

Supplementary Information

Exploring plant-derived phytochrome chaperone proteins for light-switchable transcriptional regulation in mammals

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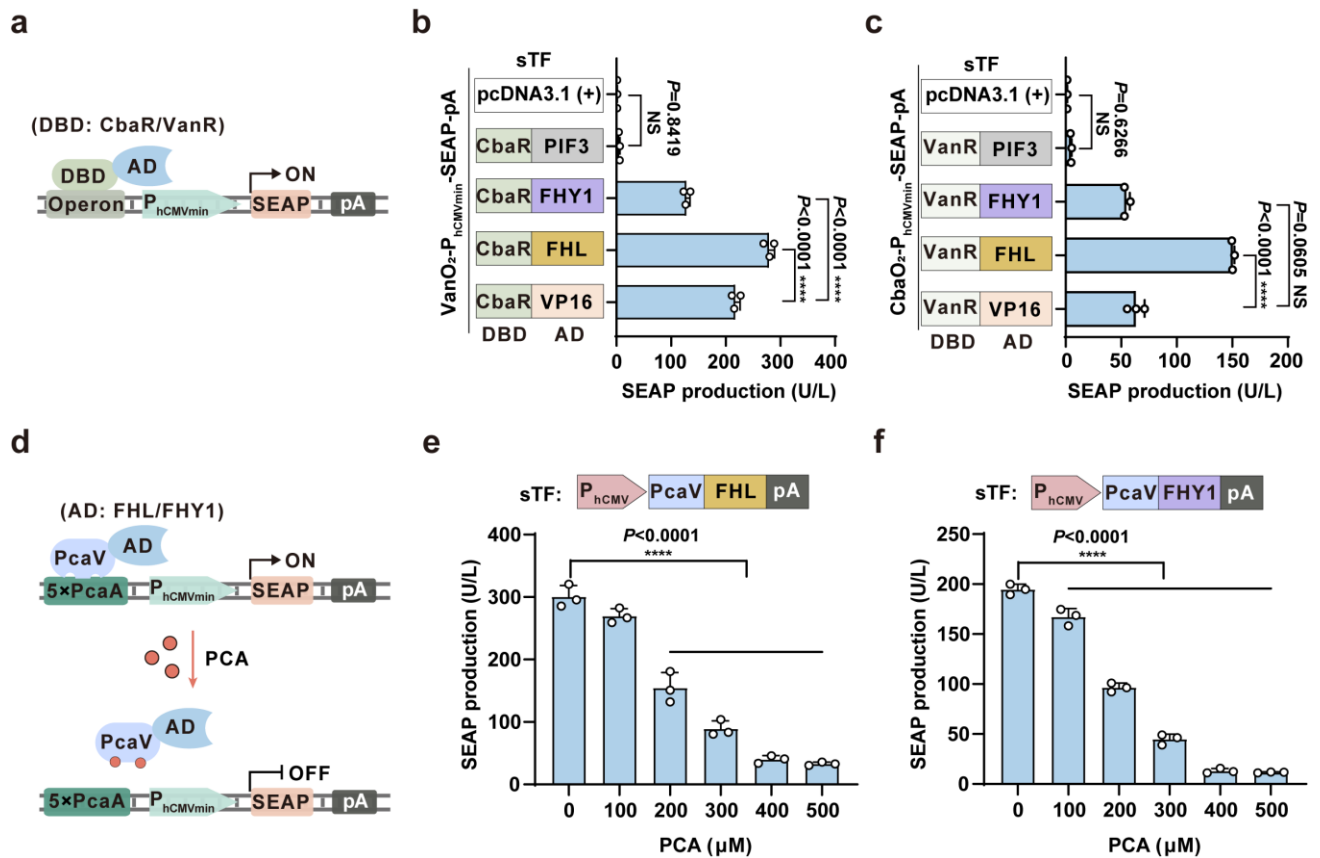
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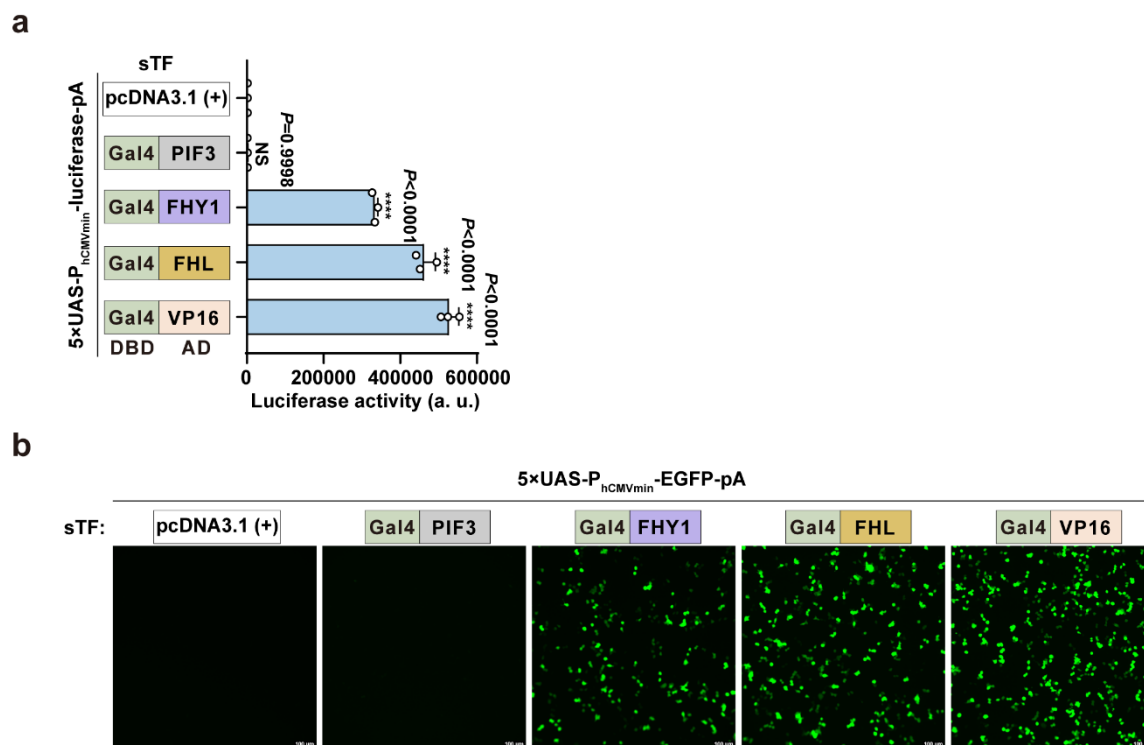
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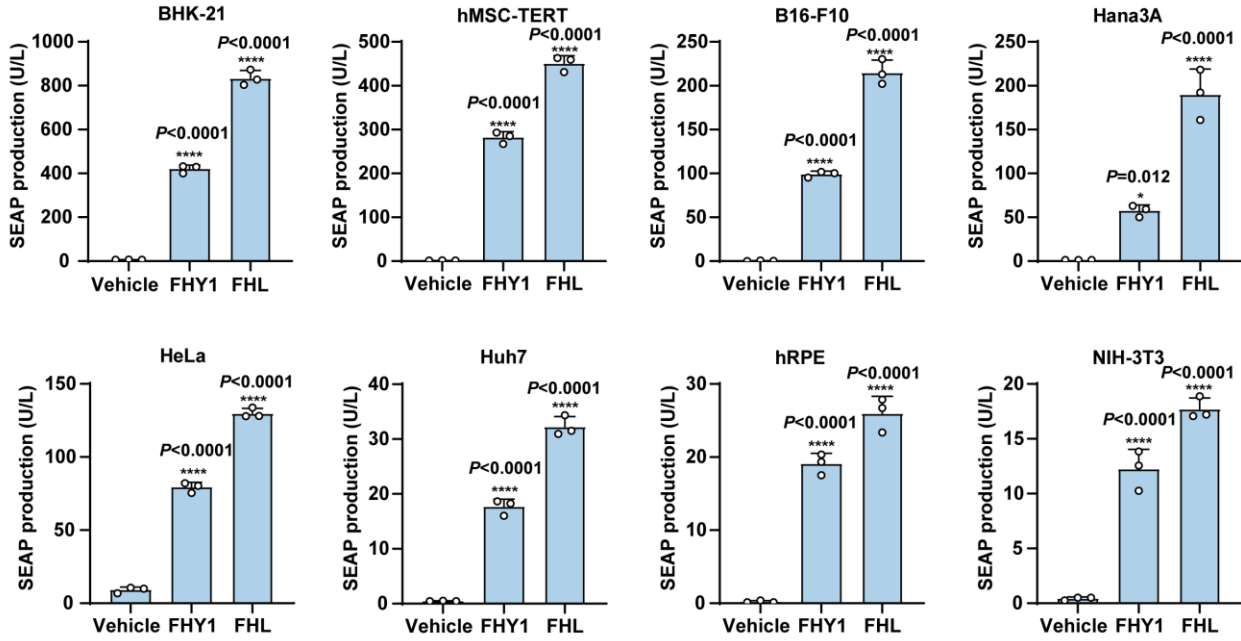


Supplementary Fig. 1 Validation of transcriptional activation functions of the *Arabidopsis*-derived phytochrome chaperone proteins in mammalian cells. **a** Schematic of the design for screening potential transactivators. The DBD (CbaR or VanR) is combined with the candidate transactivator (PIF3, FHY1, FHL, or VP16) to generate a synthetic TF (sTF), which translocates into the nucleus and binds to the synthetic specific promoter to initiate the target gene expression. **b, c** Quantification of gene expression mediated by CbaR-based (**b**) and VanR-based (**c**) sTFs. HEK293T cells (6×10^4) transfected with an sTF alongside the corresponding reporter (CbaO₂-P_{hCMVmin}-SEAP-pA or VanO₂-P_{hCMVmin}-SEAP-pA) were grown for 24 hours before quantifying SEAP production. Control cells were transfected with the reporter and pcDNA3.1(+). **d** Schematic of a protocathechuic acid (PCA)-triggered transcriptional inhibition system using FHL/FHY1. Without PCA, the sTF (PcaV-FHL/FHY1) binds to a chimeric P_{hCMVmin} promoter, with a specific DBD binding site positioned upstream of the promoter to initiate target gene expression. In the presence of PCA, PcaV-FHL/FHY1 is released from the chimeric promoter, terminating target gene expression. **e, f** Dose-dependent gene expression dynamics facilitated by a PCA-responsive sTF. HEK293T cells (6×10^4) transfected with PcaV-FHL (**e**) or PcaV-FHY1 (**f**), along with the SEAP reporter (O_{PcaA}-P_{hCMVmin}-SEAP-pA), were

grown using varying amounts of PCA for 24 hours, before measuring SEAP production. Data are presented as means $\pm$ s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by one-way ANOVA; NS, not significant, **** $p < 0.0001$.



Supplementary Fig. 2 Expression of genes of interest facilitated by different sTFs. a Quantification of luciferase expression mediated by various sTFs. HEK293T cells (6×10^4) transfected with an sTF and the corresponding reporter ($5 \times \text{UAS-P}_{\text{hCMVmin}}$ -luciferase-pA) were grown for 24 hours before quantifying the luciferase production. Data are presented as means $\pm$ s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by one-way ANOVA; NS, not significant, *** $p < 0.001$, **** $p < 0.0001$. **b** Representative fluorescence micrographs of HEK293T cells transfected with different sTFs. HEK293T cells transfected with an sTF and the corresponding reporter ($5 \times \text{UAS-P}_{\text{hCMVmin}}$ -EGFP-pA) were grown for 24 hours before obtaining fluorescence micrographs. Data are representative of three independent experiments. Scale bar, 100 μm . Control cells were transfected with the reporter and pcDNA3.1(+).

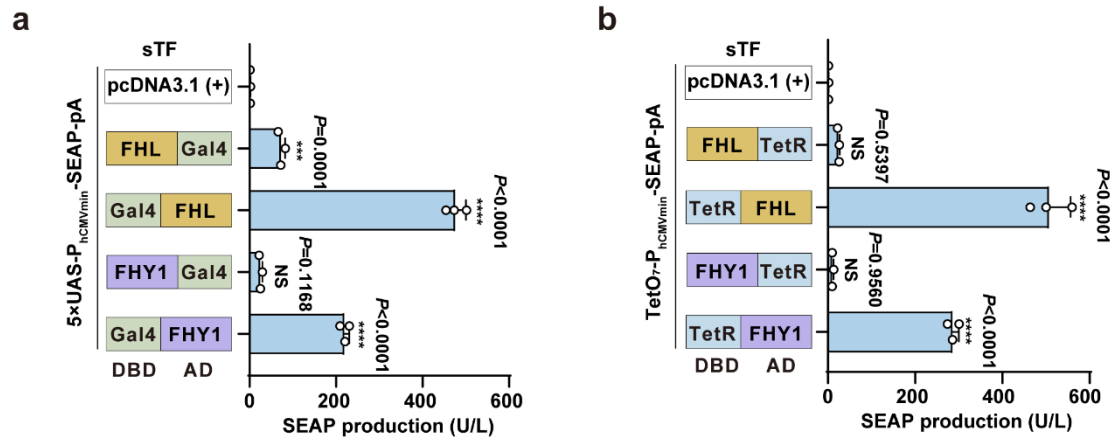


Supplementary Fig. 3 FHL/FHY1-mediated gene expression in diverse mammalian cell lines.

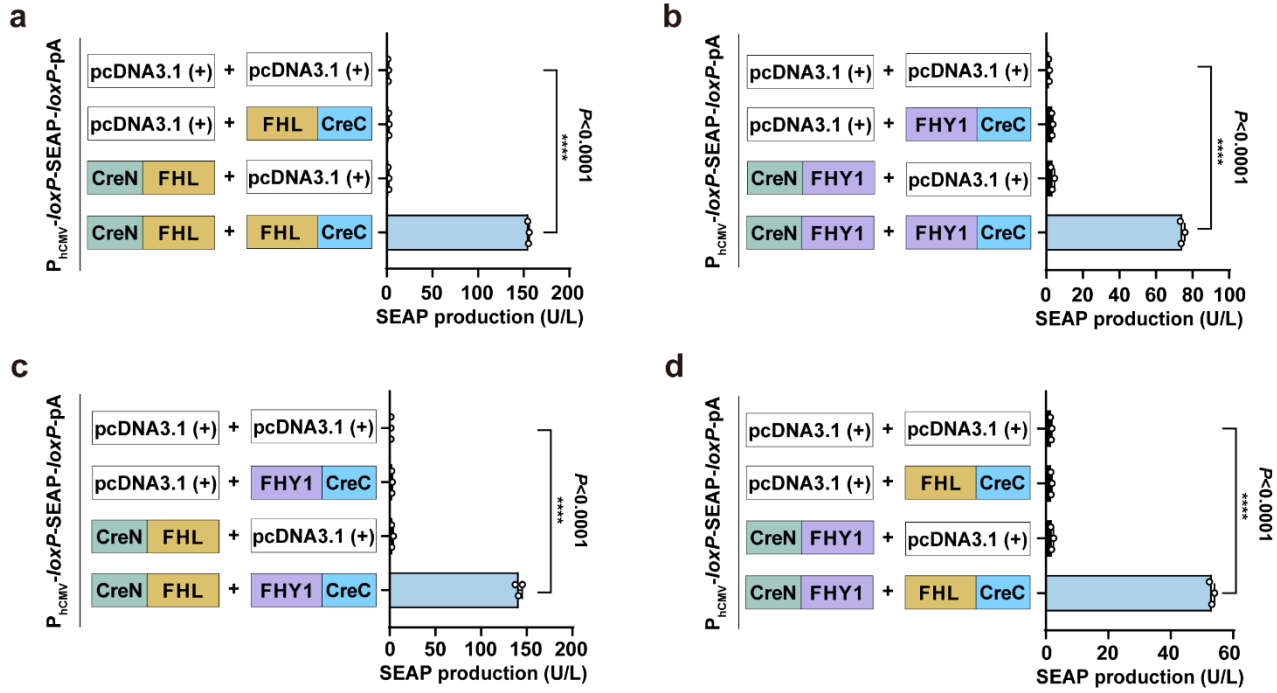
Mammalian cells including BHK-21, hMSC-TERT, B16-F10, Hana3A, HeLa, Huh7, hRPE, and NIH-3T3 were transfected using an sTF (Gal4-FHY1 or Gal4-FHL) as well as the corresponding reporter (5×UAS-P_{hCMVmin}-SEAP-pA), and SEAP production was quantified 24 hours following transfection. Control cells were transfected with the reporter and pcDNA3.1(+). Data are presented as means ± s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by one-way ANOVA; * $p < 0.05$, **** $p < 0.0001$.

FHL	M DDADK	SCSP	SLD	HSD	INDP	MIVAV	ESLDT	SK	32
FHY1	MPEVEVDNNEKP	SEINS	SFH	HMI	ISSSKNV	LKM	EVEV	SK	40
Consensus	m	s	s	h	i		e	s k	
									26
									33
									50
FHL	KRK	LHAEESD	. . .	LLPLP	KHF	CSEHQASLV	NSS	CPSSVID	69
FHY1	KRK	FQTDQSD	ELSL	LLPLS	KHTC		FANVA	CSENTNG	74
Consensus	k r k	s d		l l p l	k h c		n	c	61
FHL	YAE	CSYAMENTKT	SDEAS	SSAS	SFTGPS	LYMFK	DSIYSTGS	109	
FHY1	NSE	IDTEYS . . .	MSYVNS	TTT	SMECNND	IEMK	EESGSCG	111	
Consensus	e		s	s	s		k		
									117
FHL	SSSGYAAT	SS	IEQCF	SKVDHKT	QEDTQ	DFTHMEFI	YHDS	E	149
FHY1	EDKMISFE	SH	LDYIYG . .	TQNLED	FSEKVIEN	ILYLDEQ	E	149	
Consensus		s						e	
									117
									165
FHL	FAVED	LQEV	LNPNVESY	I	LSSAR	WSVSNQD	.SKEAT	TTKPTID	189
FHY1	EEEED	AKGC	SSNAAKFV	LSSGR	WTVN	QDDSTL	HETK	KPTID	190
Consensus	e d				l s s r w v	d		t k p t i d	
									165
FHL	QEF	EQYFSTLM	M						201
FHY1	QEF	EQYFSTLM	L						202
Consensus	q e f	e q y f s t l m							

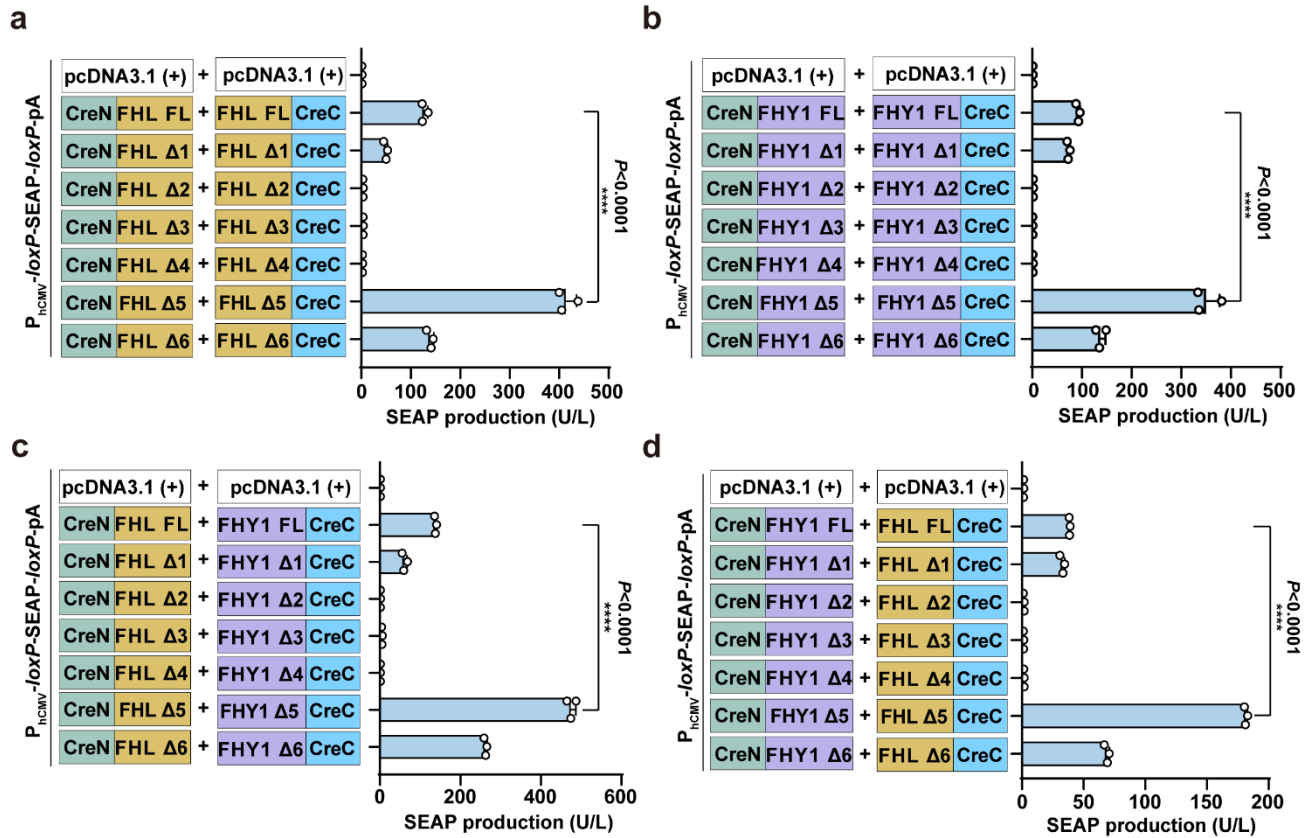
Supplementary Fig. 4 Alignment of amino acid sequences of FHL and FHY1. Identical amino acid residues are indicated in black, and the boundary residue coordinates for each protein are listed at the end. The truncation sites are indicated above (FHL) or below (FHY1) the sequence.



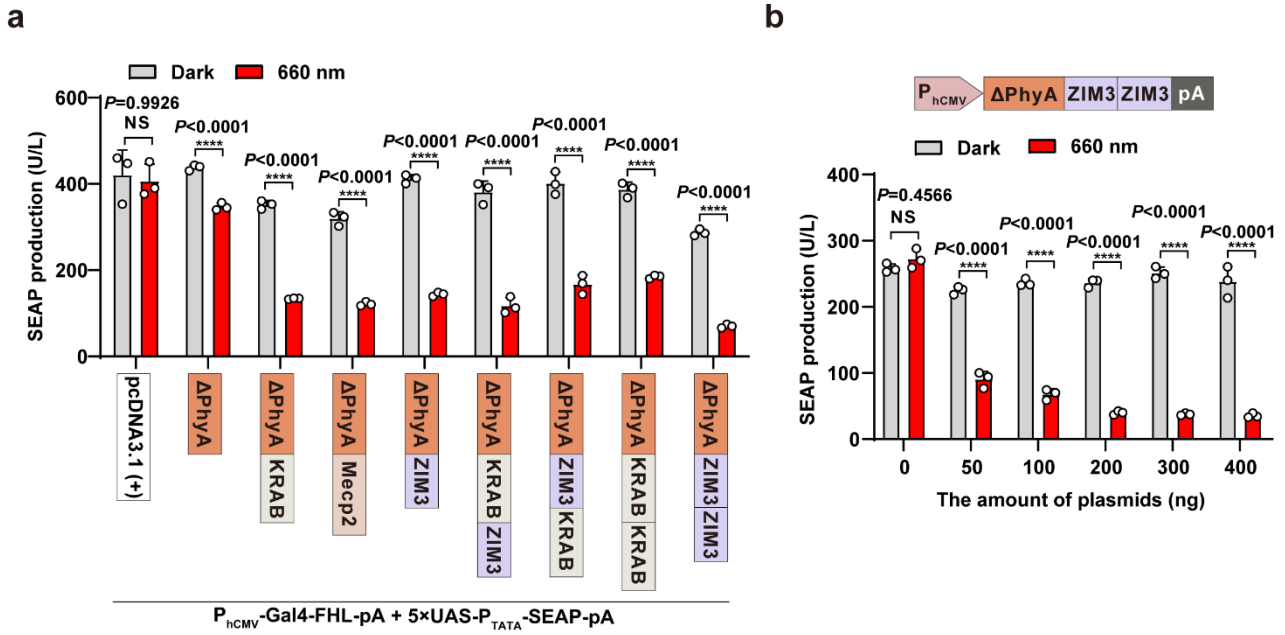
Supplementary Fig. 5 Design and characterization of various sTF constructions for transcriptional activation. sTFs were developed by fusing FHL/FHY1 to the N-terminus or C-terminus of Gal4 (**a**) and TetR (**b**). HEK293T cells (6×10^4) transfected with respective sTFs and the corresponding reporter (5×UAS-P_{hCMVmin}-SEAP-pA or TetO₇-P_{hCMVmin}-SEAP-pA) were grown for 24 hours prior to quantifying SEAP production. Control cells were transfected with the reporter and pcDNA3.1(+). Data are presented as means $\pm$ s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by one-way ANOVA; NS, not significant, *** $p < 0.001$, **** $p < 0.0001$.



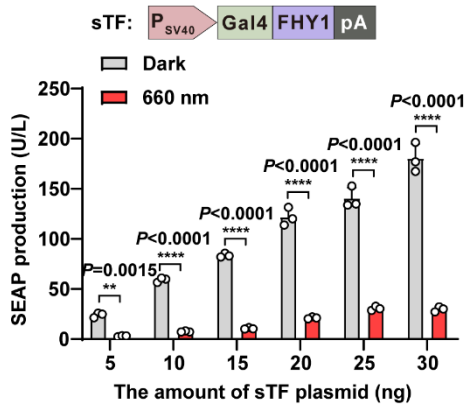
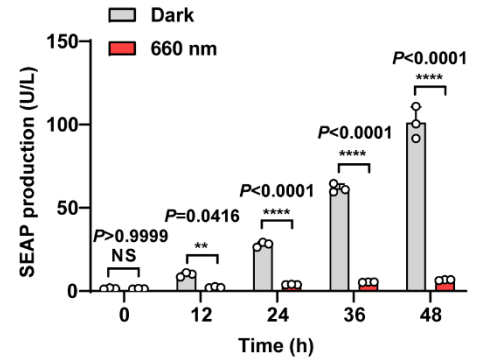
Supplementary Fig. 6 Design and characterization of FHY1/FHL-based split-Cre recombinase systems. **a, b** DNA recombination mediated by homodimerization of full-length FHL (**a**) and FHY1 (**b**). **c, d** DNA recombination induced by heterodimerization of full-length FHL and FHY1. HEK293T cells (6×10^4) transfected with CreN/CreC-FHY1/FHL fusions and the corresponding reporter ($P_{hCMV-loxP-SEAP-loxP-pA}$) were grown for 24 hours prior to quantifying SEAP production. Control cells were transfected with the reporter and pcDNA3.1(+). Data are presented as means $\pm$ s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by two-tailed Student's t -test; **** $p < 0.0001$.



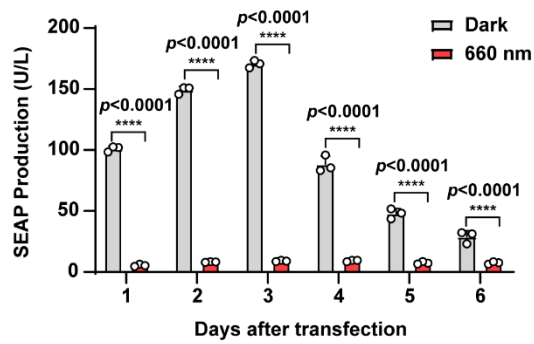
Supplementary Fig. 7 Confirmation of the homo- and/or hetero-dimerization activity of FHY1/FHL truncations with the split-Cre recombinase system. Various FHL and FHY1 truncations were combined with different fragments of split-Cre. HEK293T cells (6×10^4) transfected with respective fusions and the corresponding reporter (P_{hCMV} -*loxP*-SEAP-*loxP*-pA) were grown for 24 hours before quantifying SEAP production. Control cells were transfected with the reporter and pcDNA3.1(+). Data are presented as means $\pm$ s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by two-tailed Student's *t*-test; **** $p < 0.0001$.



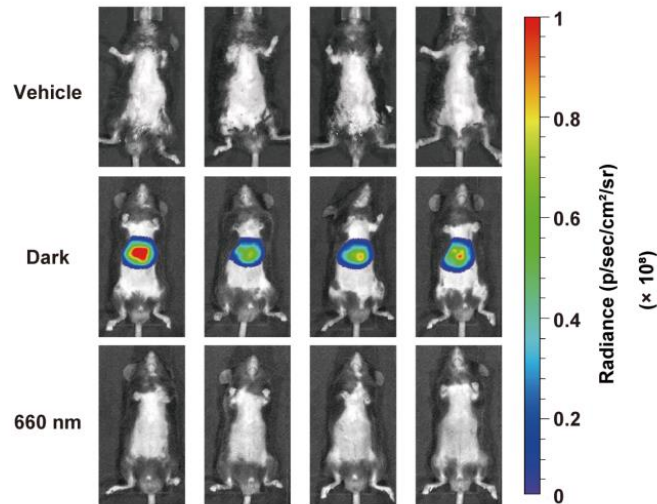
Supplementary Fig. 8 PTRC-mediated transcriptional repression. **a** Transcriptional repression mediated by a light-responsive transcriptional repressor in different configurations. HEK293T cells (6×10^4) transfected with Gal4-FHL, ΔPhyA fused to different transcription repressors, and the corresponding reporter ($5\times\text{UAS-P}_{\text{TATA}}\text{-SEAP-pA}$) were illuminated by red light (660 nm, 1 mW/cm^2) or maintained in darkness for 24 hours prior to quantifying SEAP production. **b** Quantification of PTRC-mediated gene expression with varying amounts of plasmids encoding $\Delta\text{PhyA-2}\times\text{ZIM3}$. Transfected HEK293T cells (6×10^4) were illuminated using red light (660 nm, 1 mW/cm^2) or maintained in darkness for 24 hours before quantifying SEAP production. Control cells were transfected with plasmids encoding Gal4-FHL, the corresponding reporter, and pcDNA3.1(+). Data are presented as means $\pm$ s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by two-way ANOVA; NS, not significant, **** $p < 0.0001$.

a**b**

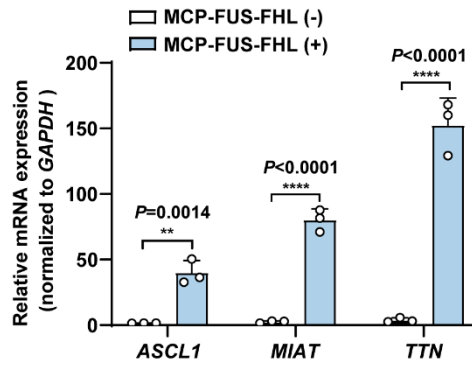
Supplementary Fig. 9 Characterization of PTRC-mediated transcriptional regulation system founded on FHY1. a Quantification of PTRC-mediated gene expression using varying levels of plasmids encoding GAL4-FHY1. Transfected HEK293T cells (6×10^4) were illuminated with red light (660 nm, 1 mW/cm²) or maintained in darkness for 24 hours before quantifying SEAP production. **b** Quantification of PTRC-mediated gene expression dynamics. Transfected HEK293T cells (6×10^4) were illuminated using red light (660 nm, 1 mW/cm²) for different time periods as outlined before quantifying SEAP production. Data are presented as means $\pm$ s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by two-way ANOVA; NS, not significant, $**p < 0.01$, $****p < 0.0001$.



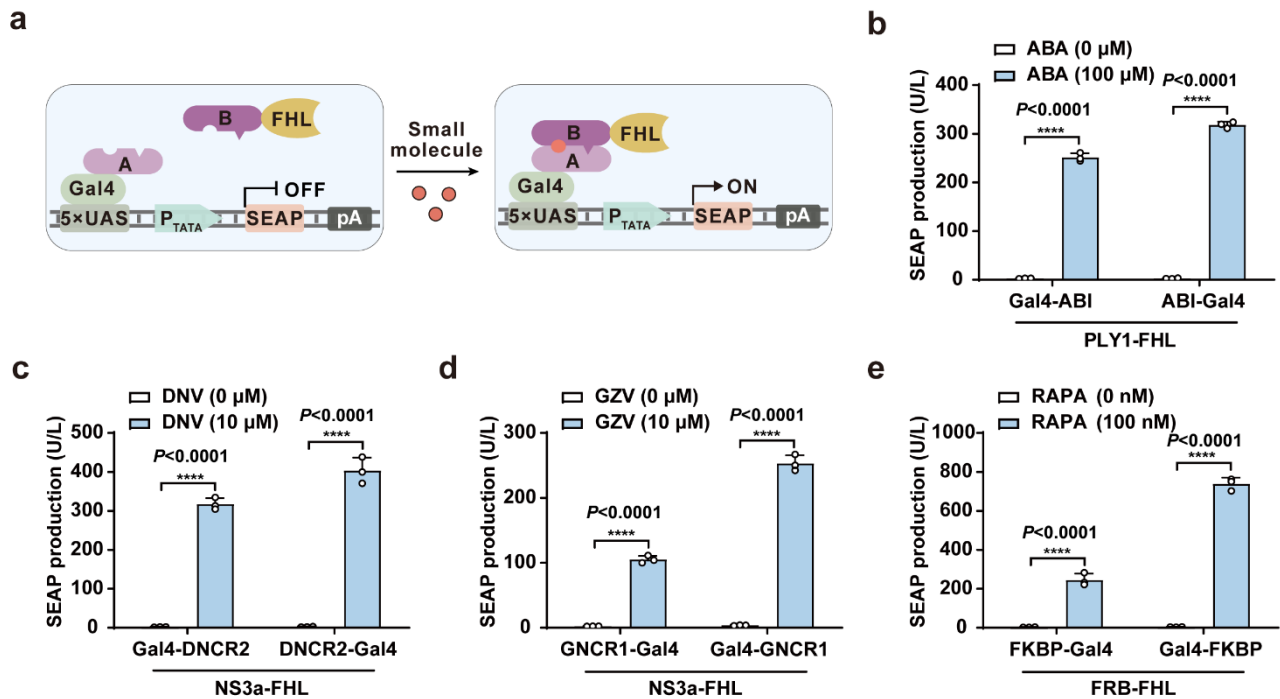
Supplementary Fig. 10 Long-term dynamics of light-responsive transcriptional regulation by the PTRC system. The PTRC transcriptional regulation system was introduced into HEK293T cells using INVI DNA Transfection Reagent. After transfection, the cells were either kept in darkness or exposed to red light (660 nm, 1 mW/cm²). SEAP production was quantified daily. Data are presented as means $\pm$ standard deviation (SD); $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by two-way ANOVA; **** $p < 0.0001$.



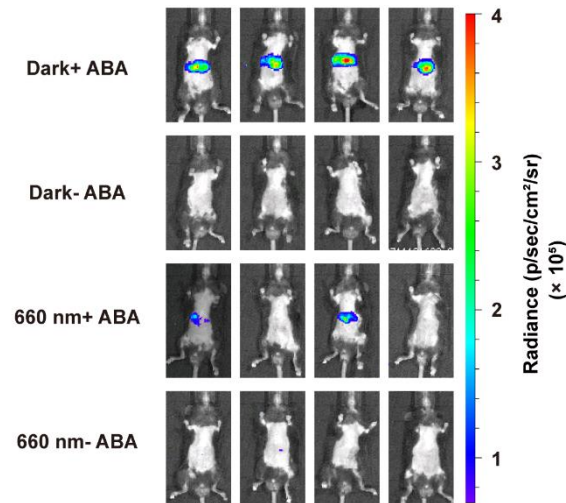
Supplementary Fig. 11 Detailed images of bioluminescence in mice possessing a PTRC-mediated transcriptional regulation system. Plasmids encoding Δ PhyA-2 $\times$ ZIM3, Gal4-FHL, and the corresponding luciferase reporter were delivered into the mouse liver by hydrodynamic injection of the tail vein. Control mice were administered pcDNA3.1(+) instead of Gal4-FHL. The mice were intraperitoneally injected with PCB and exposed to red light (660 nm, 5 mW/cm², 1 minute on, 5 minutes off, alternating) or continuous darkness for 16 hours before the bioluminescence images of mice were captured using an IVIS.



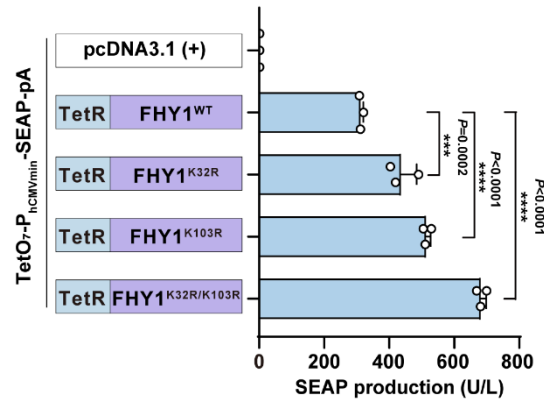
Supplementary Fig. 12 MCP-FUS-FHL-mediated endogenous gene activation. HEK293T cells (6×10^4) were transfected with plasmids encoding dCas9, MCP-FUS-FHL, and sgRNA targeting different endogenous genes (*ASCL1*, *MIAT*, or *TTN*). The relative mRNA expression was determined using qPCR at 48 hours after transfection. Data are presented as means $\pm$ s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by two-way ANOVA; ** $p < 0.01$, **** $p < 0.0001$.



Supplementary Fig. 13 Design and characterization of small molecule-induced transcriptional regulation systems based on FHL. **a** Schematic of small molecule-induced transcriptional regulation systems founded on FHL. Gal4-based synthetic DBD (ABI-Gal4, DNCR2-Gal4, Gal4-GNCR1, or Gal4-FKBP) binds to the chimeric promoter (5×UAS-P_{TATA}). In the presence of small molecules, including ABA (abscisic acid), DNV (Danoprevir), GZV (Grazoprevir), or RAPA (Rapamycin), the small molecule-responsive transactivator (PYL1-FHL, NS3a-FHL, or FRB-FHL) is recruited to the corresponding promoter according to interactions with the synthetic DBD to initiate target gene expression. **b-e** Quantification of FHL-mediated transcriptional activation mediated by ABA (abscisic acid) (**b**), DNV (Danoprevir) (**c**), GZV (Grazoprevir) (**d**), and RAPA (Rapamycin) (**e**). Transfected HEK293T cells (6×10^4) were grown for 24 hours in the presence or absence of small molecule ligands before quantifying SEAP production. Data in (**b**, **c**, **d**, **e**) are presented as means $\pm$ s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by two-way ANOVA; **** $p < 0.0001$.



Supplementary Fig. 14 Detailed images of bioluminescence in mice possessing a PTRC_{CIP-ABA}-mediated transcriptional regulation system. Plasmids encoding the PTRC_{CIP-ABA} system (ABI-Gal4, PYL1-FHL, and Δ PhyA-2 $\times$ ZIM3) and luciferase reporter (5 $\times$ UAS-P_{hCMVmin}-luciferase-pA) were administered to the mouse liver via hydrodynamic tail vein injection. The mice were intraperitoneally injected with ABA eight hours later and illuminated with red light for 16 hours or maintained in darkness. The bioluminescence signal was measured using an IVIS 24 hours after hydrodynamic injection.



Supplementary Fig. 15 Transcriptional activation of FHY1 variants. Mutations were introduced into various SUMOylation sites of FHY1, resulting in K32R and/or K103R. These FHY1 variants were linked to TetR and transfected into HEK293T cells alongside the corresponding SEAP reporter. After 24 hours of incubation, the production of SEAP was quantified. SUMOylation motifs (LK³²ME and MK¹⁰³EE) were identified using JASSA (<http://www.jassa.fr/index.php?m=jassa>). Data are presented as means $\pm$ s.d.; $n = 3$ independent experiments, each with 3 technical replicates. Statistical comparisons were performed by one-way ANOVA; *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Table 1. DNA sequence data.

FHL
atgGACGACGCCGACAAGAGCTGCAGCCCCTCTTTAGACCACTCCGACATCAACGATCCCATGATC GTGGCCGTGGAGTCTTTAGACACAAGCAAGAAGAGGAAGCTGCACGCCGAAGAGTCCGATTTACT GCCTTTACCCAAGCACTTCTGCAGCGAGCACCAAGCTTCTTTAGTGAACAGCAGCTGCCCCAGCA GCGTGATCGACTACGCCGAGTGCAGCTACGCCATGGAGAACACCAAAACCAGCGACGAGGCCTCC AGCAGCGCTAGCTTCACCGGACCCTCTTTATACATGTTCAAGGACAGCATCTACAGCACTGGTAG CAGCTCCTCCGGCTACGCTGCCACCAGCAGCATCGAGCAGTGCTTCTCCAAGGTGGACCACAAGA CCCAAGAAGACACCCAAGATTTACCCACATGGAGTTCATCTACCACGACAGCGAGTTTGCCGTG GAGGATTTACAAGAAGTGCTGAACCCCGTTGAGTCCTACATTTTAAGCAGCGCCAGATGGAGCGT CTCCAACCAAGATAGCAAGGAGGCCACCACCAAAACCCACCATCGACCAAGAATTCGAGCAGTACT TCAGCACTTTAATGATGtaa
FHY1
atgCCCGAGGTGGAGGTCGACAACAACAACGAGAAGCCCAGCGAGATCAACAGCTTCCACCACATG ATTATCAGCAGCAGCAAGAACGTGCTGAAGATGGAGGAGGTGGAGGTGTCCAAGAAGAGGAAGT TCCAGACCGACCAGAGCGACGAGCTGAGCTTATTACCTTTATCCAAACACACATGCTTTGCCAACG TGGCTTGTAGCGAGAACACCAACGGCAACAGCGAGATCGATACCGAGTACAGCATGTCCAGCTAC GTGAACTCCACCACCTCCATGGAGTGCAACAACGACATCGAGATGAAGGAAGAATCCAGCGGCAG CTGCGGAGAGGACAAGATGATCAGCTTCGAGTCCCATTTAGACTATATCTACGGCACCCAGAATC TGGAGGACTTCTCCGAGAAGGTGATCGAGAACATTCTGTACCTCGACGAGCAAGAAGAGGAGGAG GAGGACGCCAAGGGCTGTAGCAGCAACGCTGCCAAGTTCGTGCTGTCCTCTGGTCGTTGGACCGT GAACCAAGATGATAGCACTTTACACGAAACCAAGAAGCCCACCATCGACCAAGAATTCGAGCAGT ACTTTAGCACTTTAATGCTGtaa
ΔPhyA
atgGAGAAGAAGATGAGCGGATCTCGTCCCACACAGTCCAGCGAGGGATCTCGTAGATCTCGTCAC TCCGCTCGTATCATCGCTCAGACCACCGTGGACGCCAACTGCACGCCGATTTTCGAGGAGAGCGG CTCCTCCTTTGATTACTCCACCAGCGTGAGGGTGAAGTGGTCCCGTGGTCGAGAACCAGCCTCCTA GGAGCGACAAGGTCACCACAACCTACCTCCATCATATCCAGAAGGGCAAGCTGATCCAGCCCTTT GGATGTTTACTGGCTTTAGACGAAAAGACCTTCAAGGTCATCGCCTACTCCGAGAACGCCTCCGA ACTGCTCACAATGGCTTCCCACGCCGTGCCTAGCGTGGGAGAGCACCCCGTTCTGGGCATCGGCA CCGATATCAGATCTTTATTTACCGCTCCCAGCGCTTCCGCTTTACAGAAGGCTCTCGGCTTCGGCG ACGTGTCTTTACTGAACCCTATCCTCGTCCATTGTCTGATACATCCGCCAAGCCCTTCTACGCCATTA TCCATAGGGTGACCGGATCCATCATCATCGACTTCGAACCCGTTAAGCCCTATGAAGTGCCCATG ACAGCCGCCGAGCTTTACAGAGCTATAAGCTGGCCGCCAAGGCCATCACAGACTCCAGTCTTT ACCCAGCGGAAGCATGGAGAGGCTGTGCGACACCATGGTGCAAGAAGTCTTCGAGCTGACTGGTT ACGATCGTGTATGGCCTACAAGTTCCACGAGGACGACCACGGCGAGGTGGTGAGCGAAGTGAC CAAGCCCGGTTTAGAACCCTATTTAGGTTTACACTATCCCGCCACAGACATCCCCAAGCTGCCAG ATTTTTATTTATGAAGAACAAGGTGAGAATGATCGTGGACTGCAACGCCAAGCACGCTAGGGTTTT ACAAGATGAGAAGCTGAGCTTCGATTTAACCCTCTGCGGCAGCACTTTAAGAGCTCCCCACTCTTG TCATCTGCAATACATGGCCAATATGGACAGCATCGCTTCTTTAGTGATGGCCGTGGTGGTGAATG AGGAGGATGGAGAGGGCGATGCTCCCGATGCCACCACACAGCCTCAGAAGAGAAAGAGGCTGTG GGGTTTAGTGCTGCCACAACACCACCCCCAGATTTGTCCCTTTTCCCTTACGTTATGCTTGTGA ATTTTTAGCCCAAGTGTTTGCCATCCACGTCAACAAGGAGGTGAGCTGGACAACCAGATGGTGG

AGAAGAACATTTTACGTACCCAGACACTCCTCTGCGACATGCTCATGAGGGACGCTCCCCTCGGC
ATCGTGAGCCAGTCCCCAATATTATGGATTTAGTCAAGTGCGACGGCGCCGCCTTATTATACAAG
GACAAGATCTGGAAGCTGGGCACCACCCCTAGCGAGTTCCATTTACAAGAAATCGCTTCTTGGCT
GTGTGAGTACCACATGGATTCCACCGGTTTAAGCACCGATTCTTTACACGACGCTGGCTTTCCTAG
GGCTTTATCTTTAGGCGACAGCGTCTGCGGAATGGCTGCCGTTCTGTATCAGCAGCAAAGATATGA
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GATAGGGACGATGCCAGAAGGATGCACCCCAGATCCTCCTTCAAGGCTTTTCTGGAGGTGGTGAA
GACCAGAAGCTTACCTTGGAAGGACTACGAAATGGATGCCATCCACTCTTTACAGCTCATCTTACG
TAACGCCTTTAAGGACAGCGAGACCACCGACGTGAACACAAAGGTGATCTATAGCAAGCTCAACG
ATCTCAAGATTGATGGAATCCAAtaa

ZIM3

atgAACAATCCCAGGGAAGAGTGACCTTCGAGGATGTCACTGTGAACTTCACCCAGGGGGAGTGG
CAGCGGCTGAATCCCGAACAGAGAACTTGTACAGGGATGTGATGCTGGAGAATTACAGCAACCT
TGCTCTGTGGGACAAGGGGAAACCACCAAACCCGATGTGATCTTGAGGTTGGAACAAGGAAAGG
AGCCATGGTTGGAGGAAGAGGAAGTGCTGGGAAGTGGCCGTGCAGAAAAAAATGGGGACATTGG
AGGGCAGATTTGGAAGCCAAAGGATGTGAAAGAGAGTCTtaa

Supplementary Table 2. Target sequences of sgRNAs employed in this study.

Gene name	Guide sequence of sgRNA (5'-3')
<i>ASCL1</i>	GGCTGGGTGTCCCATTGAAA
	ATGGAGAGTTTGCAAGGAGC
<i>RHOXF2</i>	ACGCGTGCTCTCCCTCATC
	CTGTGGGTGTTGGGCCTGCTG
<i>TTN</i>	CCTTGGTGAAGTCTCCTTTG
	ATGTTAAAATCCGAAAATGC
<i>MIAT</i>	GCGCCCATGAAATTTTAATG
	GCTTCTGCGCCCCTGGTCCG
<i>Ascl1</i> (mouse)	GCAGCCGCTCGCTGCAGCAG
	AGCTGAGGAGGTGGGGGAAG
<i>Hbb</i> (mouse)	AGAGAGTCTGGGCAAGACAG
<i>Ins2</i> (mouse)	GTTAAGACTCTAATTACCCT
<i>Dkk1</i> (mouse)	CCCAAAGCTTTGAAATCCCA

Supplementary Table 3. Oligonucleotide sequences used for qPCR analysis.

Gene name	Primer name	Primer sequence (5'-3')
<i>ASCL1</i> (human)	Forward	CGCGGCCAACAAGAAGATG
	Reverse	CGACGAGTAGGATGAGACCG
<i>GAPDH</i> (human)	Forward	CGAGATCCCTCCAAAATCAA
	Reverse	ATCCACAGTCTTCTGGGTGG
<i>RHOXF2</i> (human)	Forward	AGTGTAGCCAGTATATGACCAGC
	Reverse	TGACCTCTTCAGTAAGCGACAG
<i>TTN</i> (human)	Forward	CCCCATCGCCCATAAGACAC
	Reverse	CCACGTAGCCCTCTTGCTTC
<i>MIAT</i> (human)	Forward	TGGCTGGGGTTTGAACCTTT
	Reverse	AGGAAGCTGTTCCAGACTGC
<i>Ascl1</i> (mouse)	Forward	GGAACAAGAGCTGCTGGACT
	Reverse	GTTTTTCTGCCTCCCCATTT
<i>Gapdh</i> (mouse)	Forward	ATGACATCAAGAAGGTGGTG
	Reverse	CATACCAGGAAATGAGCTTG
<i>Hbb</i> (mouse)	Forward	CACTTGAGATCATCTCCAAGC
	Reverse	TAACCCCCAAGCCCAAGGATG
<i>Ins2</i> (mouse)	Forward	GGCTTCTTCTACACACCCAT
	Reverse	CCAAGGTCTGAAGGTCACCT
<i>Dkk1</i> (mouse)	Forward	TCCGTCTGCCTCCGATCATC
	Reverse	GCCTTTCCGTTTGTGCTTGG
<i>tdTomato</i> (mouse)	Forward	GACACCAAGCTGGACATCAC
	Reverse	ACCTTGAAGCGCATGAACTC