Title

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TAEL 2.0: An Improved Optogenetic Expression System for Zebrafish

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Abstract

Inducible gene expression systems are valuable tools for studying biological processes. We previously developed an optogenetic gene expression system called TAEL that is optimized for use in zebrafish. When illuminated with blue light, TAEL transcription factors dimerize and activate gene expression downstream of the TAEL-responsive C120 promoter. By using light as the inducing agent, the TAEL/C120 system overcomes limitations of traditional inducible expression systems by enabling fine spatial and temporal regulation of gene expression. Here, we describe ongoing efforts to improve the TAEL/C120 system. We made modifications to both the TAEL transcriptional activator and the C120 regulatory element, collectively referred to as "TAEL 2.0." We demonstrate that TAEL 2.0 consistently induces higher levels of reporter gene expression and at a faster rate, but with comparable background and toxicity as the original TAEL system. With these improvements, we were able to create functional stable transgenic lines to express the TAEL 2.0 transcription factor either ubiquitously or with a tissue-specific promoter. We demonstrate that the ubiquitous line in particular can be used to induce expression at late embryonic and larval stages, addressing a major deficiency of the original TAEL system. We believe this improved optogenetic expression system will be a useful resource for the zebrafish community.

Introduction

Inducible gene expression systems are valuable tools for studying biological processes as they enable user-defined control over the timing, location, and level of expression. In zebrafish and other model organisms, the most widely used inducible expression systems fall into two broad categories – those that rely on the heat shock response[1] and those using small molecule inducing agents[2]. More recently, optogenetic approaches have been developed based on light-sensitive transcription factors[3-6]. One such system is based on EL222, a naturally occurring blue light-activated transcription factor found in the bacterium *Erythrobacter litoralis HTCC2594*. The endogenous transcription factor contains a light-oxygen-voltage-sensing (LOV) domain that in response to blue light (450 nm) undergoes a conformational change and dimerizes, allowing it to bind and initiate transcription from a regulatory element termed C120[7]. EL222 was the basis for an inducible expression system designed for mammalian cell culture[8]. Our group previously designed EL222 for use in zebrafish by fusing it to a KalTA4 transcriptional activation domain, which minimized toxicity in zebrafish embryos while still maintaining functionality[6]. We demonstrated that this KalTA4-EL222 fusion protein, which we termed TAEL, could be combined with C120-containing transgenes to achieve light-inducible expression of multiple genes of interest. We also validated multiple approaches for delivering patterned blue light illumination to spatially and temporally control induction in zebrafish embryos. However, we were unable to establish stable transgenic lines for TAEL expression that could induce expression from our C120 reporter lines, suggesting that TAEL and/or the C120 promoter could be further optimized.

In this study, we present ongoing efforts to improve the function of the TAEL/C120 system. We made changes to both the TAEL transcriptional activator and the C120 promoter, collectively termed TAEL 2.0, that produce significantly higher levels of light-induced expression at a faster rate. Importantly, these improvements allowed us to address a major deficiency of our

previously published system (referred to here as TAEL 1.0), namely the lack of functional, stable transgenic lines for both TAEL and C120 components. Here, we describe the generation of transgenic lines that express functional TAEL 2.0 components either ubiquitously or in the developing endoderm. We demonstrate that the ubiquitous line in particular can be used to induce expression at late embryonic and larval stages, extending the use of this system beyond early embryo stages.

Materials and Methods

Vector construction and mRNA synthesis

pμTol2 backbone. For expression plasmids and transgenes created for this study, we generated a minimal plasmid backbone called pμTol2, which can be used for both Tol2-based transgenesis and in vitro mRNA synthesis. Its short length of 2520 base pairs enables modification of inserts by PCR through the backbone, thus eliminating the need to subclone. In brief, pμTol2 was constructed by Gibson assembly, fusing the Tol2 sites for genomic integration[9] with the commonly used expression cassette of pCS2 including polylinkers and SV40 polyadenylation site[10,11] and a plasmid backbone derived from pUC19[12]. To ensure efficient protein synthesis, all plasmids newly constructed for this study contain the zebrafish-optimized Kozak sequence 5'-GCAAACatgG-3', where the lower case "atg" denotes the start codon[13].

Expression plasmids. pCS2-TAEL has been described previously[6]. To construct expression plasmids pμTol2-N-TAEL, Optologix, Inc. (Dallas, TX) provided synthesized oligomers containing the SV40 large T-antigen nuclear localization signal. We fused these to the 5′ end of the TAEL ORF and to the pμTol2 backbone by Gibson assembly[14]. Similarly, pμTol2-TAEL-N was constructed by fusing synthesized oligomers containing the nucleoplasmin nuclear

localization signal (also provided by Optologix, Inc.) to the 3′ end of the TAEL ORF by Gibson assembly[14]. Capped messenger RNA was synthesized using mMESSAGE mMACHINE SP6 kit (Ambion) with plasmids cut with NotI as linear template. For experiments in Fig. 1–3, Tg(C120:mCherry;cryaa:Venus) or Tg(C120F:mCherry) males were crossed to wild-type females and resulting embryos were each injected with ~50 pg of TAEL, N-TAEL, or TAEL-N mRNA at the 1-cell stage.

Transgene plasmids. To construct pμTol2-C120F:mCherry, the mouse *Fos* basal promoter sequence: 5'-CCAGTGACGTAGGAAGTCCATCCATTCACAGCGCTTC-TATAAAGGCGCCAGCTGAGGCGCCTACTACTCCAACCGCGACTGCAGCGAGCAACT -3'[15] was synthesized by Integrated DNA Technologies and the C120 sequence[6] was amplified by PCR. These sequences were fused together and inserted into pμTol2 by Gibson assembly. The transgene plasmid pμTol2-C120F:GFP was constructed by separate PCR amplification of the C120F promoter and GFP ORF which were then cloned into pμTol2 by Gibson assembly. pμTol2-sox17:TAEL-N was constructed by separate PCR amplification of the *sox17* promoter[16] and TAEL-N ORF which were then cloned into pμTol2 by Gibson assembly. pμTol2-ubb:TAEL-N was constructed by separate PCR amplification of the *ubb* promoter[17] and TAEL-N ORF, which were then cloned into pμTol2 by Gibson assembly.

Zebrafish Strains

Adult Danio rerio zebrafish were maintained under standard laboratory conditions. Zebrafish in an outbred AB, TL, or EKW background were used as wildtype strains.

Tg(C120:mCherry;cryaa:Venus)^{sfc14}, referred to here as *Tg(C120T:mCherry)*, has been previously described[6]. *Tg(C120-Mmu.Fos:mCherry)^{ucm104}*, *Tg(C120-Mmu.Fos:GFP)^{ucm107}*, *Tg(ubb:TAEL-N)^{ucm113}*, and *Tg(sox17:TAEL-N)^{ucm114}* were generated using standard

transgenesis protocols[9,18]. This study was performed with the approval of the Institutional Animal Care and Use Committee (IACUC) of the University of California Merced.

Global light induction

Global light induction was provided by a MARS AQUA-165-55 110W LED aquarium hood. Actual power of light received by embryos (lids of plates removed) was measured as ~1.6 mW/ cm^2 at 456 nm. For experiments in Fig. 1–2, 4 hpf (hours post-fertilization) embryos were illuminated with constant blue light for 1–3 hours. For experiments in Fig. 3–6, a timer was used to apply constant or pulsed light (NEARPOW Timer Switch). Dark controls were placed in a light proof box in the same 28.5°C incubator as the light-treated samples.

Real-time quantitative PCR

To quantify light-induced expression, total RNA from 30–50 light-treated or dark control embryos was extracted using the illustra™ RNAspin Mini kit (GE Healthcare). 1 µg total RNA was used for reverse transcription with qScript XLT cDNA SuperMix (Quantabio). Each qPCR reaction contained 2X PerfeCTa® SYBR green fast mix (Quantabio), 5-fold diluted cDNA and 325 nM each primer. Reactions were carried out on a QuantStudio3 (Applied Biosystems) real time PCR machine using the following program: initial activation at 95°C for 10 min, followed by 40 cycles of 30 s at 95°C, 30 s at 60°C and 1 min at 72°C. Once the PCR was completed, a melt curve analysis was performed to determine reaction specificity. Data represent averages from 3–5 biological replicates, each with three technical replicates. The housekeeping gene *ef1a* was used as a reference. Fold change was calculated using the $2^{(-\Delta\Delta CT)}$ method[19]. Statistical significance was determined using Prism software (GraphPad). qPCR primers used are: mcherry forward: 5'-GACCACCTACAAGGCCAAGA-3'; mcherry reverse: 5'-CTCGTTGTGGGAGGTGATGA-3'; ef1a forward 5'-CACGGTGACAACATGCTGGAG-3'; ef1a reverse: 5'-CAAGAAGAGTAGTACCGCTAGCAT-3'

Microscopy and image processing

Fluorescence and brightfield images were acquired on a Leica dissecting stereomicroscope or Olympus dissecting stereomicroscope. Dechorionated embryos or larvae were embedded in 1.5% low-melting agarose (ISC BioExpress) containing 0.01% tricaine (Sigmal-Aldrich) within glass-bottom Petri dishes (MatTek Corporation). Standard filter settings were applied and brightfield and fluorescence images were merged after acquisition. Identical exposure settings for fluorescence images were used for all embryos from the same set of experiments. All image processing and analysis was performed using ImageJ software[20]. Illustrations were created with BioRender (https://biorender.com/).

Results

TAEL-induced expression is increased by coupling the C120 regulatory element to a *Fos* basal promoter

In our previously published system, the TAEL-responsive C120 regulatory sequence was coupled to a minimal TATA box[6,8]. Because this minimal TATA box originated from a mammalian expression vector, we reasoned that using a zebrafish-optimized basal promoter instead would improve performance of the TAEL system. The basal promoter from the mouse *Fos* gene was previously shown to function well in zebrafish transgenes, allowing for high expression levels with minimal background[15,21]. Therefore, we constructed a new TAEL-responsive promoter consisting of 5 repeats of the C120 regulatory sequence coupled to the mouse *Fos* basal promoter (*C120-Mmu.Fos*, abbreviated throughout as *C120F*). We then determined whether this new C120 promoter improves light-induced expression compared to the previous TATA box-containing version (Fig. 1). First, we generated a stable transgenic zebrafish line using *C120F* to control expression of an mCherry reporter (*Tg(C120F:mCherry)*)

to make direct comparisons to our previously published reporter line[6], referred to here as *Tg(C120T:mCherry)*. We injected both *Tg(C120T:mCherry)* and *Tg(C120F:mCherry)* embryos with ~50 pg TAEL mRNA then globally illuminated them with blue light starting at 3 hpf. qRT-PCR analysis showed that compared to sibling control embryos kept in the dark, mCherry expression was induced 43.5 $^{+10.6}_{-8.5}$-fold in *Tg(C120F:mcherry)* embryos, which was significantly higher than the 2.9 $^{+1.2}_{-0.8}$-fold induction in *Tg(C120T:mCherry)* embryos (p=0.0009) (Fig. 1C). Consistent with these results, mCherry fluorescence was qualitatively brighter in *Tg(C120F:mCherry)* embryos compared to *Tg(C120T:mCherry)* embryos (Fig. 1D-E). Importantly, we did not observe mCherry fluorescence in embryos kept in the dark for either genotype (Fig. 1F-G). Together, these results suggest that coupling the C120 regulatory element with a *Fos* basal promoter instead of a minimal TATA box significantly increases TAEL-induced gene expression while maintaining low background expression.

TAEL-induced expression is increased by adding a C-terminal nuclear localization signal to TAEL

Our original TAEL construct consists of a Kal-TA4 transcription activation domain, the light-sensitive LOV domain, and a DNA-binding domain that recognizes the C120 sequence but does not contain an explicit nuclear localization signal (NLS). Although TAEL can likely enter the nucleus through diffusion, because of its relatively small size of 257 amino acids, we wanted to test whether targeting TAEL specifically to the nucleus by adding an NLS would increase the amplitude of induction and improve light-induced expression (Fig. 2).

We first generated a construct in which the SV40 large T-antigen NLS was fused to the amino terminus of TAEL (N-TAEL). When delivered by mRNA injection into *Tg(C120F:mCherry)* embryos, we were surprised to find that N-TAEL induced mCherry expression less strongly

(47.7 $^{+81.3}_{-30.0}$-fold) than the original TAEL protein (81.7 $^{+69.8}_{-37.7}$-fold; p=0.3398) (Fig. 2C). Consistent with these qPCR results, mCherry fluorescence was more variable and often dimmer in embryos injected with N-TAEL versus TAEL mRNA (Fig. 2D-E). We speculated that fusing the NLS to the N-terminus of TAEL places it directly adjacent to the KalTA4 transcriptional activation domain, which may negatively interfere with transactivation. Therefore, we generated a construct in which the nucleoplasmin NLS was fused to the carboxy terminus of TAEL (TAEL-N). By qPCR analysis, *Tg(C120F:mCherry)* embryos injected with TAEL-N mRNA showed higher levels of mCherry induction (176.5 $^{+87.6}_{-58.5}$-fold) compared to both TAEL (p=0.053) and N-TAEL (p=0.0392) (Fig. 2C). Correspondingly, mCherry fluorescence was brightest in embryos injected with TAEL-N (Fig. 2F). We did not observe mCherry fluorescence in any injected embryos kept in the dark (Fig. 2G-I). Together, these results demonstrate that adding a nuclear localization signal at the C-terminus of TAEL further increases light-induced gene expression with minimal background.

TAEL 2.0 induces higher expression levels at a faster rate

We next characterized the effects of combining the modifications we made to the C120 promoter and TAEL transcriptional activator. With our previously published TAEL system, we found that peak expression levels were reached by 3 hours post-illumination and could be sustained up to 8 hours when embryos were exposed to blue light pulsed at 1 hour on/off intervals[6]. To determine if TAEL 2.0 improves the kinetics and/or range of light-induced expression, we injected *Tg(C120T:mCherry)* embryos with TAEL mRNA ("TAEL 1.0") or *Tg(C120F:mcherry)* embryos with TAEL-N mRNA ("TAEL 2.0"). Starting at approximately 3 hpf, injected embryos were globally illuminated with pulsed blue light (1 hour on, 1 hour off) and mCherry expression was measured by qRT-PCR at various timepoints up to 9 hours post-illumination. Throughout the time course, we found that TAEL 2.0 induced significantly higher

mCherry expression compared to TAEL 1.0 (2-way ANOVA, $p<0.0001$). Induction kinetics also improved. At 1 hour post-illumination, mCherry expression was induced 738.6 $^{+749.2}_{-379.9}$-fold with TAEL 2.0, and this level of expression was maintained up to 9 hours post-illumination. In contrast, with TAEL 1.0, mCherry expression at 1 hour post-illumination was induced 89.1 $^{+54.0}_{-33.6}$-fold; induction peaked by 3 hours post-illumination to 221.6 $^{+222.2}_{-110.0}$-fold and then decreased to 56.8 $^{+138.47}_{-16.28}$-fold by 9 hours post-illumination. Together, these results demonstrate that the combined modifications we made to the TAEL system improve both the range and induction kinetics of this light-activated expression system.

TAEL 2.0 modifications enable functional stable transgenic lines of TAEL components

One notable deficiency of our previous TAEL system was the lack of functional stable transgenic lines expressing the TAEL transcriptional activator. With its greatly increased amplitude and kinetics of induction, we determined whether TAEL 2.0 could address this previous limitation.

We generated a stable transgenic line, *Tg(sox17:TAEL-N)*, to express TAEL-N under the *sox17* promoter, which drives expression in the endoderm and dorsal forerunner cells (DFCs)[16]. We crossed this line with a *Tg(C120F:GFP)* reporter line. The resulting double transgenic embryos were globally illuminated with pulsed blue light (1 hour on/off) or kept in the dark from 6–18 hpf (Fig. 4A). We observed GFP fluorescence in derivatives of the endoderm such as the gut tube and the pharyngeal endoderm as well as derivatives of the dorsal forerunner cells (DFCs) within the tail mesoderm in illuminated embryos but not those kept in the dark (Fig. 4B-E). Because activating blue light was applied globally, this result suggested that TAEL-N functions in, and is restricted to, the *sox17* expression domain. Additionally, we observed that the intensity of GFP fluorescence was brightest in the tail (Fig. 4B-C), again consistent with the known *sox17*

231 expression pattern, which is highest in the DFCs. Together, these results demonstrate
232 successful generation of a stable transgenic line for tissue-specific TAEL-N expression, which in
233 turn enables tissue-specific induction of a gene of interest even when activating blue light is
234 applied globally.
235
236 One consequence of the lack of functional stable transgenic lines for TAEL 1.0 is that its use is
237 limited to early embryonic stages. To determine if TAEL 2.0 modifications could expand the
238 range of accessible developmental stages, we generated a stable transgenic line,
239 *Tg(ubb:TAEL-N)*, to express TAEL-N under the *ubb* promoter, which has been shown to drive
240 ubiquitous expression at all developmental stages[17]. We crossed this line to *Tg(C120F:GFP)*
241 then exposed double transgenic embryos to activating blue light at several different time points
242 spanning embryonic to larval stages (Fig. 5A). In all cases, we observed increased GFP
243 fluorescence in illuminated embryos or larva but not in control siblings that had been kept in the
244 dark (Fig. 5B-G). At 4 days post-fertilization (dpf), we observed GFP fluorescence in the livers of
245 both illuminated and control larvae (arrows, Fig. 5D, G), which is likely due to insertional effects
246 of the Tg(C120F:GFP) transgene specific to this line; we did not observe similar liver
247 fluorescence in Tg(C120F:mCherry). Importantly, we could still detect light-dependent GFP
248 induction above this background expression at 4 dpf (Fig. 5D). Taken together, these results
249 demonstrate that TAEL 2.0 can be used to induce expression in a broad range of
250 developmental stages.
251
252 A recent study showed that blue light alone can increase expression of *Fos* and other activity-
253 dependent genes in cultured mouse cortical neurons[22]. Because the *C120F* promoter utilizes
254 the basal promoter from the mouse *Fos* gene, it is possible that there are endogenous factors,
255 especially in neural tissues, that can drive light-responsive expression from the *C120F* promoter
256 independent of TAEL-N and reduce the specificity of the TAEL 2.0 system. To determine

whether the *C120F* promoter can function in the absence of TAEL-N, we exposed *Tg(C120F:GFP)* zebrafish to blue light at 2 dpf or 4 dpf; the latter time point was chosen as light-driven neuronal activity likely increases over time. Apart from the ectopic liver expression at 4 dpf described above, we did not observe any appreciable GFP fluorescence either in illuminated animals or controls kept in the dark (Fig. 6A-D).

We quantified GFP expression by qPCR in *Tg(C120F:GFP)* and *Tg(C120F:GFP);Tg(ubb:TAEL-N)* double-transgenic animals with and without blue light illumination. At 2 dpf, we detected low levels of GFP expression in both *Tg(C120F:GFP)* and *Tg(C120F:GFP);Tg(ubb:TAEL-N)* embryos kept in the dark, suggesting there is a small amount of basal activity of the *C120F* promoter (Fig. 6E). Upon blue light illumination, we detected strong induction of GFP expression in *Tg(C120F:GFP);Tg(ubb:TAEL-N)* double transgenic embryos ($73\ ^{+81.2}_{-38.5}$-fold compared to controls kept in the dark, p=0.0032) (Fig. 6F). We also observed a slight but statistically significant increase in GFP expression in embryos containing only the *Tg(C120F:GFP)* transgene ($1.5\ ^{+0.3}_{-0.5}$-fold compared to controls kept in the dark, p=0.0386). However, given that we did not observe any GFP fluorescence in 2 dpf *Tg(C120F:GFP)* embryos (Fig. 6A-B), this slight increase in GFP mRNA levels is likely not functionally significant.

At 4 dpf, qPCR analysis detected elevated background GFP expression from all larvae kept in the dark (Fig. 6E), presumably due to the ectopic liver expression in this transgenic line. However, even with this higher background expression, we could detect significant induction of GFP in response to light ($6.6\ ^{+3.8}_{-0.2}$-fold compared to controls kept in the dark, p=0.0025) in *Tg(C120F:GFP);Tg(ubb:TAEL-N)* double transgenic larvae but not in *Tg(C120F:GFP)* larvae (Fig. 6F). These results suggest that in the absence of activated TAEL-N, basal activity of the

281 *C120F* promoter is low and negligibly responsive to light, demonstrating specificity of the TAEL
282 2.0 system.
283
284 **Discussion**
285
286 In this study, we describe improvements we have made to a zebrafish-optimized optogenetic
287 expression system called TAEL/C120. In the original TAEL/C120 system, a LOV domain-
288 containing transcription factor (TAEL) is used to drive expression of genes of interest
289 downstream of the C120 regulatory element in response to blue light. The improvements we
290 made include adding a C-terminal nuclear localization signal to TAEL (TAEL-N) and coupling
291 C120 regulatory elements with a basal promoter taken from the mouse *Fos* gene (*C120F*).
292 These improvements, collectively referred to as TAEL 2.0, significantly increased both the level
293 and rate of light-induced expression.
294
295 Importantly, these improvements allowed us to generate functional stable transgenic lines for
296 TAEL-N expression. Previously under TAEL 1.0, we had difficulties generating such transgenic
297 lines, possibly due to sub-optimal performance of the TAEL transcriptional activator and/or
298 sensitivity of the C120 promoter. We speculate that these deficiencies were overcome in TAEL
299 1.0 by transiently expressing TAEL by mRNA or plasmid injection, which can deliver many more
300 molecules of TAEL than can be achieved by transgene expression. However, this approach
301 limits the applications for TAEL 1.0 as injections are labor intensive, introduce experimental
302 variability, and often preclude use beyond early embryonic stages. In this study, the
303 improvements we made to both the transcriptional activator (TAEL-N) and promoter (*C120F*)
304 together allowed us to generate functional TAEL-N transgenic lines. Such lines can provide
305 additional spatiotemporal specificity to gene induction, as demonstrated with the
306 *Tg(sox17:TAEL-N)* line (Fig. 4). And, as shown with the *Tg(ubb:TAEL-N)* line (Fig. 5),

307 transgenesis enables usage beyond early embryonic stages, which is not possible with mRNA
308 delivery to the zygote.
309
310 The choice of a basal promoter is often overlooked when designing zebrafish transgenes even
311 though it can have profound effects on the function of a transgene. In our original TAEL 1.0
312 system, the C120 regulatory element is coupled to a minimal TATA box sequence taken from a
313 mammalian expression vector[6,8]. In this study, we replaced the minimal TATA box with the basal
314 promoter of the mouse *Fos* gene, which was previously used in zebrafish transgenesis[15,21]. This
315 modification alone resulted in more than 40-fold activation following illumination — a 15-fold
316 increase over the original TAEL system (Fig. 1C). Several different basal promoters have been
317 used in zebrafish transgene and enhancer trap constructs, each with different
318 characteristics[15,21,23,24]. The *Fos* basal promoter is derived from a gene well-known for its
319 activation in response to neuronal activity[25]. Our experiments indicate that coupling this basal
320 promoter to the C120 regulatory sequence imparts several desirable attributes to the TAEL
321 system (fast induction, low background, high amplitude) that extend to the whole organism. For
322 cell type-specific applications, further improvement may be possible by choosing a different
323 basal promoter optimized for that cell type.
324
325 With the improvements that we have made, the TAEL 2.0 system further expands the multitude
326 of different applications we envision, including lineage tracing and precise targeting (spatially
327 and temporally) of gene perturbations. One major advantage of TAEL 2.0 is the extension of
328 these applications beyond early embryonic stages through transgene-directed expression of the
329 TAEL-N transcription factor. This improved zebrafish-optimized light-gated gene expression
330 system should be a broadly useful resource for the zebrafish community.
331
332 **Acknowledgements**

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Competing Interests

L.B.M-M. and K.H.G were co-founders of Optologix, Inc., which developed light-gated transcription factors for research applications. As of September 2020, Optologix, Inc. has ceased business.

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Figures and Figure Legends

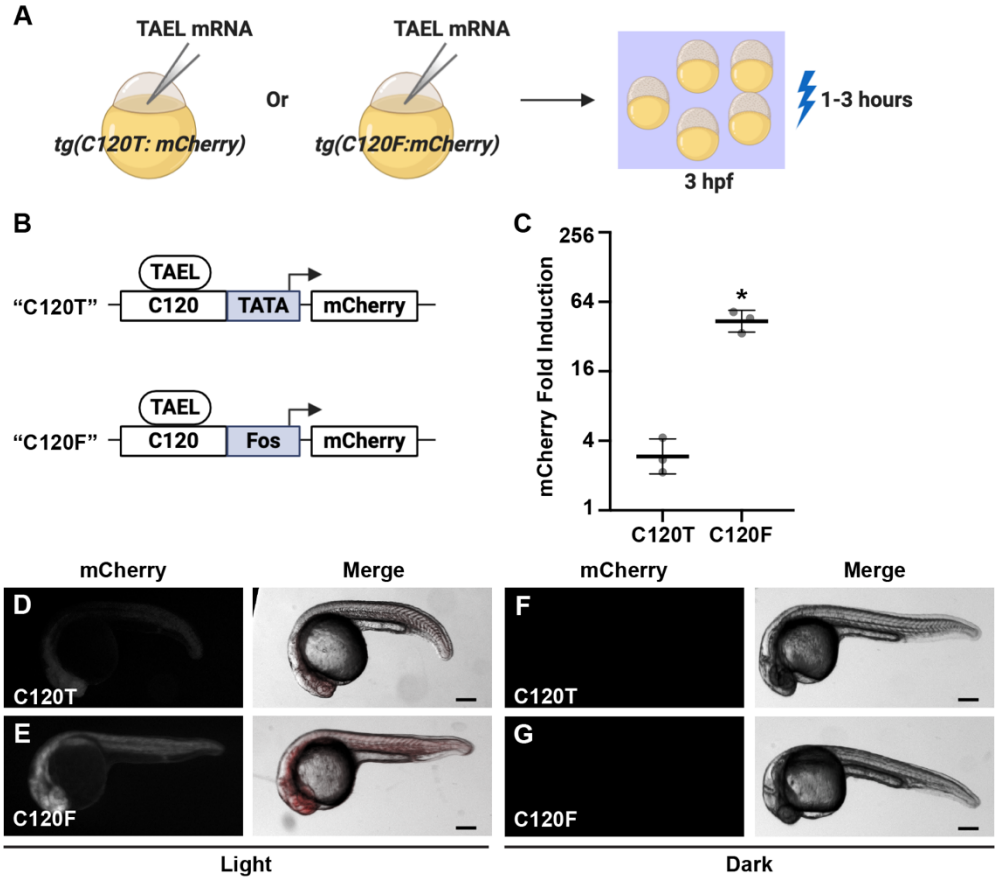

Figure 1. Coupling the C120 regulatory element to the Fos basal promoter significantly increases light-induced expression. A. Schematic of experimental design. *Tg(C120T:mCherry)* or *Tg(C120F:mCherry)* embryos were injected with TAEL mRNA. mCherry expression was induced by illuminating embryos with blue light starting at 3 hours post-fertilization (hpf). **B.** Schematic comparing different C120-based reporter constructs in which TAEL-responsive C120 sequences (C120) were coupled to either a minimal TATA box (TATA) or the basal promoter from the mouse *Fos* gene (Fos) and used to drive expression of mCherry. **C.** Comparison of light-induced mCherry expression in *Tg(C120T:mCherry)* and *Tg(C120F:mCherry)* embryos injected with TAEL mRNA. mCherry transcript levels were measured by qPCR from embryos illuminated with blue light for 1 hour and compared to sibling

429 embryos kept in the dark. Dots represent biological replicates. Solid lines represent mean. Error
430 bars represent S.D. *p<0.05. **D-G.** Representative images of mCherry fluorescence in
431 *Tg(C120T:mCherry)* (D, F) or *Tg(C120F:mCherry)* (E, G) embryos injected with TAEL mRNA
432 and illuminated with blue light for 3 hours (D, E) or kept in the dark (F, G). Images were
433 acquired between 20 and 24 hours post-illumination. Scale bars, 200 μm.
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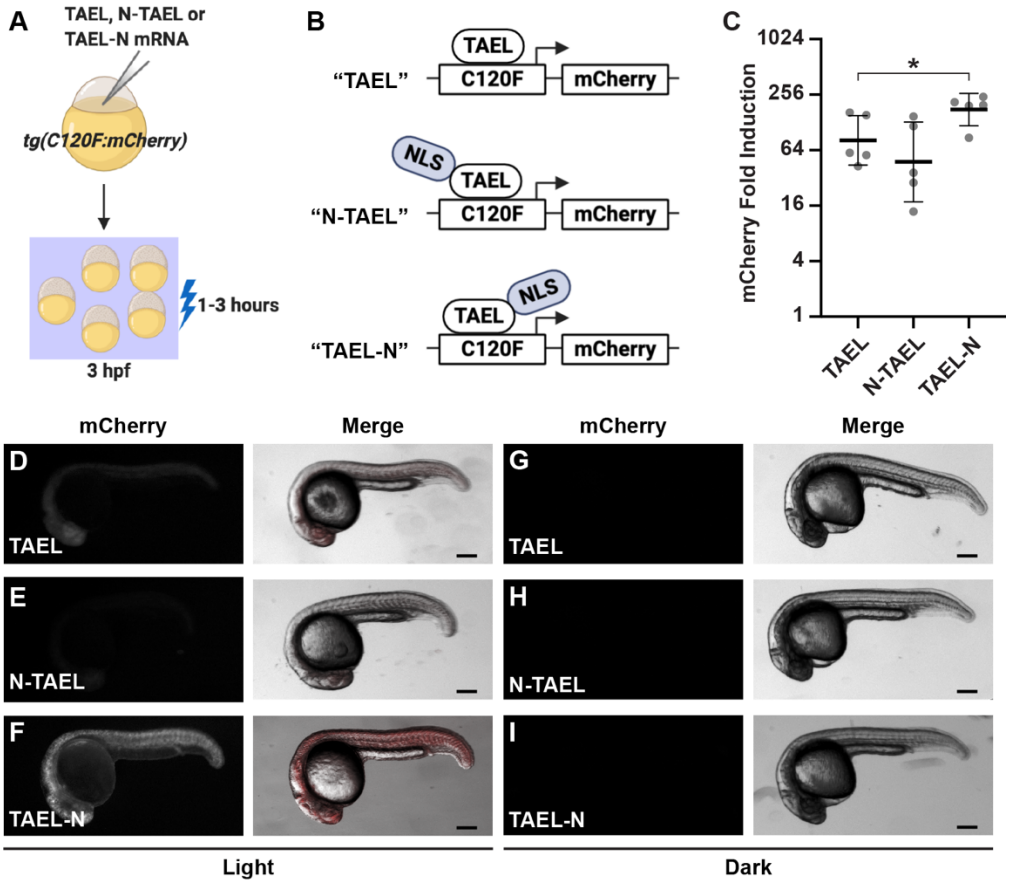

Figure 2. Adding a C-terminal nuclear localization signal (NLS) to TAEL significantly increases light-induced expression. A. Schematic of experimental design. *Tg(C120F:mCherry)* embryos were injected TAEL, N-TAEL, or TAEL-N mRNA. mCherry expression was induced by illuminating embryos with blue light starting at 3 hours post-fertilization (hpf). **B.** Schematic comparing different TAEL constructs containing no NLS (TAEL), one N-terminal NLS (N-TAEL), or one C-terminal NLS (TAEL-N). **C.** Comparison of light-induced mCherry expression in *Tg(C120F:mCherry)* embryos injected with TAEL, N-TAEL, or TAEL-N mRNA. mCherry transcript levels were measured by qPCR from embryos illuminated with blue light for 1 hour and compared to sibling embryos kept in the dark. Dots represent biological replicates. Solid lines represent mean. Error bars represent S.D. *p<0.05. **D-I.** Representative images of mCherry fluorescence in *Tg(C120F:mCherry)* embryos injected with TAEL (D, G), N-TAEL (E, H), or TAEL-N (F, I) mRNA and illuminated with blue light for 3 hours

448 (D-F) or kept in the dark (G-I). Images were acquired between 20 and 24 hours post-

449 illumination. Scale bars, 200 μm.

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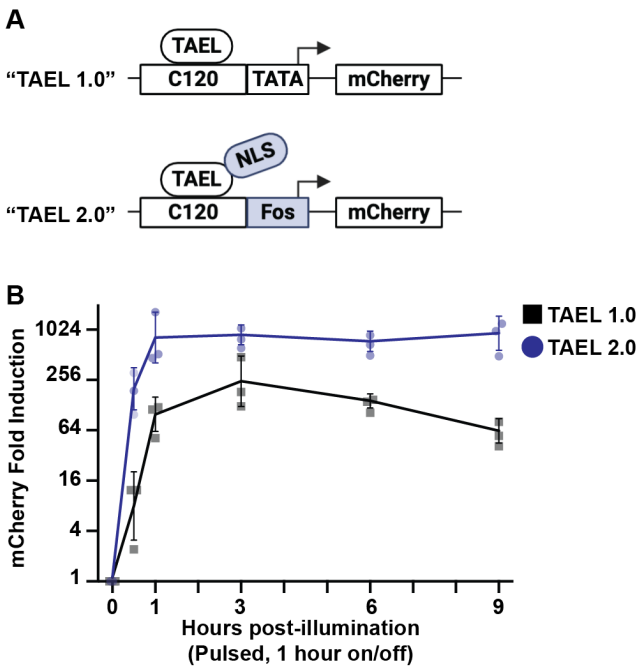

Figure 3. TAEL 2.0 modifications improve both the rate and level of light-induced expression. A. Schematic comparing TAEL 1.0 and TAEL 2.0. TAEL 1.0 consists of the TAEL transcription factor that lacks an NLS and the C120T promoter containing a minimal TATA box sequence. TAEL 2.0 consists of the TAEL-N transcription factor with a C-terminal NLS and the C120F promoter containing the basal promoter from the mouse *Fos* gene. **B.** Comparison of light-induced mCherry expression over time using TAEL 1.0 (black, dots) or TAEL 2.0 (blue, squares). *Tg(C120T:mCherry)* or *Tg(C120F:mCherry)* embryos were injected with mRNA for TAEL or TAEL-N, respectively. mCherry expression was induced by illuminating embryos with blue light (pulsed at a frequency of 1 hour on/1 hour off), starting at 3 hours post-fertilization. mCherry transcript levels were measured by qPCR at the indicated time points and normalized to 0 h post-illumination. Dots and squares represent biological replicates. Solid lines represent mean. Error bars represent S.D.

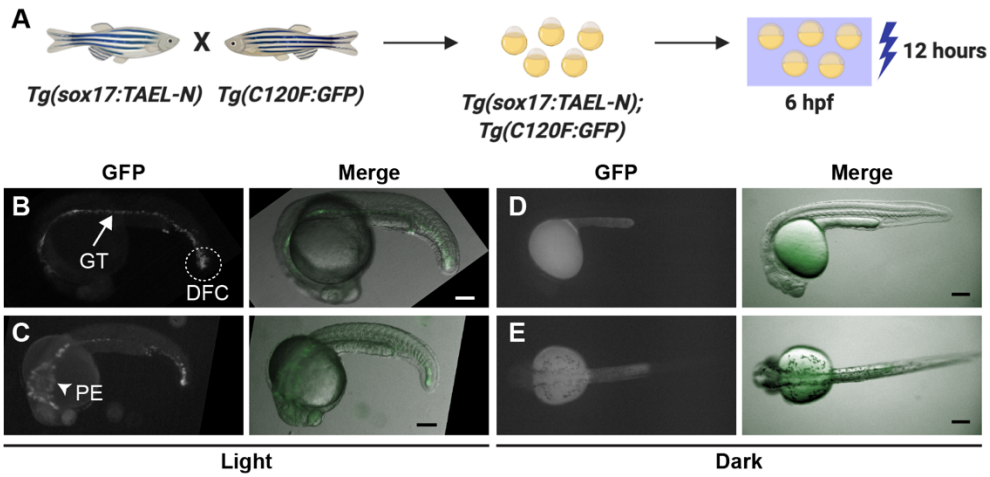

Figure 4. The stable transgenic line *Tg(sox17:TAEL-N)* restricts light-induced expression to endoderm-derived tissues. A Schematic depicting experimental design. *Tg(sox17:TAEL-N)* and *Tg(C120F:GFP)* adult zebrafish were crossed to produce double transgenic embryos. GFP expression was induced by illuminating embryos for 12 hours, starting at 6 hours post-fertilization (hpf), with blue light pulsed at a frequency of 1 hour on/1 hour off. **B-E.** Representative images of *Tg(sox17:TAEL-N);Tg(C120F:GFP)* embryos exposed to blue light (B-C) or kept in the dark (D-E). Images were acquired between 18 and 20 hours post-illumination. Arrow in (B) indicates gut tube (GT). Dashed lines in (B) indicate derivatives of the dorsal forerunner cells (DFC). Arrowhead in (C) indicates pharyngeal endoderm (PE). B, D are lateral views, anterior to the left. C, E are dorsal views, anterior to the left. Scale bars, 200 μm.

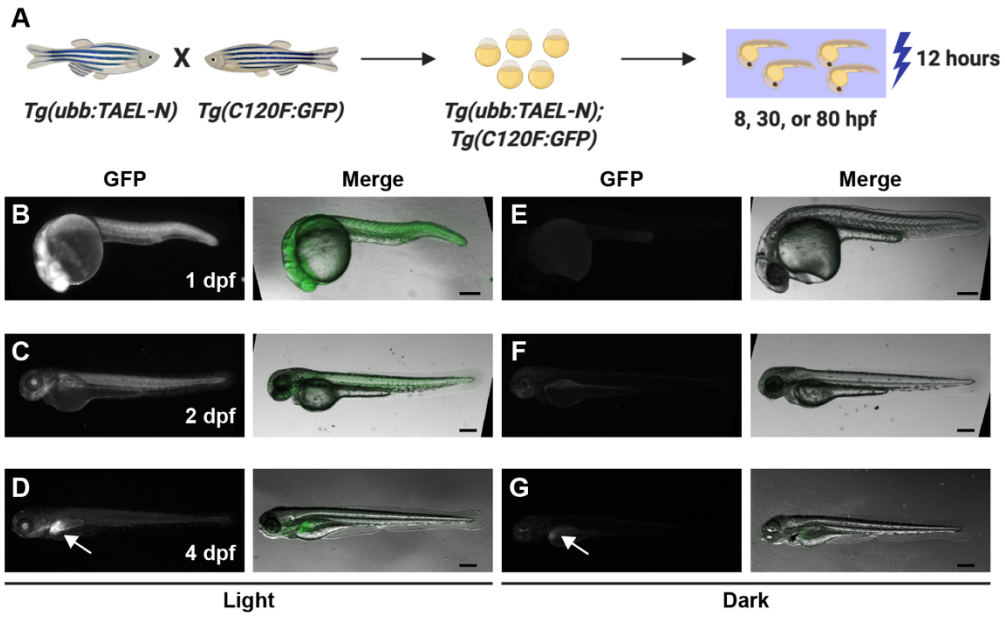

Figure 5. The stable transgenic line *Tg(ubb:TAEL-N)* enables light-induced expression at multiple developmental stages. A. Schematic depicting experimental design. *Tg(ubb:TAEL-N) and Tg(C120F:GFP)* adult zebrafish were crossed together to produce double transgenic embryos. GFP expression was induced at multiple time points by illuminating embryos for 12 hours with blue light pulsed at a frequency of 1 hour on/1 hour off. hpf, hours post-fertilization. **B-G.** Representative images of *Tg(ubb:TAEL-N);Tg(C120F:GFP)* embryos or larvae exposed to blue light (B-D) or kept in the dark (E-G). Images were acquired at the indicated stages between 18 and 20 hours post-illumination. dpf, days post-fertilization. Arrows in (D, G) point to ectopic liver expression of GFP. Scale bars, 200 nm.

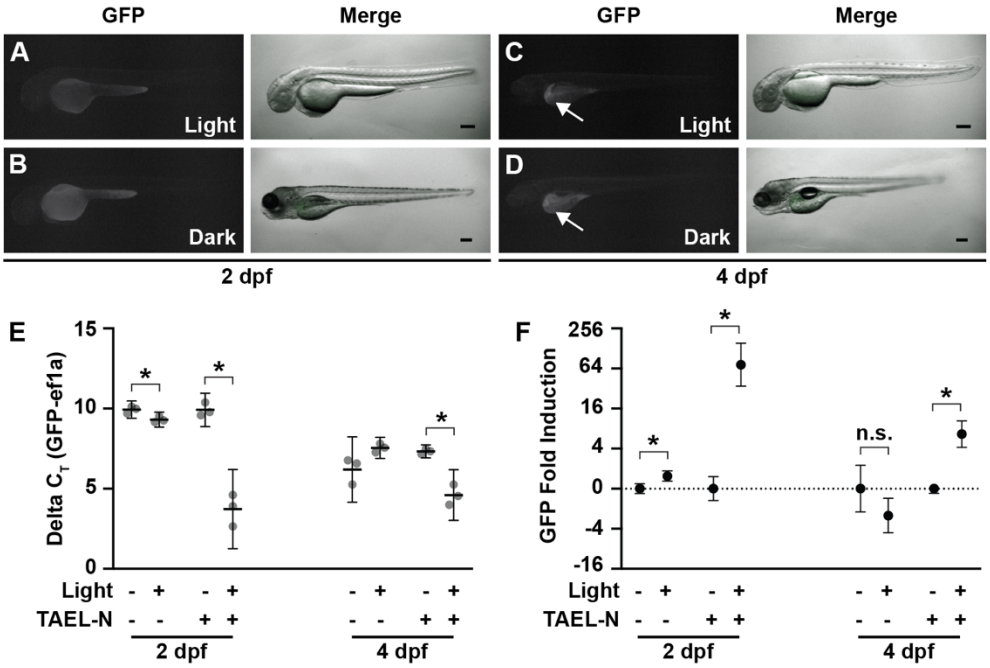

Figure 6. Basal expression from *Tg(C120F:GFP)* is not responsive to light. A-D. Representative images of *Tg(C120:GFP)* embryos at 2 days post-fertilization (dpf) (A-B) or larvae at 4 dpf (C-D). Embryos were illuminated for 12 hours with blue light pulsed at a frequency of 1 hour on/1 hour off (A, C) or kept in the dark (B, D). Images were acquired between 18 and 20 hours post-illumination. Arrows in (C, D) point to ectopic liver expression of GFP. Scale bars, 200 nm. **E.** qPCR analysis of GFP expression from *Tg(C120:GFP)* or *Tg(C120F:GFP);Tg(ubb:TAEL-N)* zebrafish at 2 or 4 dpf illuminated with constant blue light for 1 hour or kept in the dark. Data are presented as delta-C_T values normalized to the housekeeping gene *ef1a*. Dots represent biological replicates. Solid lines represent mean. Error bars represent S.D. *p<0.05. **F.** Fold induction of GFP expression in response to light calculated from the same qPCR analysis shown in (E). Data are presented as mean ± S.D. *p<0.05. n.s., not significant.