Multiple Chemical Inducible Tal Effectors for Genome Editing and Transcription Activation

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Supporting Information

ABSTRACT: Inducible modulation is often required for precise investigations and manipulations of dynamic biological processes. Transcription activator-like effectors (TALEs) provide a powerful tool for targeted gene editing and transcriptional programming. We designed a series of chemical inducible systems by coupling TALEs with a mutated human estrogen receptor (ER), which renders them 4-hydroxy-tamoxifen (4-OHT) inducible for access of the genome. Chemical inducible genome editing was achieved via fusing two tandem ER domains to customized transcription activator-like effector nucleases (TALENs), which we termed “Hybrid Inducible Technologies” (HIT-TALEN). Those for transcription activation were vigorously optimized using multiple construct designs. Most efficient drug induction for endogenous gene activation was accomplished with minimal background activity using an optimized inducible TALE based SunTag system (HIT-TALE-SunTag). The HIT-SunTag system is rapid, tunable, selective to 4-OHT over an endogenous ligand, and reversible in drug induced transcriptional activation. Versatile systems developed in this study can be easily applied for editing and transcriptional programming of potentially any genomic loci in a tight and effective chemical inducible fashion.

TALE proteins enabled a series of technologies ranging from targeted gene editing to transcription modulation. A TAL protein contains an array of repeats, each 33 or 34 amino acids in length. The repeat variable diresidues (RVDs) in the middle determine which DNA base is preferentially bound. Such a simple one-to-one “TAL code” altogether provides DNA binding specificity within a TAL array, which confers target sequence dependency when fused to various effectors for genetic and epigenetic modulation. Upon fusion with a nuclease such as FokI, TALENs can introduce double strand breaks (DSBs) that result in targeted gene editing, either through error prone non-homologous end-joining (NHEJ) or precise homology directed repair (HDR). When fusing with a transcription activator domain (AD) such as VP64, a TAL array that targets a promoter can direct the construct to activate gene transcription specifically. Despite the latest CRISPR/Cas9 based applications, further development of TALE technologies is necessary as one versus the other might be more optimal in different applications. Many biological processes are regulated by highly dynamic molecular events. Accordingly, conditional and inducible systems for manipulating these events are empowering for understanding their functions in greater precision. One of the most widely used chemical inducible technologies utilizes the ligand dependent translocation of estrogen receptor (ER) from the cytoplasm to the nucleus. ER binds heat shock protein Hsp90 and localizes in the cytoplasm without its hormone ligand. Upon ligand binding, ER is dissociated from Hsp90 and translocates to the nucleus. ER, an artificial mutated form bearing three mutations G400V/M543A/L544A, is much more sensitive to the synthetic estrogen antagonist 4-OHT over β-estradiol, the endogenous ER ligand, a property that is crucial for low background activity. ERT2 fusion to the Cre recombinase has been widely used in conditional and inducible genome engineering, which enables researchers to precisely dissect molecular functions with spatial and temporal resolutions.

Herein, we developed and optimized a series of chemical inducible systems based on the fusion of ERT2 to TALEs, which we named “Hybrid Inducible Technologies” (HIT). Tight and efficient 4-OHT inducible genome editing and transcriptional activation were achieved via combinatorial engineering of ER fusion constructs with different types of TALEs bearing distinct functional domains. These systems offer simple and versatile technologies for dynamic modulation of genetic functions for potentially any genomic loci of interest.
RESULTS AND DISCUSSION

Chemical Inducible Genome Editing with HIT-TALEN.

First, we envisioned a chemical inducible genome editing approach by subjecting TALEN constructs to 4-OHT regulation via linking them with ER\textsuperscript{T2} domains (Figure 1a,b). It was reported that fusion of two ER\textsuperscript{T2} domains to Cre resulted in lower background activity.\textsuperscript{14} The C-terminal Fok1 fusion in a TALEN construct has a strict conformational requirement for efficient DNA cleavage, thus likely to be less vulnerable for additional fusions.

Figure 1. Design and optimization of the HIT-TALEN system. (a) Schematic of ER\textsuperscript{T2}-TALEN fusion constructs. (b) Cartoon illuminating mechanism of HIT-TALEN system. (c) NHEJ frequencies were examined in Surveyor assays for ER\textsuperscript{T2}-TALENs and 2ER\textsuperscript{T2}-TALENs targeting the human AAVS1 site, with and without 4-OHT treatment. Cells transfected with a plasmid coexpressing ER\textsuperscript{T2} and GFP were used as negative controls (NC). (d) NHEJ frequencies were examined in Surveyor assays after transfection of the indicated amount of 2ER\textsuperscript{T2}-TALEN plasmids in each well of 24-well plates. (e) NHEJ efficiency was examined with mCherry fluorescence using a high content scanning system in a stable cell line where TLR was integrated into the genome through lentiviral delivery. Representative images (left) and quantifications (right) were shown. (f) HDR efficiency was examined with GFP fluorescence using flow cytometry in the TLR stable cell line. Representative plots (left) and quantifications (right) were shown. (g) Schematic of the donor template used for HDR mediated genome editing at the hAAVS1 site. (h) Representative images of GFP+ monoclones (left) and quantifications of GFP+ clones with and without 4-OHT induction before puromycin selection (right). (i) PCR genotyping for GFP+ clones with and without 4-OHT induction after puromycin selection. Top panel showed results using primers flanking the left homologous arm. The bottom panel showed results using primers flanking the right homologous arm. M, marker; +, + 4OHT; −, − 4OHT. (j) Representative Sanger sequencing results of genomic PCR amplicons showing correct genome editing. Error bars indicate SEM; n = 3. NS: nonsignificant; *p < 0.05; ***p < 0.001; student t test. Unless otherwise stated, data from three biological replicates were used for statistical analyses.
Therefore, we reasoned that fusion of two tandem ER\textsuperscript{2} domains to the N-terminus of TAL repeats might result in tighter regulation. We used TALENs targeting a human AAVS1 site\textsuperscript{15} and generated constructs with either one or two ER\textsuperscript{2} N-terminal fusions (Figure 1a). The Surveyor nuclease assay, which measures NHEJ efficiency, was first used to assess the efficiency and tightness of these constructs for cleavage of genomic DNA upon treatment with 4-OHT in human embryonic kidney 293T cells. In agreement with our reasoning, TALEN constructs fused with two tandem ER\textsuperscript{2} enabled chemical inducible genomic DNA cleavage, whereas single fusion did not (Figure 1c). However, 2ER\textsuperscript{2}-TALEN constructs introduced high background activity without 4-OHT treatment (Figure 1c). This background could be relieved while maintaining a chemical inducible effect by lowering the amount of TALEN constructs introduced (Figure 1d). Such an effect was also observed by decreasing the amount of just one 2ER\textsuperscript{2}-TALEN construct in the pair (Figure S1). We therefore introduced 2ER\textsuperscript{2}-TALEN constructs at a relatively low amount thereafter for low background activity while maintaining their efficiency. And we named the design of 2ER\textsuperscript{2}-TALEN “Hybrid Inducible TALEN Technology” (HIT-TALEN).

To further examine the drug induction effect of the HIT-TALEN constructs, we next employed a traffic-light reporter (TLR) construct that probed both NHEJ and HDR events (Figure S2).\textsuperscript{19} A stable cell line was used in which the reporter construct was stably integrated into the genome via lentiviral delivery. Thus, chemical inducible gene editing upon nucleus translocation of HIT-TALENs can be resolved by the TLR construct. The target sequence of TAL recognition is inserted into the GFP coding region of TLR. Neither GFP nor mCherry has a functional reading frame. NHEJ introduces insertions and deletions (indels) at the target sequence, a proportion of which lead to frame shifts that restore the mCherry reading frame. When provided a donor template of nonfluorescent GFP fragment that encompasses the target sequence, HDR leads to restoration of a functional GFP reading frame. Therefore, positive mCherry and GFP signals measure the activities of NHEJ and HDR, respectively. Using TLR, we found that both NHEJ and HDR events mediated by HIT-TALEN constructs were significantly induced by 4-OHT (Figure 1e,f). Importantly, no significant difference in either mCherry or GFP signals was observed between cells not treated with 4-OHT and those that received the same amount of traffic-light reporter only, an indication of undetectable background activity (Figure 1e,f).

We next further examined whether HDR mediated endogenous genome editing in a 4-OHT inducible manner can be achieved using the HIT-TALEN constructs. We codervivered HIT-TALEN constructs targeting the human AAVS1 locus and a donor template that carries GFP expression and puromycin resistant cassette flanked by homology arms (Figure 1g). Prior to puromycin selection, a significantly greater portion of monoclonal GFP positive cells were obtained upon 4-OHT treatment, while cells not treated with 4-OHT yielded only a background amount of GFP expressing clones compared to those transfected with the donor construct both render 4-OHT induction on its target gene activation, as examined by endogenous Sox2 expression and a mCherry reporter driven by the same TALE target sequence (Figure 2a,b and Figure S3).\textsuperscript{5,17} Notably, high background activity without a 4-OHT stimulus was associated with these constructs (Figure 2b and Figure S3).

It has been reported that VP64 and other ADs including P65(P), Rta(R), and HSF1(H), act in synergy and result in higher efficiency to initiate transcription.\textsuperscript{16,19} We therefore examined distinct ADs tandemly fused to the C-terminus of TALE-VP64 (Figure 2c). First, we fused P65(P) and Rta(R) to 1/2ERT2-TALE-VP64, a combination (VPR) reported previously (Figure S4).\textsuperscript{18} We found that dual ER\textsuperscript{2} fusion reduced the background activity in the absence of 4-OHT, while drug induction remained efficient albeit to a lower level (Figure S4). Given that low background is critical in chemical inducible systems, we then focused on the dual ER\textsuperscript{2} design and examined additional hybrid fusions of ADs in different combinations, all of which introduced a synergistically higher level of transcriptional activation upon drug induction (Figure 2c,d). Of the three constructs including 2ERT2-TALE-VP64-P65-Rta (2ERT2-TALE-VPVR), 2ERT2-TALE-VP64-P65-HSF1 (2ERT2-TALE-VPVH), and 2ERT2-TALE-VP64-P65-HSF1-Rta (2ERT2-TALE-VPVPHR), VPH elicited markedly higher transcriptional activation than VPR upon 4-OHT stimulation, and additional R fusion (VPVR) did not further improve its efficiency (Figure 2d). Nonetheless, background activation in the absence of 4-OHT remained high across all these constructs (Figure 2d and Figure S4).

Next, we hypothesized that linking more molecules of AD(s) to a TAL may further improve its efficiency of transcriptional activation. To this end, we generated a series of inducible TALE based SUperNova TAGging (HIT-TALE-SunTag) systems in which a TAL array is fused with 10 tandem repeats of GCN4 peptides, which recruits multiple molecules of AD(s) fused with a single-chain variable fragment (scFv) recognizing the GCN4 peptide (Figure 3a).\textsuperscript{20} When both the TAL-10xGCN4 and scFv-AD(s) constructs were subjected to 4-OHT regulation by fusing with ER\textsuperscript{2}, the background activity of gene activation without 4-OHT treatment was dramatically reduced to the base level (Figure 3b). By contrast, a scFv-VP64 construct tagged with nucleus localization signal (NLS) peptides, when cotransfected with fusion constructs of ER\textsuperscript{2} (s) to TALE-GCN4, introduced significant background activation in the absence of 4-OHT (Figure 3b), indicating essential drug control of ADs for low background activity. Taking advantage of a GFP protein inserted within the ScFv constructs, quantitative imaging analyses indicate cytoplasmic retention without drug and drug induced nuclear accumulation of the ER\textsuperscript{2} construct, in contrast with constitutive localization in the nucleus when fused with NLS (Figure S5). These observations confirm the ER based mechanism of drug regulation. Notably, tandem fusion of two ER\textsuperscript{2}’s to TALE-GCN4...
elicits much higher transcriptional activation upon drug induction in comparison against that of one ER\textsuperscript{T2} (Figure 3b). We therefore used the 2ER\textsuperscript{T2}-TALE-GCN4 construct in the following experiments.

Next, we optimized the HIT-TALE-SunTag system via changing from a singular AD(VP64) to tandem AD combinations (VPR, VPH, VPHR; Figure 3a and c). Interestingly, only VPH showed significant improvement over VP64 in activation efficiency (Figure 3e). We also observed that dual ER\textsuperscript{T2} fusions did not further improve drug induction efficiency or affect background activation for the scFv-VPH construct (Figure S6), thus using AD constructs fused with single ER\textsuperscript{T2} in the following experiments.

Multiple GCN4 repeats in this HIT-TALE-SunTag system offer an option to split the most efficient VPH combination, which, as we speculated, might result in better synergy for even higher efficiency. Indeed, a higher level of transcriptional activation was achieved by codelivering 2ER\textsuperscript{T2}-TALE-GCN4, scFv-ER\textsuperscript{T2}-VP64, and scFv-ER\textsuperscript{T2}-PH (Figure 3d), suggesting recruitment of VP64 and PH to neighboring GCN4 sites leads to higher synergy than tandem fusion within the same molecule. The same difference was observed when replacing 2ER\textsuperscript{T2}-TALE-GCN4 with a NLS tagged TALE-GCN4 construct (Figure 3e). By contrast, V+P+H did not result in better efficiency (Figure 3d), possibly due to the limited number of synergistic AD combinations that the GCN4 array can accommodate. Furthermore, when using the NLS-TALE-GCN4 construct, a marked increase over 2ER\textsuperscript{T2}-TALE-GCN4 in drug induced activation efficiency was observed without elevating the background activity (Figure 3e), which indicates that chemical inducible control of ADs is sufficient to achieve tight regulation and that enhanced delivery of TALE construct to the nucleus promotes efficiency. Consequently, our optimization of the HIT-TALE-SunTag system concluded at conjunct use of NLS-TALE-GCN4, scFv-ER\textsuperscript{T2}-VP64, and scFv-ER\textsuperscript{T2}-PH.

We then compared the most efficient constructs in each scenario side by side in the same experiment, including 2ER\textsuperscript{T2}-TALE-VP64, 2ER\textsuperscript{T2}-TALE-VPH, and HIT-TALE-SunTag. Results confirmed both the highest efficiency and the lowest background for the optimized HIT-TALE-SunTag system (Figure 3f and g).

Upon completion of optimization using Sox2 as a surrogate, we next asked whether our design can be applied to other genes in a generalizable manner. We then expanded our investigations to additional genes, including Klf4, cMyc, and Neurog2, and observed tight, potent, and specific chemical inducible transcriptional activation elicited by our optimized HIT-TALE-SunTag design across all these genes (Figure 4). Significant improvement of activation potency over TALE-VP64 was confirmed (Figure 4a,b).

In addition to tightness and effectiveness, a good chemical inducible system should be tunable, selective, rapid, and reversible in response to chemical control. Its dynamic control is particularly important for transcriptional modulation. Therefore, we next examined the performance of HIT-TALE-SunTag system according to these additional criteria. Using a luciferase assay, in which the reporter expression was driven by Sox2 TALE target sequence, we first examined dose dependent activation and observed a highly selective response to 4-OHT over \(\beta\)-estradiol, the endogenous ER ligand (Figure 5a). When cells were treated with 4-OHT at two concentrations in the selective window (Figure 5a), significant activation could be observed at as short as 1 h, and the signal was increased over time, an indication of a very rapid response that is also tunable by altering the exposure time to the ligand in addition to its concentration (Figure 5b). We also conducted a reversibility test by toggling 4-OHT treatment. Activated luciferase signal declined upon 4-OHT withdrawal and resurge upon resupply of the compound (Figure 5c and Figure S7).
Figure 3. Design and optimization of HIT-TALE-SunTag systems. (a) Schematic of constructs. The expression of endogenous Sox2 is activated by cotransfection of GCN4 and scFv plasmids. (b−f) Relative mRNA level of endogenous Sox2, as determined by quantitative RT-PCR, after transfections of indicated plasmids and 4-OHT treatments. (g) Schematic presentation of the mechanism of chemical inducible endogenous gene activation using N-TALE-GCN4, E-VP64, and E-PH constructs shown in a, the most efficient HIT-SunTag system we have developed in this study. Cells transfected with a plasmid coexpressing ER^{12} and GFP were used as negative controls. Error bars indicate SEM; n = 3. NS, nonsignificant; *p < 0.05; **p < 0.01; ***p < 0.001; student t test.
These data demonstrated our HIT-TALE-SunTag system delivered selective, tunable, rapid, and reversible actions, crucial properties for precise and dynamic control of functional modulation.

**DISCUSSION**

In summary, we have designed and vigorously optimized chemical inducible systems for genome editing and transcription activation based on 4-OHT induced cytoplasm-nucleus translocation of TALE and AD constructs fused with ER<sup>T2</sup> (Figures 1b and 3g). These systems open new opportunities for precise chemical control of dynamic biological processes, which are potentially useful for many biomedical investigations and applications. 2ER<sup>T2</sup>-TALENs enable efficient chemical inducible genome editing via both NHEJ and HDR with minimal background, as demonstrated by Surveyor assays, fluorescent reporter assays, and genome targeting (Figure 1). Further, the best chemical inducible system for transcription activation engineered in this study was the conjunct use of NLS-TALE-GCN4, scFv-ER<sup>T2</sup>-VP64, and scFv-ER<sup>T2</sup>-PH, which we termed HIT-TALE-SunTag (Figures 3e−g and 4). Multiple merits of a such system was demonstrated, including its tight, effective, tunable, selective, and reversible response to 4-OHT induction (Figures 4 and 5). Notably, no background signal was observed from the optimized HIT-TALE-SunTag system in the absence of 4-OHT, which is in strong contrast to the high background from direct fusion constructs (Figures 2, 3e−g, and 4). This points to the best approach being one that makes functional effectors subject to drug induction while maximizing TAL’s access to its target. Moreover, VPH combination showed the highest efficiency across multiple scenarios (Figures 2d and 3c) and splitting VPH to V and PH exhibited higher intermolecular synergy in the HIT-TALE-SunTag system (Figure 3d and e). In addition, tandem fusion of 2-ERT2 often results in tighter control of drug induction (Figure 1c, 2b and Figure S3). Interestingly, while dual-ERT<sup>T2</sup> fusion to TALE-AD often compromises transcriptional activation in response to 4-OHT treatment (Figure 2b and Figure S4), that to TALEN and TALE-GCN4 (Figure 1c and Figure 4b) appears to further elevate their potency upon drug induction. Such discrepancy when fusing with different C-terminal domains might reflect distinct folding or trafficking dynamics of these hybrid constructs. It also demonstrated the exciting possibilities,

**Figure 4.** Activation of multiple endogenous genes using optimized HIT-TALE-SunTag system. The relative mRNA levels of multiple endogenous genes, including Sox2, Klf4, cMyc, and Neuro2, were examined using quantitative RT-PCR upon cotransfection with their specific NLS-TALE-GCN4 targeting the promoter region in conjunction with scFv-ER<sup>T2</sup>-VP64 and scFv-ER<sup>T2</sup>-PH and upon 4-OHT treatment. NLS-TALE-VP64 constructs targeting Sox2 (a) and Klf4 (b), which were reported previously, were included for comparison. Cells transfected with a plasmid coexpressing ER<sup>T2</sup> and GFP were used as negative controls. Error bars indicate SEM; n = 3. NS, nonsignificant; **p < 0.01; ***p < 0.001; student t test.
in some cases, of lowering background noise without a drug and enhancing drug induced potency at the same time. In the future, similar designs to HIT-TALE systems can be applied for other types of functional modulations such as transcriptional repression,21 and epigenetic modulation.22,23 Further, development of 4-OHT inducible CRISPR/Cas9 systems based on ER24 fusions will greatly expand the applicability of these designs.24

## METHODS

### Construction of TALE and Reporter Plasmids.

ER72 was cloned from the pAd-CreER plasmid (a gift from T. C. He’s lab, Chicago University). One or two tandem ER72s was fused to the N-termini of a pair of TALEs targeting the human AAVS1 site (gifts from Feng Zhang, Addgene plasmids #35431 and #35432;14 Figure 1a). The donor plasmid was constructed using AAVS1 hPGK-Puro-Ra donor (a gift from Rudolf Jaenisch, Addgene plasmids #22072)25 by inserting a GFP expression cassette downstream of a puromycin resistant gene (Figure 1g). To interrogate constructs for chemical inducible transcription activation, an artificial TALE of 12.5-mer repeats to target a 14-bp DNA-binding site located on the promotor of the human Sox2 gene (a gift from Feng Zhang, Addgene plasmids #35388) was used as a surrogate. Constructs of N-terminal fusions with ER72 or 2ER72 were generated. Multiple synthetic transcription activation domains (ADs) including VP64(V), P65(P), Rta(R), and HSFI(H) were fused with the Sox2 TAL array at its C-terminus in various combinations (Figure 2a,c). Both V and H were PCR amplified from plasmid templates (gifts from Feng Zhang, Addgene plasmids #35388 and # 6142619), while P and R were synthesized (Genezwir) according to the sequences reported by Chavez et al.18 As for the TALE-SunTag designs, 10xGCN4 repeats were fused with 1/2ER72-Sox2TALE or 3xNLS-Sox2TALE constructs at its C-terminus. And a series of ADs in distinct combinations were cloned into scFv-sGFP-GBI−1/2ER72 constructs at their C-termini (Figure 3a). ScFv-sGFP-GBI and 10xGCN4 were synthesized (Genezwir) according to the sequences reported by Tanenbaum et al.19 The traffic light reporter plasmid used in this study to examine gene editing activity was constructed by replacing the Sce target with the hAAVS1 targeting sequence in the plasmid of pCVL. Traffic Light Reporter 1.1 (Sce target) EFLa Puro (a gift from Andrew Scharenberg, Addgene plasmids #31482;16 Figure S2), GFP donor plasmid was a gift from Andrew Scharenberg (Addgene plasmids #31475).13 The mCherry and gaussia luciferase reporters used in this study to examine transcription activation was generated by inserting sequences containing the Sox2 TALE binding site upstream of a minimal promoter driving the reporter expression (Figure 5, Figure S3a and 7). Key HIT-TALE and HIT-TALEN constructs will be deposited to Addgene.

### Lentivirus Production.

Lentiviral particles were produced by cotransfecting 293T cells with each of the lentiviral expression plasmids, the psPAX2 plasmid (a gift from Didier Trono, Addgene plasmids #12260), and the pCMV-VSV-G (a gift from Bob Weinberg, Addgene plasmids #8454)26 at a ratio of 10:9:1, respectively. Viral supernatants were collected 48–72 h following transfection and concentrated using the amicon ultracentrifugal filter unit (Millipore) if necessary.

### Cell Culture.

The human embryonic kidney cell line 293T (ATCC) was maintained in Dulbecco’s Modified Eagle’s Medium supplemented with 10% (v/v) FBS, 2 mM GlutaMAX (Thermo Fisher), 100 U/mL penicillin, and 100 μg/mL streptomycin under 37 °C and 5% (v/v) CO2. Transfections were done using Lipofectamine 2000 (Thermo Fisher) according to the manufacturer’s recommended protocol. Within each experiment, the molar amount of each AD and/or the total weight of transfected DNA were matched for each well. Twenty-four hours after transfection, 200 nM of 4-OHT or a matched amount of control vehicle was added into the medium, and cells were cultured for additional 48 h before examinations of genome editing or transcription activation.
The polyclonal traffic light reporter (TLR) cell line TALEN-TLR-293T was established by lentiviral delivery of the TALEN-TLR reporter into 293T cells followed by selection with 2 μg/mL of puromycin for 5 days. The polyclonal mCherry reporter cell line used in this study was established by lentiviral infection of the 293T cell line followed by selection with 2 μg/mL of puromycin for 5 days.

**Surveyor Assay.** Various amounts of ER72/2ER72-TALEN pairs were transfected into 293T cells seeded in 24-well plates. Transfected cells were then cultured at 37 °C for 48 h with or without addition of 4-OHT. Genomic DNA was then extracted using the Wizard Genomic DNA purification kit (Promega). The human AAVS1 locus was PCR amplified from 100 ng of genomic DNA using a high fidelity polymerase (Accuprime TaqHiII from Thermo Fisher) in 25 μL reactions. The sequences of primers are listed in Table S1. A mixture containing 3.5 μL of PCR products and 6 μL of 1 × Accuprime buffer II was then denatured by heating to 95 °C and slowly reannealed from 95 to 85 °C by 2 °C/s followed by 0.1 °C/s from 85 to 25 °C for rehybridization using Mastercycler nexus gradient (Eppendorf). Samples were then incubated with Surveyor nuclease (Transgenomic) for 20 min at 42 °C. The nuclease recognizes and cleaves DNA mismatches (wild type-mutant hybridization). The digested products were electrophoresed through a 15% acrylamide gel (w/v) and visualized by EB staining. Quantification of cleavage bands was performed using ImageJ software (National Institutes of Health). The genome cleavage efficiency of the tested target was calculated by the following formula:

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\text{Indel percentage (\%) = 100 \times (1 - (1 - \text{fraction cleaved})^{1/2})}
\]

The fraction cleaved was determined by dividing the average intensity of cleavage products (185–295 bps) by the sum of the intensities of the uncleaved PCR product (480 bp) and the cleavage product.

**TLR Assay.** A total of 200 ng of each 2ER72-TALEN with or without 600 ng of GFP donor template (a gift from Andrew Scharenberg, Addgene plasmids #31475(4)) was transfected into each well of TLR reporter cells preseeded on 24-well plates. After 48 h of incubation with or without 4-OHT, cells for HDR assessment were resuspended by 0.5% trypsin (w/v; Thermo Fisher) and applied for flow cytometry using CytoFLEX cell analyzer (Beckton Coulter). At least 50,000 cells from each well were analyzed. The HDR rates were determined by the percentiles of GFP positive cells. As for NHEJ measurements, cells were transferred to 96-well plates. Cells were then fixed by 4% (w/v) paraformaldehyde and stained with Hoechst 33342 (Thermo Fisher). The cell images were collected using the Operetta High Content Screening system (PerkinElmer). The mCherry fluorescence was quantified using Harmony 3.5 (PerkinElmer).

**HDR Mediated GFP Knock-in.** To evaluate HDR mediated precise genome editing by 2ER72-TALENS, 293T cells were seeded in 24-well plates and cotransfected with TALENS and a donor template containing hPGK driven puromycin and EF1α driven GFP. The cells were transfected with donor only were used as a negative control. One day after transfection, cells were passaged to 10 cm culture dishes at a density of 200 cells/dish. After 7 day culture in the presence or absence of 4-OHT, cells were maintained in culture medium supplemented with 2 μg/mL puromycin for 2 weeks. The numbers of GFP+ monolones and the total numbers of monolones were counted before and after puromycin selection. Genomic DNA of surviving monolones after puromycin selection was extracted and employed for PCR amplification using primers specific to puromycin and GFP (Tables S1 and S2). The PCR products were examined by electrophoreses and Sanger sequencing.

**Transcription Activation Assays.** The polyclonal mCherry reporter cell line was cultured and transfected using standard protocols. And mCherry fluorescence was scanned using the Operetta High Content Screening system (PerkinElmer) and quantified by Harmony 3.5 (PerkinElmer).

For endogenous gene activation, at least 500,000 cells were harvested from each transfection and subsequently processed for total RNA extraction using the Direct-zol RNA MiniPrep Kit (Zymo Research). cDNA was generated using the GoScript cDNA Synthesis Kit (Promega) according to the manufacturer’s recommended protocol. mRNA expression levels were quantitated using SYBR Green Gene Expression Assays (Toyobo). GAPDH was used as an internal control for normalization. The sequences of qPCR primers and TALEN targets were listed in Tables S2 and S3.

To examine the dose dependence, speed of response, and reversibility upon drug induction (Figure 5 and Figure S7), a stable cell line was generated via lentiviral infection, in which a gausia luciferase reporter driven by the Sox2 TALE target sequence was integrated into the genome. HIT-TALE-SunTag constructs based on Sox2 TALE were stably expressed upon sequential delivery. A cell line in which ER72-GFP replaced scFv-ER72-ADs served as normalization basis for fold of activation. All gausia luciferase readout was normalized against cell numbers probed by cell counting kit (ckc-8).

**ASSOCIATED CONTENT**

* Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschembio.7b00606.

Figures S1–S7 and Tables S1–S3 (PDF)

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**Author Contributions**


**Notes**

The authors declare the following competing financial interest(s): Patents covering the novel designs in this work have been filed.

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