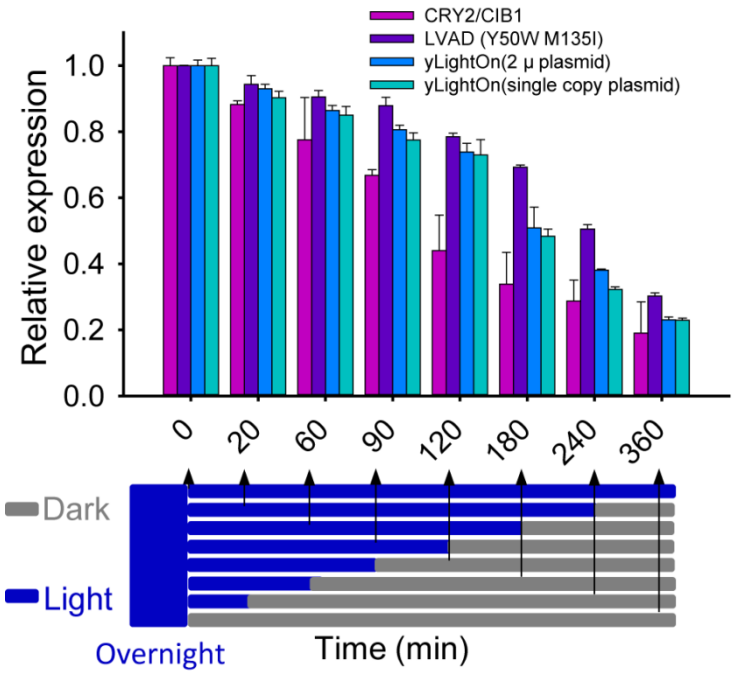

Figure S5 Decay kinetics of the light-switchable gene expression systems from a pulse experiment. The engineered cells were treated with different durations of dark conditions and mCherry fluorescence was measured at 6 h. Data are means of three independent experiments, and error bars indicate the standard deviation.

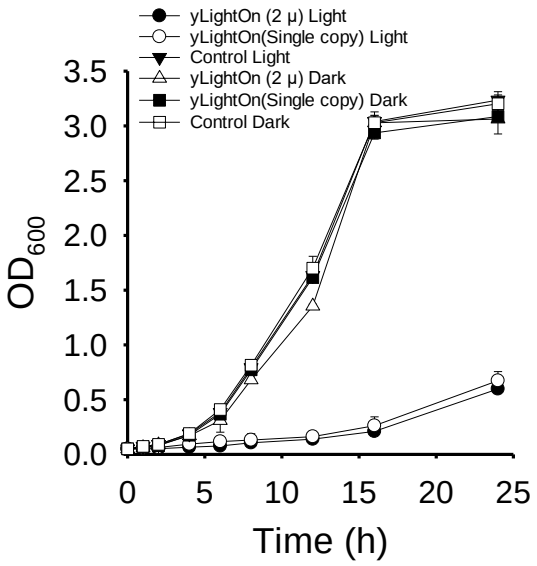

Figure S6 Growth rate of the engineered cells expressing $^{\Delta N}$Sic1 in light and dark conditions. Data are means of three independent experiments, and error bars indicate the standard deviation.

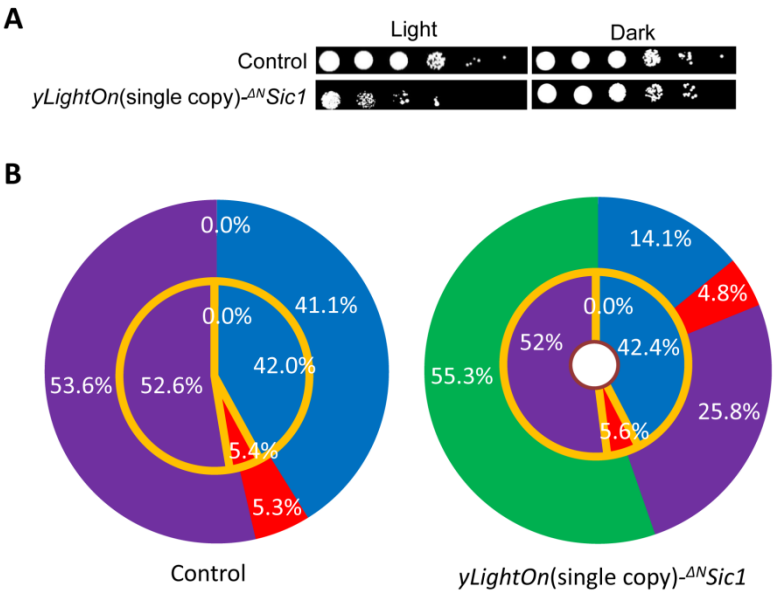

Figure S7 Cell cycle progression controlled by yLightOn system in single copy plasmids. (**A**) The engineered TUB1/YML085C (*Tub*-GFP) cells transformed with yLightOn system in single copy plasmids using $^{\Delta N}$Sic1 as the reporter were serially diluted (1:10; first spot approximately 10^5 cells) and grown in solid medium under light or dark conditions. (**B**) The same yeast cells as in (**A**) were cultured in light or dark conditions for 10 h before imaging. Circular graphs (inner ring, dark conditions; outer ring, light conditions) show the mean distribution of cell cycle stages obtained from six biological replicates counting at least 100 cells for each replicate.

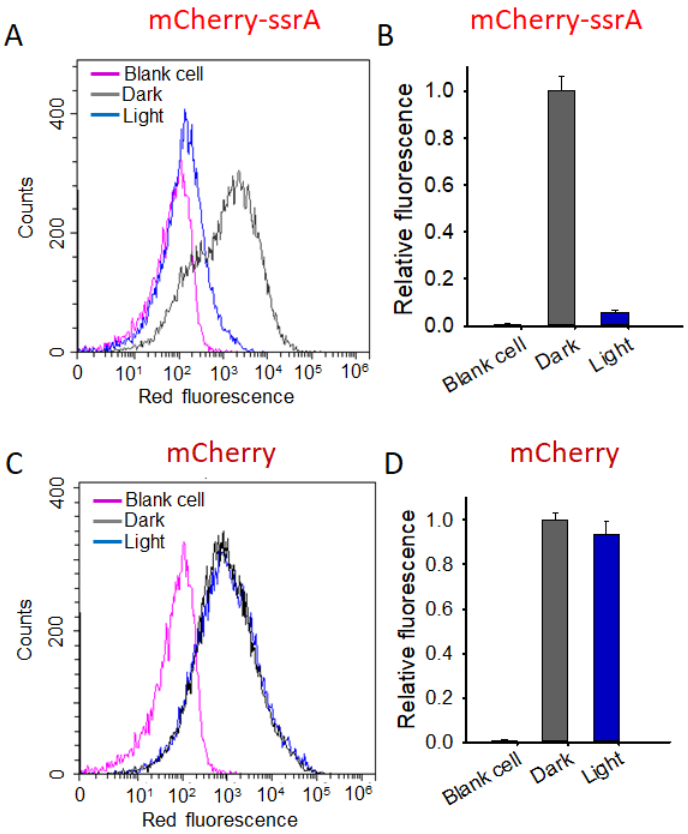

Figure S8 FACS analysis of degradation of ssrA-tagged mCherry. The BY4742 cells transformed with pBi-ClpX-ClpP and pADH1(410)-LVADO-ADH1-mCherry-ssrA **(A)** or pADH1(410)-LVADO-ADH1-mCherry **(C)** were cultured in light or dark conditions for 24 h before analysis of mCherry fluorescence by flow cytometry. **(B)** and **(D)** The statistics of the FACS results in **(A)** and **(C)**, respectively**.** Data are means of three independent experiments, and error bars indicate the standard deviation.

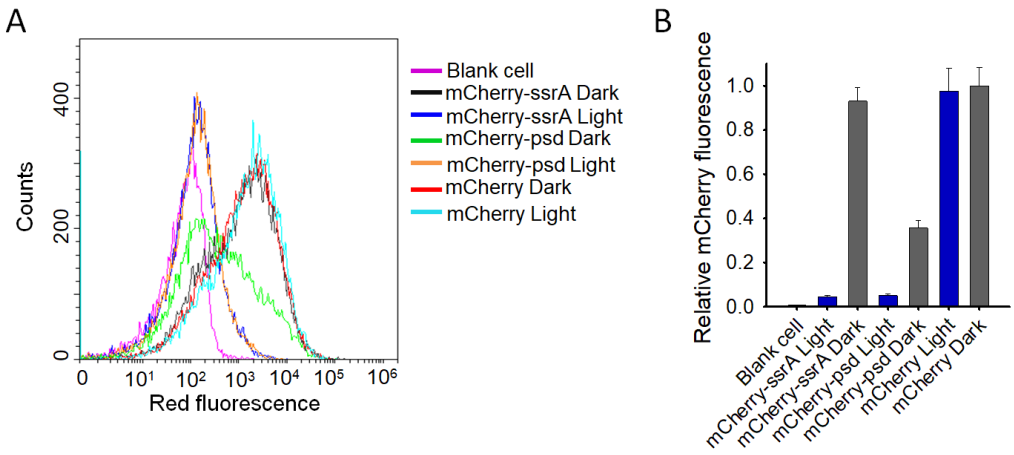

Figure S9 Comparison of ClpX/ClpP and psd degradation systems. **(A)** The BY4742 cells were transformed with pADH1(410)-LVADO-ADH1-mCherry-ssrA and pBi-ClpX-ClpP, or with pADH1-mCherry-psd. The engineered cells were cultured in light or dark conditions for 24 h before analysis of mCherry fluorescence by flow cytometry. mCherry without degradation tag driven by ADH1 promoter were used as the controls. **(B)** The statistics of the FACS results in **(A).** Fluorescence was normalized to mCherry without degradation tag driven by ADH1 promoter in dark conditions. Data are means of three independent experiments, and error bars indicate the standard deviation.

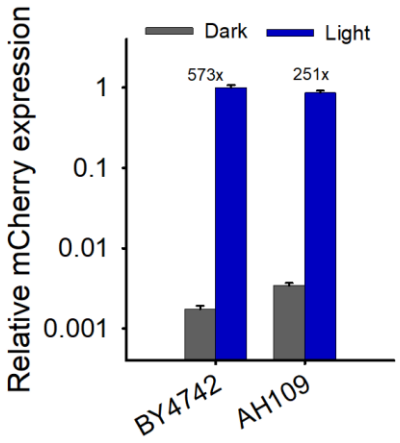

Figure S10 Light-switched gene expression by yLightOn system in different yeast strains. The engineered BY4742 and AH109 cells transformed with yLightOn system using mCherry as the reporter were cultured in light or dark conditions for 24 h before mCherry fluorescence was determined. Fluorescence was normalized to yLightOn system in BY4742 cells upon light illumination. Data are means of three independent experiments, and error bars indicate the standard deviation.

Table S1 Hill function fitting results for gene expression activation by light-switchable gene expression systems.

Parameter	LVADO		LVAD (Y50W M135I)		CRY2/CIB1		Unit
	Best-fit value	Fit standard error	Best-fit value	Fit standard error	Best-fit value	Fit standard error	
a	1.0298	0.1433	1.0219	0.0947	1.2246	0.2400	
b	0.0059	0.0048	0.0036	0.0005	0.0434	0.0040	
n	2.6027	0.7457	0.9811	0.0711	0.7856	0.0912	
k	0.6537	0.1254	0.0608	0.0164	0.2732	0.0869	W/m^2

Data fitted to activating Hill function $[b + (a * Ir^n)/(Ir^n + k^n)]$ using the method of least squares[1].

b represents the leak expression in darkness.
a+b represents the maximal expression when cells were illuminated with light of infinite intensity
n represents Hill coefficient.
k represents the light intensity for half-maximal response.

Table S2 Hill function fitting results for protein degradation

	mCherry-ssrA		mCherry-psd		
Parameter	**Best-fit value**	**Fit standard error**	**Best-fit value**	**Fit standard error**	**Unit**
a	0.9794	0.1015	1.0135	0.2046	
b	0.0339	0.0010	0.0401	0.0659	
n	2.0518	0.1422	0.6596	0.1652	
k	0.0664	0.0109	0.2926	0.1742	W/m^2

Data fitted to repressing Hill function $[b + (a * k^n)/(Ir^n + k^n)]$ using the method of least squares[1].

b represents the background expression when cells were illuminated with light of infinite intensity.
a+b represents the maximal expression in darkness.
n represents Hill coefficient.
k represents the light intensity for half-maximal response.

Table S3 Plasmids designed and used in this study

Plasmids	Description	Replication region	Selectable markers	Reference or source
pSH18-34	A reporter vector containing eight copies of LexA operator sequence and Gal1 minimal promoter driven expression of LacZ	2μ	URA3	Invitrogen
pGal4BD-CRY2	Constitutive expression of Gal4BD-CRY2 under the control of ADH1 promoter.	2μ	TRP1	Addgene
pGal4AD-CIB1	Constitutive expression of Gal4AD-CIB1 under the control of ADH1 promoter.	2μ	LEU2	Addgene
pLEVI-mCherry	Constitutive light-switchable-transcription factor LEVI expression vector.	CDF(E.coli)	—	Ref. 2
pLEVI-sfGFP	Constitutive light-switchable transcription factor LEVI expression vector.	CDF(E.coli)	—	Ref. 2
pGAVPO	Constitutive light-switchable transactivation factor GAVPO expression vector.			Ref. 3
pU5-Gluc	A reporter vector for LigthOn system containing five copies of UAS_G and E1b minimal promoter driven expression of Gluc.	pUC(E.coli)	—	Ref. 3
pGADT7	A commercialized plasmid vector containing ADH1 promoter.	2μ	LEU2	Clontech
pYES2.1 TOPO	A commercialized plasmid vector containing Gal1 promoter.	2μ	URA3	Invitrogen
pGBKT7	A commercialized plasmid vector containing ADH1 promoter and Gal4 activation domain.	2μ	TRP1	Clontech
pL8Gal1-mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and Gal1 minimal promoter driven expression of mCherry. Eight copies of LexA operator sequence was synthesized and cloned into pYES2.1 TOPO vector whose UAS_G sequence was removed by PCR, resulting in pL8Gal1 plasmid. mCherry encoding gene was amplified from pLEVI-mCherry and cloned into pL8Gal1 using BamHI and EcoRI digestion to obtain pL8Gal1-mCherry.	2μ	URA3	This work
pL4Gal1-mCherry	A reporter vector for yLigthOn system containing four copies of LexA operator	2μ	URA3	This work

	sequence and Gal1 minimal promoter driven expression of mCherry. Four copies of LexA operator sequence was synthesized and cloned into pL8Gal1-mCherry using Acc651 and XhoI digestion to obtain pL4Gal1-mCherry.			
pL2Gal1-mCherry	A reporter vector for yLigthOn system containing two copies of LexA operator sequence and Gal1 minimal promoter driven expression of mCherry. pL4Gal1-mCherry was amplified using primers containing two copies of LexA operator sequences; the linearized vector was phosphoresced and ligated.	2μ	URA3	This work
pL1Gal1-mCherry	A reporter vector for yLigthOn system containing single copy of LexA operator sequence and Gal1 minimal promoter driven expression of mCherry. pL4Gal1-mCherry was amplified using primers containing single copy of LexA operator sequences; the linearized vector was phosphoresced and ligated.	2μ	URA3	This work
pL8CYC-mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and CYC minimal promoter driven expression of mCherry. CYC promoter was PCR-amplified from the yeast BY4742 genome, digested and ligated into XhoI and BamHI sites of pL8Gal1-mCherry.	2μ	URA3	This work
pL8cyc100-mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and cyc100 minimal promoter driven expression of mCherry. Single mutation by reverse PCR was performed on the basis of pL8CYC-mCherry.	2μ	URA3	This work
pL8cyc70-mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and cyc70 minimal promoter driven expression of mCherry. Single mutation by reverse PCR was performed on the basis of pL8CYC-mCherry.	2μ	URA3	This work
pL8cyc43-mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and cyc43 minimal promoter driven expression of mCherry. Mutations	2μ	URA3	This work

	by reverse PCR was performed on the basis of pL8CYC-mCherry.			
pL8cyc28-mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and cyc28 minimal promoter driven expression of mCherry. Mutations by reverse PCR was performed on the basis of pL8CYC-mCherry.	2μ	URA3	This work
pL8cyc16-mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and cyc16 minimal promoter driven expression of mCherry. Mutations by reverse PCR was performed on the basis of pL8CYC-mCherry.	2μ	URA3	This work
pL8Gal1(10)- mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and Gal1 minimal promoter with a 10 bp spacer between them. pL8Gal1-mCherry was amplified using primers containing 10 bp random sequences; the linearized vector was phosphoresced and ligated.	2μ	URA3	This work
pL8Gal1(20)- mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and Gal1 minimal promoter with a 20 bp spacer between them. pL8Gal1-mCherry was amplified using primers containing 20 bp random sequences; the linearized vector was phosphoresced and ligated.	2μ	URA3	This work
pL8Gal1(50)- mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and Gal1 minimal promoter with a 50 bp spacer between them. pL8Gal1-mCherry was amplified using primers containing 50 bp random sequences; the linearized vector was phosphoresced and ligated.	2μ	URA3	This work
pL8Gal1(100)- mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and Gal1 minimal promoter with a 100 bp spacer between them. A 100 bp random sequence spacer was synthesized and inserted into pL8Gal1-mCherry using Hieff Clone^{TM} One Step Cloning Kit (YEASEN).	2μ	URA3	This work

pL8Gal1(200)- mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and Gal1 minimal promoter with a 200 bp spacer between them. A 200 bp random sequence spacer was synthesized and inserted into pL8Gal1-mCherry using Hieff Clone^{TM} One Step Cloning Kit (YEASEN).	2μ	URA3	This work
pL8Gal1(500)- mCherry	A reporter vector for yLigthOn system containing eight copies of LexA operator sequence and Gal1 minimal promoter with a 500 bp spacer between them. A 500 bp random sequence spacer was synthesized and inserted into pL8Gal1-mCherry using Hieff Clone^{TM} One Step Cloning Kit (YEASEN).	2μ	URA3	This work
pL8Gal1(truncation)-mCherry	A reporter vector for yLightOn system containing eight copies of LexA operator sequence and Gal1(truncation) minimal promoter. Truncation was performed by reverse PCR on the basis of pL8Gal1-mCherry.	2μ	URA3	This work
pADH1-LVAD	Constitutive expression of LVAD under the control of ADH1 promoter. LEVI amplified from pLEVI-mCherry was fused to Gal4AD-NLS by overlap PCR to obtain LVAD gene fragment. pGADT7 vector was linearized using reverse PCR to remove the original Gal4AD sequence and the multiple cloning site (MCS), and to introduce new EcoRI and XhoI sites at the two ends. The LVAD gene fragment was then ligated into EcoRI and XhoI sites of the linearized pGADT7.	2μ	LEU2	This work
pADH1(410)-LVAD	Constitutive expression of LVAD under the control of ADH1(410) promoter. ADH1(410) promoter was PCR-amplified from pGADT7 vector, digested and ligated into XbaI and EcoRI sites of pADH1-LVAD.	2μ	LEU2	This work
pADH1(210)-LVAD	Constitutive expression of LVAD under the control of ADH1(210) promoter. ADH1(210) promoter was PCR-amplified from pGADT7 vector, digested and ligated into XbaI and EcoRI sites of pADH1-LVAD.	2μ	LEU2	This work

pPMA1-LVAD	Constitutive expression of LVAD under the control of PMA1 promoter. PMA1 promoter was PCR-amplified from the yeast BY4742 genome, digested and ligated into XbaI and EcoRI sites of pADH1-LVAD.	2μ	LEU2	This work
pL8Gal1-His3	A reporter vector for yLigthOn system using His3 as the reporter. His3 gene was synthesized and ligated into BamHI and EcoRI of pL8Gal1-mCherry.	2μ	URA3	This work
pL8Gal1-$^{\Delta N}$Sic1	A reporter vector for yLigthOn system using $^{\Delta N}$Sic1 as the reporter. $^{\Delta N}$Sic1 was PCR-amplified from the yeast BY4742 genome, digested and ligated into BamHI and EcoRI of pL8Gal1-mCherry.	2μ	URA3	This work
pADH1(410)-LVAD (mutants)	Constitutive expression of LVAD variants under control of ADH(410) promoter. Mutations by reverse PCR was performed on the basis of pADH1(410)-LVAD.	2μ	LEU2	This work
pBi-mCherry-sfGFP	A bidirectional module using mCherry and sfGFP as the reporters. Gal1 minimal promoter amplified from pL8Gal1-mCherry was fused to sfGFP-ADH terminator sequence. The fused fragment was digested and ligated into Acc65I and SacI sites of pL8Gal1-mCherry.	2μ	URA3	This work
pBi-ClpX-ClpP	A bidirectional module using ClpP and ClpX as the reporters. ClpP and ClpX amplified from the *E.coli* genome, were digested and ligated into BglII/HindIII and BamHI/EcoRI sites of pBi-mCherry-sfGFP, respectively.	2μ	URA3	This work
pYE-ADH1-mCherry	Vector constitutive expression of mCherry under the control of ADH1 promoter. ADH1-mCherry was PCR-amplified from pADH1-mCherry, digested and ligated into the linearized pYES2.1 TOPO vector using BamHI and EcoRI digestion.	2μ	URA3	This work
pADH1(410)-LVADO-ADH1-mCherry-ssrA	Vector containing ADH1(410)-driven expression of LVADO and ADH1-driven expression of mCherry-ssrA. ADH1-mCherry-ssrA amplified from pYE-ADH1-mCherry was fused to CYC terminator sequence amplified from pL8CYC100-mCherry; the obtained fragment was digested and ligated into XbaI	2μ	LEU2	This work

	and SalI of plasmid pADH1(410)-LVADO.			
pADH1(410)-LVADO-ADH1-mCherry	Vector containing ADH1(410) driven expression of LVADO and ADH1-driven expression of mCherry.	2μ	LEU2	This work
pU5Gal1-mCherry	A reporter vector containing five copies of UAS_G, Gal1 minimal promoter and mCherry reporter. Five copies of UAS_G was amplified from pU5-Gluc and inserted into Acc65I and XhoI sites of pL8Gal1-mCherry.	2μ	URA3	This work
pPMA1-mCherry	Vector constitutive expression of mCherry under the control of PMA1 promoter. PMA1 promoter amplified from the BY4742 genome was fused to mCherry gene; the obtained fragment was digested and ligated into the linearized pYES2.1 TOPO vector using BamHI and EcoRI digestion.	2μ	URA3	This work
pU5Gal1-mCherry	A reporter vector for CRY2/CIB1 system containing five copies of UAS_G sequence and Gal1 minimal promoter driven expression of mCherry. $5xUAS_G$-Gal1 promoter sequence amplified from pYES2.1 TOPO was fused to mCherry by overlapping PCR to obtain $5xUAS_G$-Gal1-mCherry gene fragment that was then inserted into pSH18-34 vector using Hieff Clone™ One Step Cloning Kit.	2μ	URA3	This work
pADH1-mCherry-ssrA	Vector containing ADH1 promoter driven expression of mCherry-ssrA. pADH1(410)-LVADO-ADH1-mCherry-ssrA was amplified using reverse PCR to remove ADH1(410)-LVADO sequence. The linearized fragment was phosphorylated and ligated.	2μ	LEU2	This work
pADH1-mCherry-psd	Vector containing ADH1 promoter driven expression of mCherry-psd. ssrA sequence in pADH1-mCherry-ssrA was then replaced by the synthesized AtLOV2-cODC (psd) sequence using Hieff Clone™ One Step Cloning Kit.	2μ	LEU2	This work
pCUP1-mCherry	Vector containing copper ion inducible CUP1 promoter driven the expression of mCherry reporter. CUP1 promoter was PCR-amplified from the yeast BY4742	2μ	URA3	This work

	genome, and then was digested and ligated into Acc651 and BamHI sites of pL8Gal1-mCherry.			
pGal1-mCherry	Vector containing galactose inducible Gal1 promoter driven the expression of mCherry reporter. Gal1 promoter was PCR-amplified from pYES2.1 TOPO, and was then digested and ligated into Acc651 and BamHI of pL8Gal1-mCherry.	2μ	URA3	This work
pRS313	A single copy plasmid using HIS3 as a selective screening marker.	CEN/ARS	HIS3	ATCC
pRS315	A single copy plasmid using LEU2 as a selective screening marker.	CEN/ARS	LEU2	ATCC
pRS316	A single copy plasmid using URA3 as a selective screening marker.	CEN/ARS	URA3	ATCC
pRS315-ADH1(410)-LVADO	A single copy vector containing a constitutive promoter ADH1(410) driven the expression of transcription factor LVADO. ADH1(410)-LVADO was PCR-amplified from pADH1(410)-LVADO-ADH1-mCherry-ssrA, and then was digested and ligated in NotI and SmaI of pRS315.	CEN/ARS	LEU2	This work
pRS315-PMA1-LVADO	A single copy vector containing a constitutive promoter PMA1 driven the expression of transcription factor LVADO. PMA1 promoter was PCR-amplified from pPMA1-LVAD and inserted into pRS315-ADH1(410)-LVADO using Hieff Clone™ One Step Cloning Kit (YEASEN).	CEN/ARS	LEU2	This work
pRS315-ADH1-LVADO	A single copy vector containing a constitutive promoter ADH1 driven the expression of transcription factor LVADO. ADH1 promoter was PCR-amplified from pADH1-LVAD and inserted into pRS315-ADH1(410)-LVADO using Hieff Clone™ One Step Cloning Kit (YEASEN).	CEN/ARS	LEU2	This work
pRS316-L8Gal1-mCherry	A reporter vector with single copy containing eight copies of LexA operator sequence and Gal1 minimal promoter driven expression of mCherry. L8Gal1-mCherry was PCR-amplified from pL8Gal1-mCherry and digested and ligated in NotI and SmaI of pRS316.	CEN/ARS	URA3	This work
pRS316-L8Gal1-$^{\Delta N}$Sic1	A reporter vector with single copy using $^{\Delta N}$Sic1 as the reporter. L8Gal1-$^{\Delta N}$Sic1 was PCR-amplified from pL8Gal1-$^{\Delta N}$Sic1 and cloned into pRS316 using NotI and SmaI	CEN/ARS	URA3	This work

	digestion to obtain pL8Gal1-mCherry.			
pRS315-U5Gal1-mCherry	A reporter vector with single copy containing five copies of UAS_G, Gal1 minimal promoter and mCherry reporter. U5Gal1-mCherry was PCR-amplified from pU5Gal1-mCherry and inserted into pRS315 using Hieff Clone^{TM} One Step Cloning Kit (YEASEN).	CEN/ARS	LEU2	This work
pRS316-ACT1-GEV	A single copy vector containing a constitutive *ACT1* promoter, Gal4 DNA binding domain, hormone binding domain(ER), and herpes simplex virus protein VP16(activation domain). *ACT1* promoter sequence was synthesized and digested and ligated in NotI and SpeI of pRS316, resulting in pRS316-ACT1 plasmid. Gal4 DNA binding domain PCR-amplified from pGAVPO was fused with hormone binding domain(ER) and VP16 synthesized to obtain GEV fragment, and then digested and ligated in SpeI and EcoRI of pRS316-ACT1.	CEN/ARS	URA3	This work

Supplementary Note

LVADO protein sequence

MKALTARQQEVFDLIRDHISQTGMPPTRAEIAQRLGFRSPNAAEEHLKALARKGVIEIVSGASRGIRLLQEE
EEGLPLVGRVAAGEPQHTLYAPGGYDIMGWLIQIMKRPNPQVELGPVDTSVALILCDLKQKDTPVVYASEA
FLYMTGYSNAEVLGRNCRFLQSPDGMVKPKSTRKYVDSNTINTIRKAIDRNAEVQVEVVNFKKNGQRFV
NFLTMIPVRDETGEYRYSMGFQCETEGSGGGGSGGGGSNFNQSGNIADSSLSFTFTNSSNGPNLITTQTN
SQALSQPIASSNVHDNFMNNEITASKIDDGNNSKPLSPGWTDQTAYNAFGITTGMFNTTTMDDVYNYLF
DDEDTPPNPKKESTSSELPKKKRKVEDP

8xLexA$_{op}$-Gal1 minimal promoter-mCherry DNA sequence

CTGTATATAAAACCAGTGGTTATATGTACAGTACTGCTGTATATAAAACCAGTGGTTATATGTACAGTACGGCG
CGCCTGCTGTATATAAAACCAGTGGTTATATGTACAGTACTGCTGTATATAAAACCAGTGGTTATATGTACAGTA
CGGCGCGCCGCTAGCGGGGGGCTATAAACTCGAGAACAATAAAGATTCTACAATACTAGCTTTTATGGTTAT
GAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAACGAATCAAATTAACAACCATAGG
ATGATAATGCGATTAGTTTTTTAGCCTTATTTCTGGGGTAATTAATCAGCGAAGCGATGATTTTTGATCTATTAA
CAGATATATAAATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCGGTTTGTATTACTTCTTAT
TCAAATGTAATAAAAGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGAGGGATCCAAAA
AAATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACAT
GGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCA
CCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAG
TTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCC
GAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCC
TCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGT
AATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAA
GGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTA
CAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACA
ACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGA
GCTGTACAAGTAA

sfGFP-Gal1 minimal promoter-8xLexA$_{op}$-Gal1 minimal promoter-mCherry DNA sequence

TTACTTGTACAGCTCGTCCATGCCGTGAGTGATCCCGGCGGCGGTCACGAACTCCAGCAGGACCATGTGATC
GCGCTTCTCGTTGGGGTCTTTGCTCAGCACGGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACG
GGGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGCACGCTGCCGTCCTCCACGTTGTG
GCGGATCTTGAAGTTGGCCTTGATGCCGTTCTTCTGCTTGTCGGCCGTGATATAGACGTTGTGGCTGTTGAA
GTTGTACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTTC
ACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTATGTGCCGTCGTCCTTGAAACTGATGGTG
CGCTCCTGGACGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGGCGCTTCATGTGGTCGGGGTAGC
GGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACGGGCAGCTTGCCGG
TGGTGCAGATGAACTTCAGGGTCAGCTTGCCATTGGTGGCATCGCCCTCGCCCTCGCCTCTCACGCTGAACT
TGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTCGCCCTTG
CTCACCATAGATCTTTGACGTTAAAGTATAGAGGTATATTAACAATTTTTTGTTGATACTTTTATGACATTTGAA
TAAGAAGTAATACAAACTGAAAATGTTGAAAGTATTAGTTAAAGTGGTTATGCAGTTTTTGCATTTATATATCT

GTTAATAGATCAAAAATCATCGCTTCGCTGATTAATTACCCCAGAAATAAGGCTAAAAAACTAATCGCATTATC
ATCCTATGGTTGTTAATTTGATTCGTTCATTTGAAGGTTTGTGGGGCCAGGTTACTGCCAATTTTTCCTCTTCA
TAACCATAAAAGCTAGTATTGTAGAATCTTTATTGTTCTCGAGTTTATAGCCCCCCGGTACCTGCTGTATATAAA
ACCAGTGGTTATATGTACAGTACTGCTGTATATAAAACCAGTGGTTATATGTACAGTACGGCGCGCCTGCTGTA
TATAAAACCAGTGGTTATATGTACAGTACTGCTGTATATAAAACCAGTGGTTATATGTACAGTACGGCGCGCCG
CTAGCGGGGGGCTATAAACTCGAGAACAATAAAGATTCTACAATACTAGCTTTTATGGTTATGAAGAGGAAA
AATTGGCAGTAACCTGGCCCCACAAACCTTCAAATGAACGAATCAAATTAACAACCATAGGATGATAATGCG
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ATGCAAAAACTGCATAACCACTTTAACTAATACTTTCAACATTTTCAGTTTGTATTACTTCTTATTCAAATGTCAT
AAAAGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGATCCAAAAAAATGGTGAGCAAG
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ACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGC
TGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCA
AGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGG
GAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGC
GAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGAC
CATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCA
GAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCC
CGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCAT
CGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAA

ClpP-Gal1 minimal promoter-8xLexA$_{op}$-Gal1 minimal promoter-ClpX DNA sequence

TCAATTACGATGGGTCAGAATCGAATCGACCAGACCGTATTCCACCGCTTCAGGGGCGGAAAGGAAGCGAT
CGCGCTCGGTATCACGTTCAATCTGTTCTAATGATTGACCCGTATGAAGCGCCATAAGTTCATTCATGCGCCCT
TTAACTTTCAGAATTTCACGGGCATGAATTTCGATATCGGTCGCCTGGCCCTGGTAGCCGCCCAACGGTTGG
TGAATCATCACGCGCGAATTCGGCAGGCAAAAACGTTTACCTTTTGCCCCTGCGGTCAGCAAGAAAGCGCC
CATCGAGGCCGCCTGGCCCATACAGATGGTGCTGACATCAGGCTTGATAAACTGCATGGTGTCATAGATAGA
CATCCCGGCAGTGATCACCCCGCCTGGGGAGTTAATGTACAGATAGATATCTTTTTCTGGGTTTTCCGCTTCC
AGGAACAGCATCTGCGCCACAATCAGGTTAGCCATGTGGTCTTCAACCTGGCCAGTCAGAAAAATGACGCG
TTCCTTAAGTAGACGAGAATAGATATCAAAAGAGCGCTCACCGCGTGAGGTCTGTTCAATGACCATCGGCAC
CAGCGCCATATGGGGTGCAAAGTTATCTCGTTCGCCGCTGTATGACATAGATCTTTGACGTTAAAGTATAGAG
GTATATTAACAATTTTTTGTTGATACTTTTATGACATTTGAATAAGAAGTAATACAAACTGAAAATGTTGAAAG
TATTAGTTAAAGTGGTTATGCAGTTTTTGCATTTATATATCTGTTAATAGATCAAAAATCATCGCTTCGCTGATTA
ATTACCCCAGAAATAAGGCTAAAAAACTAATCGCATTATCATCCTATGGTTGTTAATTTGATTCGTTCATTTGAA
GGTTTGTGGGGCCAGGTTACTGCCAATTTTTCCTCTTCATAACCATAAAAGCTAGTATTGTAGAATCTTTATTG
TTCTCGAGTTTATAGCCCCCCGGTACCTGCTGTATATAAAACCAGTGGTTATATGTACAGTACTGCTGTATATAA
AACCAGTGGTTATATGTACAGTACGGCGCGCCTGCTGTATATAAAACCAGTGGTTATATGTACAGTACTGCTGT
ATATAAAACCAGTGGTTATATGTACAGTACGGCGCGCCGCTAGCGGGGGGCTATAAACTCGAGAACAATAAA
GATTCTACAATACTAGCTTTTATGGTTATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAA
TGAACGAATCAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATTTCTGGGGTAATTAATC
AGCGAAGCGATGATTTTTGATCTATTAACAGATATATAAATGCAAAAACTGCATAACCACTTTAACTAATACTT
TCAACATTTTCAGTTTGTATTACTTCTTATTCAAATGTCATAAAAGTATCAACAAAAAATTGTTAATATACCTCTA
TACTTTAACGTCAAGGATCCAAAAAAATGACAGATAAACGCAAAGATGGCTCAGGCAAATTGCTGTATTGCT
CTTTTTGCGGCAAAAGCCAGCATGAAGTGCGCAAGCTGATTGCCGGTCCATCCGTGTATATCTGCGACGAAT

GTGTTGATTTATGTAACGACATCATTCGCGAAGAGATTAAAGAAGTTGCACCGCATCGTGAACGCAGTGCGC
TACCGACGCCGCATGAAATTCGCAACCACCTGGACGATTACGTTATCGGCCAGGAACAGGCGAAAAAAGTG
CTGGCGGTCGCGGTATACAACCATTACAAACGTCTGCGCAACGGCGATACCAGCAATGGCGTCGAGTTGGG
CAAAAGTAACATTCTGCTGATCGGTCCGACCGGTTCCGGTAAAACGCTGCTGGCTGAAACGCTGGCGCGCC
TGCTGGATGTTCCGTTCACCATGGCCGACGCGACTACACTGACCGAAGCCGGTTATGTGGGTGAAGACGTT
GAAAACATCATTCAGAAGCTGTTGCAGAAATGCGACTACGATGTCCAGAAAGCACAGCGTGGTATTGTCTAC
ATCGATGAAATCGACAAGATTTCTCGTAAGTCAGACAACCCGTCCATTACCCGAGACGTTTCCGGTGAAGGC
GTACAGCAGGCACTGTTGAAACTGATCGAAGGTACGGTAGCTGCTGTTCCACCGCAAGGTGGGCGTAAACA
TCCGCAGCAGGAATTCTTGCAGGTTGATACCTCTAAGATCCTGTTTATTTGTGGCGGTGCGTTTGCCGGTCTG
GATAAAGTGATTTCCCACCGTGTAGAAACCGGCTCCGGCATTGGTTTTGGCGCGACGGTAAAAGCGAAGTC
CGACAAAGCAAGCGAAGGCGAGCTGCTGGCGCAGGTTGAACCGGAAGATCTGATCAAGTTTGGTCTTATC
CCTGAGTTTATTGGTCGTCTGCCGGTTGTCGCAACGTTGAATGAACTGAGCGAAGAAGCTCTGATTCAGATC
CTCAAAGAGCCGAAAAACGCCCTGACCAAGCAGTATCAGGCGCTGTTTAATCTGGAAGGCGTGGATCTGGA
ATTCCGTGACGAGGCGCTGGATGCTATCGCTAAGAAAGCGATGGCGCGTAAAACCGGTGCCCGTGGCCTGC
GTTCCATCGTAGAAGCCGCACTGCTCGATACCATGTACGATCTGCCGTCCATGGAAGACGTCGAAAAAGTGG
TTATCGACGAGTCGGTAATTGATGGTCAAAGCAAACCGTTGCTGATTTATGGCAAGCCGGAAGCGCAACAG
GCATCTGGTGAATAA

ADH1 promoter-mCherry-ssrA

ATCCTTTTGTTGTTTCCGGGTGTACAATATGGACTTCCTCTTTTCTGGCAACCAAACCCATACATCGGGATTCC
TATAATACCTTCGTTGGTCTCCCTAACATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATACCAGACA
AGACATAATGGGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTGGTACATAACGAACTAATACT
GTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTCACTACCCTTTTTCCATTTGCCATCTATTGAGGTAA
TAATAGGCGCATGCAACTTCTTTTCTTTTTTTTTTCTTTTCTCTCTCCCCCGTTGTTGTCTCACCATATCCGCAAT
GACAAAAAAATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTGT
TCCAGAGCTGATGAGGGGTATCTCGAAGCACACGAAACTTTTTCCTTCCTTCATTCACGCACACTACTCTCTA
ATGAGCAACGGTATACGGCCTTCCTTCCAGTTACTTGAATTTGAAATAAAAAAAAGTTTGCTGTCTTGCTATC
AAGTATAAATAGACCTGCAATTATTAATCTTTTGTTTCCTCGTCATTGTTCTCGTTCCCTTTCTTCCTTGTTTCTT
TTTCTGCACAATATTTCAAGCTATACCAAGCATACAATCAACTAGCTTTGCAAAGATGGCCATGGCTTCCAGAT
CTGGTTCTTCTAGAGGTTCTGGATCCGCTATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAG
GAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGG
GCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTT
CGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCC
CGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGC
GTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCA
CCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATG
TACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTAC
GACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACA
TCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGC
CACTCCACCGGCGGCATGGACGAGCTGTACAAGACTATGGGCGGCGGTTCTGCTGCGAACGATGAAAACTA
CGCACTGGCTGCGTAA

Reference

1. Olson, E.J., Hartsough, L.A., Landry, B.P., Shroff, R. and Tabor, J.J. (2014) Characterizing bacterial gene circuit dynamics with optically programmed gene expression signals. *Nat Methods*. 11, 449-455.
2. Chen, X., Liu, R., Ma, Z., Xu, X., Zhang, H., Xu, J., Ouyang, Q. and Yang, Y. (2016) An extraordinary stringent and sensitive light-switchable gene expression system for bacterial cells. *Cell Res*. 26, 854-857.
3. Wang, X., Chen, X. and Yang, Y. (2012) Spatiotemporal control of gene expression by a light-switchable transgene system. *Nat Methods*. 9, 266-269.