

Real-time observation of light-controlled transcription in living cells

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Abstract

Gene expression is tightly regulated in space and time. To dissect this process with high temporal resolution, we introduce an optogenetic tool termed BLInCR (Blue Light-Induced Chromatin Recruitment) that combines rapid and reversible light-dependent recruitment of effector proteins with a real-time readout for transcription. We used BLInCR to control the activity of a reporter gene cluster in the human osteosarcoma cell line U2OS by reversibly recruiting the viral transactivator VP16. RNA production was detectable ~2 minutes after VP16 recruitment and readily decreased when VP16 dissociated from the cluster in the absence of light. Quantitative assessment of the activation process revealed biphasic activation kinetics with a pronounced early phase in cells treated with the histone deacetylase inhibitor SAHA. Comparison with kinetic models for transcription activation suggests that the gene cluster undergoes a maturation process when activated. We anticipate that BLInCR will facilitate the study of transcription dynamics in living cells.

Introduction

Tools that enable the accurate control, visualization and quantitation of the transcription process have driven recent progress in the field and are key to dissecting its molecular underpinnings. Tracing RNA by live cell imaging has provided a wealth of information on gene regulation and RNA processing (Cho et al., 2016; Darzacq et al., 2009; Larson et al., 2013; Martin et al., 2013). A system used in several of these studies is a reporter gene array integrated into the human U2OS cell line (Janicki et al., 2004). It has been applied to investigate transcription activation and the associated changes in the chromatin environment (Janicki et al., 2004; Rafalska-Metcalf et al., 2010) as well as elongation and pausing of RNA polymerase II (Darzacq et al., 2007). However, it is difficult to interrogate the early steps of the activation process and to assess its reversibility with existing techniques, which rely on chemicals for transcription initiation and therefore depend on diffusion and uptake/release of the respective compounds. To control protein interactions with high temporal precision a variety of optogenetic methods have been adapted for use in mammalian cells (Tischer and Weiner, 2014). These include the CIBN-PHR system derived from the *Arabidopsis thaliana* proteins CIB1 and CRY2. Rapid binding of PHR-fused effector proteins to tethered CIBN can be induced by illumination with blue light (Kennedy et al., 2010). Different variations of the CIB1-CRY2 system have been exploited to recruit the viral transactivator VP64 to gene promoters marked by transcription activator-like effectors (TALEs) or dCas9 fusion constructs (Koneremann et al., 2013; Polstein and Gersbach, 2015). These systems did not include live-cell readouts for transcriptional activity and were therefore not suited to study the kinetics of transcription activation with high time resolution in single cells. Here, we introduce a tool termed **Blue Light-Induced Chromatin Recruitment (BLInCR)**, which combines rapid and reversible binding of effectors with a real-time transcription readout in living cells. We used BLInCR to dissect the transcription activation process at a gene cluster and to probe the persistence of its activated state.

Results

Implementation of a light-inducible chromatin recruitment system

The BLInCR system is based on the PHR and CIBN domains of the *Arabidopsis thaliana* proteins CRY2 and CIB1 (Kennedy et al., 2010) that interact with each other when illuminated with blue light (**Fig. 1A**). Accordingly, CIBN fusion proteins that localize to nuclear structures or genomic loci of interest ('localizers') reversibly bind PHR fusion proteins ('effectors') upon exposure to blue light. To test the versatility of the BLInCR system

we triggered and visualized the targeting of fluorescently labeled effector proteins to different nuclear subcompartments in the human U2OS 2-6-3 cell line (Janicki et al., 2004). It carries a stably integrated array of ~200 reporter construct units with promoter-proximal repeats of the *tetO* bacterial operator sequence. BLInCR robustly induced accumulation of PHR-mCherry as a mock effector at the reporter array, telomeres, nucleoli, PML bodies or the nuclear lamina (**Fig. 1B**, **Supplementary Table S1**).

We then tested whether BLInCR is compatible with the U2OS 2-6-3 reporter system that allows the visualization of RNA transcripts in living cells. Their detection is based on a fluorescently labeled MS2 coat protein (MCP) that binds MS2 stem-loop sequences contained within the RNA transcripts that are produced at the reporter array (**Fig. 2A**). The protein products encoded by these transcripts contain a cyan fluorescent protein (CFP) domain and localize to peroxisomes. We transfected cells with CIBN-TetR and PHR-YFP-VP16, which contains the strong viral VP16 transcription activation domain, and illuminated them with blue light overnight, thereby constantly recruiting the activator to the array. We observed MS2-RNA accumulation at the reporter array and peroxisomal CFP expression in almost all illuminated cells (RNA: 92%, CFP: 81%, $n = 37$; see **Fig. 2B** for a representative cell). We confirmed RNA production at these arrays by RNA FISH with a probe directed against the MS2 loop sequences of the transcript (**Supplementary Fig. S1A,B**). Furthermore, RNA polymerase II (**Fig. 2C**) and its Ser5-phosphorylated form (**Supplementary Fig. S2**) were present at reporter arrays activated by PHR-YFP-VP16 but not at silent arrays in cells that expressed the PHR-YFP-NLS control. To assess if activation affects other genes beyond the reporter we performed RNA-seq in non-transfected cells and in cells transfected either with CIBN-TetR and PHR-YFP-VP16 or, as a control, with RFP-TetR and YFP. All cells were illuminated with blue light following transfection. Transfection with CIBN-TetR and PHR-YFP-VP16 induced a robust activation of the reporter. Virtually no additional changes of gene expression were observed beyond those seen in the control sample that was transfected with RFP-TetR and YFP (**Supplementary Fig. S3**). Accordingly, we conclude that BLInCR behaves like other techniques that involve TetR in this respect.

Kinetics of light-induced chromatin association and dissociation

To characterize the kinetics of light-induced association and subsequent dissociation of the VP16 effector protein at the reporter array, we transfected cells with a CIBN-TetR construct and a fluorescently labeled PHR-VP16 fusion. To quantitate the association reaction, we first identified the array using RFP-tagged CIBN-TetR excited by green laser light, which does not induce optogenetic recruitment. Subsequently, the binding of YFP-labeled PHR-VP16

was induced with blue laser light and monitored at 200 ms time resolution (**Supplementary Fig. S4A, Movie 1**). Half-maximal levels were reached after 13.6 ± 4.9 s (**Fig. 2D**), which is about one and two orders of magnitude faster than doxycycline- and tamoxifen-induced recruitment, respectively (Normanno et al., 2015; Rafalska-Metcalf et al., 2010). These estimates refer to moderate expression levels of the recruited component of ~ 100 nM. YFP-labeled PHR fused to a nuclear localization sequence (NLS) or to the human histone acetyltransferase GCN5 accumulated with similar kinetics (**Supplementary Fig. S4B, Supplementary Table S2**). Induced accumulation at the array occurred fast with two characteristic rates (**Supplementary Table S2**). These might reflect the kinetics of the CIBN-PHR interaction and the previously described PHR oligomerization (Bugaj et al., 2013), which are both triggered by blue light. To quantitate the dissociation reaction, PHR-mCherry-VP16 was targeted to the reporter array in cells expressing CIBN-TetR by illumination with blue light for 38 s (**Supplementary Fig. S4C**). Subsequently, the loss of mCherry signal from the array was monitored over time, yielding a characteristic half-life of 4.9 ± 0.8 min (**Fig. 2E**), which was identical for PHR-mCherry-NLS (4.8 ± 0.6 min, **Supplementary Fig. S4D, Supplementary Table S3**). Notably, the effectors were not detectable at the array 15-20 min after removing the light trigger.

Quantitation of transcription activation

Next, we used BLInCR to follow the transcription activation kinetics of the reporter array in U2OS 2-6-3 cells with high temporal resolution. RNA polymerase II was present at the array only a few seconds after activation (**Movie 2**). Low RNA levels were already detectable after about two minutes (**Fig. 3A**), followed by a lag phase with little or no additional RNA accumulation (**Fig. 3B**, black line). After 20-30 minutes, a second phase of rapid accumulation of reporter RNA was observed, indicating that the activation process involves at least two distinct time scales. To assess the influence of activating chromatin marks on the activation kinetics we treated cells with the histone deacetylase inhibitor SAHA, which led to globally elevated histone acetylation levels (**Fig. 3C**). Local enrichment of histone acetylation has previously been shown to coincide with transcriptional activation of the reporter array (Rafalska-Metcalf et al., 2010) and was also observed when activating transcription using BLInCR overnight (**Supplementary Fig. S5A-C**). Cells that had been pretreated with SAHA exhibited a much more pronounced fast activation phase (**Fig. 3B**, red line), suggesting that preexisting histone acetylation might facilitate the activation process. These results are consistent with the notion that some of the ~ 200 copies of the reporter array are poised for immediate activation, and that this fraction can be increased by hyperacetylation of histones in SAHA-treated cells. It is noted that SAHA elicits a complex

cellular response beyond histone hyperacetylation (Johnstone and Licht, 2003). In particular, it has been reported that a variety of non-histone targets are affected with respect to their acetylation state (Choudhary et al., 2009). Those effects also influence the cell cycle, which we assessed in the U2OS cell line studied here by FACS. The fraction of cells in S phase was reduced by about 17% with a corresponding increase of the G1 fraction upon SAHA treatment. Thus, SAHA induces other effects than histone hyperacetylation that might also contribute to changes of the transcription activation kinetics.

The produced reporter RNA was not homogeneously distributed across the array (**Fig. 3A**, insets), suggesting that activated transcription sites might be clustered and co-regulated. The size of the reporter array remained unchanged within the first 50 minutes after PHR-YFP-VP16 recruitment (**Fig. 3B**, bottom). Thus, the global chromatin decondensation on the μm scale observed previously (Janicki et al., 2004; Rafalska-Metcalf et al., 2010) was not a prerequisite for PHR-YFP-VP16 mediated activation, but might rather occur downstream of transcription initiation (**Supplementary Fig. S1C**). This conclusion is in line with other studies showing that decondensation is not required for transcription (Carpenter et al., 2005; Tumber et al., 1999), suggesting that both processes can occur independently from each other. The transcriptional response to VP16 recruitment was heterogeneous among cells. Without treatment, the response time, after which transcripts were first detectable, varied from less than two minutes to more than 30 minutes, whereas it was always less than 5 minutes in SAHA-treated cells (**Fig. 3D**, top). In addition, the extent of early transcription as estimated from the level of the first plateau at ~9-12 min (see **Fig. 3B**) varied greatly for both treated and untreated cells (**Fig. 3D**, bottom) and heterogeneity was also observed among constitutively activated cells (**Supplementary Fig. S1A,B**). This heterogeneity might reflect differences in epigenetic promoter signals and other chromatin features.

Modeling of transcription activation kinetics

To interrogate the transcription activation mechanism, we analyzed the activation kinetics measured for treated and untreated cells (**Fig. 3B**) according to the theoretical framework described in the Methods section. In a comparison of different models, we found that a previously derived two-state model (Shahrezaei and Swain, 2008) did not fit the early kinetics observed here (**Fig. 4A**, left). This model was recently found to describe the reactivation kinetics of a single-copy reporter that was silenced by recruitment of a histone deacetylase beforehand (Bintu et al., 2016). In contrast, a model with the same number of parameters that included positive feedback and a fraction of promoters that acted as independent transcription units fitted the data well (**Fig. 4A**, center). Positive feedback could originate from spatial interactions among cooperatively transcribed reporter genes (Li et al.,

2012; Papantonis and Cook, 2013) and might include the transcription-induced relocalization of promoters during the activation process (Therizols et al., 2014). A third model that represents the activation process as a series of multiple sequential reaction steps with the same transition rate also yielded a good but slightly worse fit (**Fig. 4A**, right). These steps might represent nucleosome repositioning events (Boeger et al., 2008; Nocetti and Whitehouse, 2016), histone modification reactions as well as recruitment of different components of the transcription machinery. Fit parameters for the different models are listed in **Supplementary Table S4**. Both the feedback and the sequential model predict a rate of $k_d \sim 0.5\text{-}0.6 \text{ min}^{-1}$ for the loss of transcripts from the array. Thus, RNA molecules dissociate relatively quickly after synthesis.

To assess the influence of the recruitment speed of the activator on the observed activation kinetics we conducted simulations for different recruitment rates (**Fig. 4B**). To this end we used the sequential model that serves as a proxy for the experimental data as shown above. Slow recruitment of the activator ($k \leq 0.1 \text{ min}^{-1}$, black/blue lines in **Fig. 4B**) would mask the early activation phase. In contrast, fast recruitment ($k > 1 \text{ min}^{-1}$, red/yellow lines) with rates similar to those achieved by BLInCR ($k > 10 \text{ min}^{-1}$ and $\tau_{1/2} \sim 12 \text{ s}$, **Table S2**) could resolve both activation phases. Accordingly, the early activation phase might not be visible when using tamoxifen ($k \sim 0.04 \text{ min}^{-1}$, $\tau_{1/2} \sim 17 \text{ min}$ (Rafalska-Metcalf et al., 2010)) or doxycycline ($k \sim 0.4 \text{ min}^{-1}$, $\tau_{1/2} \sim 100 \text{ s}$, (Normanno et al., 2015)) to recruit transcriptional activators present at low concentrations. Slow recruitment in combination with moderately fast RNA detection (e.g., every 5 minutes) would yield data that could be fitted with the two-state model (**Fig. 4B**, right). Thus, both fast recruitment of the activator and fast readout of RNA production are critical to resolve the biphasic activation kinetics and thereby distinguish between different activation models.

Persistence of transcriptional activity

We next asked if transcriptional activity persisted after removal of the activating stimulus (**Fig. 5A**). Such persistence might, for example, arise from activating histone modifications that maintain the reporter array in a permissive state (Ng et al., 2003; Zhang et al., 2015). When we analyzed the RNA signal at the array during and after VP16 recruitment (**Fig. 5B**) we found that RNA production was readily detected upon VP16 binding and decreased upon VP16 dissociation in the absence of blue light (**Fig. 5C**). As expected, the reporter could be reactivated 40 minutes after the first activation phase (**Fig. 5C**). Accordingly, we conclude that transcriptional activity at the array requires continuous recruitment of an activator. To validate these findings, we used real-time quantitative PCR to follow the total amount of reporter RNA in cells that had been illuminated for 50 minutes or 3 hours. Total RNA levels

should increase over time and reach a plateau if transcriptional activity persisted and decrease over time otherwise (**Fig. 5A**). For both pulse durations, RNA levels decreased after blue light was switched off (**Fig. 5D**), supporting the conclusion that most of the transcriptional activity is lost when VP16 is removed.

Discussion

The BLInCR method presented here can rapidly and precisely trigger light-induced recruitment of transcription regulators to genomic loci in single living cells to measure the RNA output with high temporal resolution. It combines the capability of plant-based optogenetic systems for transcriptional control, e.g., (Koneremann et al., 2013; Motta-Mena et al., 2014; Niopek et al., 2014; Polstein and Gersbach, 2015), with the power of real-time microscopy readouts, e.g., (Janicki et al., 2004). In this manner, cellular function can be dissected at subcellular resolution over time. Our approach can be easily adapted to study the activities of other PHR-fused effectors within any nuclear subcompartment that can be marked by CIBN-fused targeting factors. While the present study applied only confocal fluorescence microscopy imaging, BLInCR is compatible with several fluorescence microscopy-based techniques like wide-field microscopy with regular filter sets, super-resolution microscopy, single particle tracking and other approaches for mobility imaging. We focused here on the measurement of RNA production via fluorescent MS2 coat proteins as a prototypical functional readout to monitor cellular processes in real-time. Further, we exploited the high temporal resolution of BLInCR to distinguish between conceptionally different models for transcriptional activation. Our kinetic analysis revealed that even strong transcriptional activators like VP16 cannot readily activate an entire gene cluster in one step (**Fig. 5E**). Rather, full activity is only reached after a pronounced maturation phase, which might allow actively transcribed genes to contact and activate promoters in close spatial proximity (Cremer et al., 2015; Papantonis and Cook, 2013). Notably, a similar mechanism has been proposed to explain the function of actively transcribed enhancers (Li et al., 2016). Furthermore, we found that the lifetime of the activated state is limited, suggesting that even a cluster containing several active genes requires the continuous presence of transcriptional activators to maintain its activity. We anticipate that BLInCR will facilitate the study of the dynamic regulation of transcription and other nuclear processes for which the induction kinetics as well as the persistence of the output signal are crucial parameters to understand the underlying molecular mechanisms.

Material and Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Plasmids

Effector and localizer plasmids were constructed based on sequences coding for the PHR domain of cryptochrome 2 and the CIBN domain of CIB1. pCRY2PHR-mCherryN1 and pCIBN(deltaNLS)-pmGFP described in (Kennedy et al., 2010) were obtained from Addgene (plasmids #26866 and #26867). LacI and TetR constructs are based on the fluorescently tagged proteins described in (Lau et al., 2003; Pankert et al., 2017). Human GCN5 was cloned from pAdEasy Flag GCN5 (Lerin et al., 2006) (Addgene plasmid #14106). The near-infrared fluorescent protein iRFP713 was from piRFP (Filonov et al., 2011) (Addgene plasmid #31857). The nuclear localization signal (NLS) used in some of the BLInCR constructs (**Supplementary Table S1**) is the peptide PKKKRKV from SV40 large T antigen (Kalderon et al., 1984) and was inserted by PCR. The MS2 coat protein (MCP) was derived from previously described vectors (Auslander et al., 2012; Pankert et al., 2017). Other human plasmids were derived from previously described vectors containing sequences coding for TRF1 (Chung et al., 2011), TRF2 (Jegou et al., 2009), PMLIII (Jegou et al., 2009), NCL (Caudron-Herger et al., 2015), LaminB1 (Muller-Ott et al., 2014) the viral transactivator VP16 (Gunther et al., 2013) and GFP-Pol II (Sugaya et al., 2000). Plasmids associated with this study are available at the Addgene plasmid repository.

Cell culture and transfection

U2OS 2-6-3 cell has been described previously (Janicki et al., 2004) and was kindly provided by Susan Janicki and David Spector. Cells were cultured in DMEM medium containing 1 g l⁻¹ glucose, supplemented with 10% doxycycline-free fetal bovine serum, 1x penicillin/streptomycin and 2 mM stable glutamine. For live cell experiments, cells were seeded in matrigel-coated (1:100 in serum-free medium for at least 30 min at room temperature) slides (Cellview, Greiner Bio-One, Austria) and cells were seeded on glass coverslips or in 4-well culture inserts (Ibidi, Germany) for IF and RNA FISH. Cells were transfected with the appropriate constructs using Effectene (Qiagen, Germany) or Xtreme Gene 9 transfection reagent (Roche) in case of subsequent RNA isolation (for RT-qPCR or RNA-Seq). Medium was changed four hours after transfection and 2 μM SAHA (Millipore)

was added to the fresh medium if applicable. The FACS analysis of the cell cycle distribution was conducted as described previously (Osterwald et al., 2015).

Fluorescence microscopy and image processing

Cells were kept in the dark overnight and mounted on a Leica TCS SP5 confocal microscope equipped with a HCX PL APO lambda blue 63x/1.4 NA oil immersion objective. A red flashlight was used to avoid premature exposure to blue light. The following excitation and emission wavelengths were used: CFP (405 nm/415-475 nm), GFP/YFP (488 nm/500-550 nm), tagRFP/tagRFP-T (561 nm/575-630 nm), mCherry/mKate2 (594 nm/605-750 nm), iRFP713 (633 nm/645-780 nm). Images were acquired as described below for the different experiments. Image analysis was done using the Fiji distribution (Schindelin et al., 2012) of ImageJ (Schneider et al., 2012). The enrichment $E(t)$ of a given protein at the *tetO* or *lacO* arrays was calculated from single images or the maximum intensity projections of image stacks. To quantify enrichments, the intensity difference between the array region (I_{array}) and the nuclear reference region (I_{nuc}) was computed for each time point:

$$E(t) = I_{array}(t) - I_{nuc}(t)$$

The nuclear reference region was selected to be close to the array region to account for uneven illumination of the cell if necessary, but outside of nucleoli, which generally showed some depletion of the constructs. To correct for bleaching, the decay of the mean intensity difference at the nuclear reference region and a background region outside of the cell $I_{nuc}(t) - I_{background}(t)$ was fitted with a single exponential term $a \cdot e^{-k \cdot t}$. The enrichment E at the array was then calculated as the intensity difference between array and nuclear reference region divided by the bleach contribution $e^{-k \cdot t}$, which was assumed to be the same for the array and the nucleus:

$$E(t) = (I_{array}(t) - I_{nuc}(t)) / e^{-k \cdot t}$$

BLInCR recruitment kinetics

U2OS 2-6-3 cells were transfected with CIBN-TetR-tagRFP-T and a PHR-YFP construct (i.e. PHR-YFP-VP16, PHR-YFP-NLS or PHR-YFP-hGCN5). Transfected cells and reporter arrays were identified in the tagRFP-T channel. Subsequently, an image series of 400 frames with 256 x 256 px images was recorded in the YFP channel at a scan speed of 1400 Hz corresponding to 204.3 ms per image and a total of ~81 s. Excitation of YFP caused optogenetic switching, resulting in accumulation of the respective PHR-YFP construct at the CIBN site, i.e. the array seen in the tagRFP-T channel (**Supplementary Fig. S4A**). To quantitatively analyze the enrichment of PHR-YFP constructs at the array site,

a maximum intensity projection was used to select the array region (diameter: ~15 px) and a nuclear reference region (diameter: 30 px) at which the mean fluorescence intensities were measured (**Supplementary Fig. S4A**) to compute $E(t)$ as described above. For PHR-YFP accumulation, all curves could be fitted well with a model containing two exponentials:

$$E(t) = a - b \cdot \exp(-k_1 \cdot t) - c \cdot \exp(-k_2 \cdot t)$$

This equation describes a reaction model with two parallel first-order reactions to the same product (Steinfeld et al., 1989). The characteristic rates k_1 and k_2 were fitted globally, whereas the plateau value a and the contributions of the two reactions b and c were fitted for each single curve. From the fit, the characteristic recruitment time $\tau_{1/2}$ was calculated as given below:

$$E(\tau_{1/2}) = E(0) + \frac{E(\infty) - E(0)}{2}$$

$E(0)$ and $E(\infty)$ were calculated from the fit and the plateau value $E(\infty) = a$ was used for normalization according to $E_{\text{norm}}(t) = E(t)/a$. Finally, all normalized curves and fits were averaged, yielding curves as in **Fig. 2D** and **Supplementary Fig. S4B**. Cells that moved in z-direction leading to fluctuations in the enrichment curves as well as cells with very low YFP signal and bad signal-to-noise ratio were excluded from the analysis.

BLInCR dissociation kinetics

U2OS 2-6-3 cells were transfected with CIBN-TetR, GFP-LacI and a PHR-mCherry construct, i.e. PHR-mCherry-VP16 or PHR-mCherry-NLS. Transfected cells were identified in the mCherry channel and a pre-recruitment stack (seven slices with $\Delta z = 0.5 \mu\text{m}$, 2x line average, 512 x 512 px, 400 Hz scan speed) was recorded. To recruit the PHR-mCherry constructs to the array, two stacks were recorded in the GFP channel corresponding to 38 s illumination with blue light. The first post recruitment stack was recorded in the mCherry channel immediately afterwards and constitutes the time point $t = 0$ s. Subsequent stacks were acquired every ~30 s for the first ~5 min and then at longer intervals for about 30 min (**Supplementary Fig. S4C**). The focus was readjusted if necessary.

To quantify the reversibility kinetics, the z-stacks for each time point were registered using the StackReg plugin (Thevenaz et al., 1998). For each time point, a maximum projection of the registered z slices was made, resulting in a time series of maximum projections. Generally, cell shapes changed considerably over the 30 min acquisition, preventing registration of the time series. Consequently, the array region (diameter: 20-40 px) and a nuclear reference region (diameter: 60 px) were manually selected for each time point and mean fluorescence intensities were measured (**Supplementary Fig. S4C**). Importantly, the sizes of the regions were kept constant over all images of one time series.

All $E(t)$ curves could be fitted well with a single exponential with a time-dependent (i.e. concentration-dependent) reaction rate similar to the model proposed by Sing *et al.* (Sing *et al.*, 2014):

$$E(t) = a \cdot \exp(-k \cdot t^m) + c$$

From the fit, the characteristic half-life $t_{1/2}$ was calculated:

$$E(t_{1/2}) = E(0) + \frac{E(0) - E(\infty)}{2}$$

$E(0)$ and $E(\infty)$ were calculated from the fit and used for normalization:

$$E_{\text{norm}}(t) = \frac{E(t) - E(\infty)}{E(0) - E(\infty)}$$

The normalized curves were averaged to yield the data shown in **Fig 2E** and **Supplementary Fig. S4D**.

Light-induced transcription activation

U2OS 2-6-3 cells were transfected with mKate2-MCP for RNA readout, CIBN-TetR as recruitment platform, CFP-LacI as array marker and PHR-YFP-VP16 for recruitment to CIBN-TetR and subsequent transcriptional activation. Image acquisition was similar to that used for the reversibility kinetics as described (**Supplementary Fig. S4C**) except for an additional third scan (CFP) and a 4x line average instead of 2x. After constitutive recruitment with a blue LED overnight, 4 slices ($\Delta z = 0.5 \mu\text{m}$) were recorded. For the time series, the number of slices recorded was between 5 and 8 ($\Delta z = 0.5 \mu\text{m}$) depending on cell size and shape. This was done to assure that the array was within the range recorded since it cannot be seen in the pre-recruitment image. For the first stack (pre/recruit), the channels were switched between stacks with the red channel (mKate2) being recorded first (yielding the reference image for mKate2-MCP before VP16 recruitment), then the YFP channel and last the CFP channel. For all subsequent stacks (one stack every 2-4 min) as well as after constitutive activation, all three channels were recorded sequentially using the “between lines” mode. Hence, different color images did not need to be registered with respect to one another. However, different z slices had to be registered since the recording of an entire image stack with three colors lasted longer than one minute, so that movement of the cell was occasionally observed. The different z slices were transformed to RGB Stacks and then registered to one of the central slices based on the YFP channel using the TurboReg (Thevenaz *et al.*, 1998) plugin.

To quantify the RNA amount at the array, maximum intensity projections were made for each time point resulting in a stack of three maximum intensity projections (mKate2, YFP and CFP). The quantification was also done manually for each time point as described

above for the reversibility kinetics. Note that the array area was selected in the YFP and/or CFP channel and was kept at the same size (diameter: 20-40 px) across all time points for a single cell. The $E(t)$ curves for RNA/mKate2-MCP enrichment at the array were calculated as described above, normalized to the enrichment values before and after 50 min of VP16 recruitment, and averaged (**Fig. 3B**). The average curve was fitted using a simple two-state model, a model assuming positive feedback or a sequential activation model (**Fig. 4A**) as described below.

To calculate the RNA enrichment values at the first plateau (**Fig. 3D**), the inflection points were calculated from the fit of the average curves (feedback model) of untreated and treated cells. The time course of array sizes was determined from the PHR-YFP-VP16 or the CFP-Lacl signal with ImageJ. The local area around the array (diameter: 60 px/3.2 μ m) was selected and converted to a binary image of the array using Otsu's method (Otsu, 1979) for thresholding. The measured array sizes at each time point were normalized to the array size at $t = 2.5$ min to assure that PHR-YFP-VP16 was fully recruited.

To compare RNA levels after constitutive activation, cells were transfected with PHR-YFP or PHR-YFP-VP16 and CFP-Lacl as well as CIBN-TetR, exposed to a blue LED overnight or via expression of a co-transfected GFP-TetR-VP16 fusion protein (**Supplementary Fig. S1**). CLSM imaging was conducted with the same laser intensities on the same day. The fluorescence intensity enrichment of RNA/mKate2-MCP was calculated from the background corrected intensities and the array area size A_{array} according to

$$E = (I_{\text{array}} - I_{\text{nuc}}) \cdot A_{\text{array}}.$$

BLInCR transcription activation and reversibility

To test the reversibility of transcription activation, cells were transfected with tagRFP-MCP for RNA detection, CFP-Lacl as array marker as well as CIBN-TetR and PHR-iRFP713-VP16 for light-induced recruitment of VP16 to the reporter array. That way, both RNA and VP16 can be separated spectrally and monitored without triggering PHR recruitment. The imaging parameters size, speed and line averaging were the same as for the light-induced transcription activation described above. Prior to recruitment and activation, a two-color stack (iRFP713 and tagRFP, sequential scan in "between lines" mode) was recorded, ensuring that neither VP16 nor RNA were detectable at the array. For light-induced recruitment of VP16, two three-color stacks (iRFP713, tagRFP and CFP, sequential scan in "between lines" mode) were recorded and the beginning of the first stack is the time point $t = 0$ s. Each stack acquisition exposed the cells to blue light for 78 s, and both stacks were recorded ~ 3 min apart. Subsequently, two-color stacks (iRFP713 and tagRFP) were recorded every 2-4 min to monitor PHR-iRFP713-VP16 dissociation and tagRFP-MCP

accumulation and dissociation from the array. After 30-40 min, PHR-iRFP713-VP16 was recruited again by switching back to three-color imaging (iRFP713, tagRFP and CFP). RNA and PHR-iRFP713-VP16 quantification at the array and bleach correction was done as described above. The maximum enrichment value and the enrichment value before VP16 recruitment $E(0)$ were used for normalization.

RNA fluorescence *in situ* hybridization (FISH) and immunofluorescence (IF)

RNA production from the reporter array was analyzed by RNA FISH. Cells were seeded on cover slips and transfected with CIBN-TetR, CFP-LacI and PHR-YFP or PHR-YFP-VP16 as described above. After illumination with a blue LED overnight, cells were permeabilized on ice with CSK buffer (100 mM NaCl, 300 mM sucrose, 3 mM MgCl₂, 10 mM PIPES, 0.5% Triton X-100) containing 10 mM vanadyl ribonucleoside complex (VRC, New England Biolabs) for 5 minutes. Further processing was done at room temperature unless noted otherwise: Cells were washed once with PBS, fixed with paraformaldehyde (12 min) and washed again with PBS. Subsequently, they were incubated with 70%, 85% and 100% ethanol (3 min each) and air-dried. For MS2 stem loop RNA detection, 50 ng of the 5'-Atto-565 labeled antisense probe 5'-GTC GAC CTG CAG ACA TGG GTG ATC CTC ATG TTT TCT AGG CAA TTA-3' (Goodier et al., 2010) per slide were mixed with 10 µg salmon sperm DNA and 5 µl formamide. The mixture was heated to 37°C for 10 min and 74°C for 7 min before 5 µl hybridization buffer (0.6 M NaCl and 60 mM trisodium citrate, pH 7.0, 20% dextran sulfate and 2 mg/ml BSA) and 10 mM VRC was added to a total volume of 11 µl. After hybridization overnight at 37°C cover slips were washed as follows: Twice with 2x SSC (0.3 M NaCl, 30 mM trisodium citrate, pH 7.0) supplemented with 50% formamide (15 min), once with 0.2x SSC/0.1% Tween (10 min, 40°C), once with 2x SSC (5 min), and once with PBS. Subsequently, YFP was visualized by immunofluorescence staining with an anti-GFP antibody since the fluorophore was destroyed during RNA-FISH. Cells were permeabilized with 0.1% ice-cold Triton X-100/PBS (5 min), washed once with PBS (5 min) and blocked with 10% goat serum in PBS for 30 min. The samples were incubated with the primary antibody (in 5% goat serum/PBS) for 1-2 h or overnight at 4°C. After washing three times with PBS supplemented with 0.002% NP40, samples were incubated with an Alexa488-coupled secondary antibody (Life Technologies) for 45 min and washed with PBS (3 x 5 min). Lastly, the slides were rinsed with water, 75% ethanol and 100% ethanol before mounting them with prolong gold antifade mountant including DAPI (Life Technologies). For IF staining of RNA polymerase II and H3K9ac (**Supplementary Fig. S2, S5**), cells were fixed after 19-24 h exposure to blue light (LED) using 4% paraformaldehyde (7 min) and washed three times with PBS. Next, cells were permeabilized and processed as described

above. The primary antibody used against H3K9ac was from ActiveMotif (#39917). The secondary antibody was Alexa568-coupled (**S5A,B**) or an Alexa633-coupled (**S5C**) anti-rabbit IgG antibody (Life Technologies). For IF staining of RNA polymerase II phosphorylated at serine 2 or 5, the primary antibody clones 3E10 and 3E8 were used (ActiveMotif, #61083 and #61085, respectively). The secondary antibody was Alexa647-coupled (Life Technologies). Additional details on the antibodies used is given in **Supplementary Table S5**.

Histone extraction and western blotting

U2OS 2-6-3 cells were cultured with 2 μ M SAHA (Millipore, 1:1000 from 2 mM stock solution in ethanol) or 1:1000 Ethanol for 24 h. Histones were extracted from $\sim 1 \times 10^6$ cells using 0.25 M HCl as described previously^{13 14}, separated by electrophoresis on a precast 4-20% polyacrylamide gel (Bio-Rad) and blotted semi-dryly onto a nitrocellulose membrane. After blocking in Tris-buffered saline (TBS) with 5% milk modified histones were detected with primary antibodies against H3K27ac and H3K9ac (both diluted in TBS with 1% BSA) and H4ac (in TBS with 3% milk) and HRP-linked anti-rabbit IgG (in TBS with 5% milk). Bands were detected by chemoluminescence using clarity western ECL substrate (Bio-Rad). After stripping the membranes with stripping buffer (Carl Roth), total H3 levels were detected with an antibody against H3 (in TBS with 1% BSA) and a secondary HRP-linked antibody against mouse IgG (in TBS with 5% milk). Additional information on the used antibodies is found in **Supplementary Table S5**.

RNA sequencing

For RNA-seq, RNA (≥ 200 nt) was isolated with the NucleoSpin RNA Plus kit (Macherey-Nagel), digested by DNase I (Promega) for 30 min at 37°C and depleted from rRNA with RiboZero (Illumina). Finally, directional cDNA synthesis and sequencing library preparation was conducted according to the NEBNext Ultra II Directional RNA Library Prep Kit (NEB). All libraries were sequenced as 50 bp single-end reads on an Illumina HiSeq 4000 system. Reads were mapped to the sequences of the respective transfected constructs and the inserted reporter using bowtie (Langmead et al., 2009). Subsequently, the remaining reads were mapped to the hg38 assembly of the human genome using STAR (Dobin et al., 2013). Normalization and differential expression analysis were performed using DESeq2 (Love et al., 2014).

RT-qPCR

For RT-qPCR, RNA was isolated and DNA was digested as described above for RNA-seq, cDNA was synthesized using Superscript IV reverse transcriptase (ThermoFisher, USA) and RNA was digested using RNase H. The qPCR reaction was performed using SYBR Green mix (Applied Biosystems, USA) and primers specific for the ECFP-SKL reporter (fwd: 5'-GTC CGG ACT CAG ATC TCG A-3' and rev: 5'-TTC AAA GCT TGG ACT GCA GG-3', designed with Primer3 (Untergasser et al., 2012)) with the following program: 10 min, 95°C and 40 cycles of 1 min, 60°C. RNA was quantitated using a standard dilution and normalized to average "pre" levels before illumination. An untransfected sample ("mock") was included as a control.

Theoretical framework for kinetic analysis

Kinetic two-state model for transcriptional activation. Curves for the relative RNA levels in **Fig. 3B** were fitted with a two-state model according to the scheme depicted below the plot. The differential equations for the activated state (A) and the RNA level (R) read

$$\frac{dA(t)}{dt} = [1 - A(t)] \cdot k_a - A(t) \cdot k_i$$
$$\frac{dR(t)}{dt} = A(t) \cdot k_r - R(t) \cdot k_d$$

Here, k_a and k_i are the transition rates into the activated and inactive state, respectively. k_r is the RNA production rate, and k_d is the RNA dissipation rate from the array. The total number of active and inactive promoter is normalized to 1, so that the initial concentration A_0 can take on values between 0 and 1. The solution for these equations is given by

$$A(t) = A_0 e^{-(k_a+k_i)t} + \frac{k_a}{k_a+k_i} (1 - e^{-(k_a+k_i)t})$$
$$R(t) = k_r \left[\frac{k_a}{k_d(k_a+k_i)} + \frac{[k_a - A_0(k_a+k_i)]e^{-(k_a+k_i)t}}{(k_a+k_i)(k_a-k_d+k_i)} - \frac{[k_a - A_0k_d]e^{-k_d t}}{k_d(k_a-k_d+k_i)} \right]$$

The fit parameters corresponding to the fit functions in **Fig. 4A** are listed in **Supplementary Table S4**. The decay rate k_d was set to be equal for untreated and SAHA-treated cells. Furthermore, fits were constrained not to exceed relative RNA levels of 5 a.u. (reached in steady state at $t = \infty$), reflecting the fact that measured RNA levels remained below this level also for late time points (up to 24 hours post-induction).

Kinetic three-state model with feedback for transcriptional activation. Curves for the relative RNA levels in **Fig. 3B** were fitted with a three-state model involving feedback according to the scheme shown in below the plot. The differential equations for the activated state (A_1) and the RNA level (R) read

$$\begin{aligned}\frac{dA_1(t)}{dt} &= [1 - A_1(t) - A_2] \cdot A_1(t) \cdot k_a \\ \frac{dR(t)}{dt} &= [A_1(t) + A_2] \cdot k_r - R(t) \cdot k_d\end{aligned}$$

Here, k_a is the transition rate into the activated state A_1 , and A_2 is the population residing in the activated state A_2 . Again, the total number of promoters is normalized to 1. The solution for these equations is given by

$$\begin{aligned}A_1(t) &= \frac{C(1 - A_2)}{C + e^{-k_c t}} \\ R(t) &= \frac{C(A_2 - 1)k_r e^{-k_d t}}{k_c + k_d} \left[{}_2F_1(1, K + 1, K + 2, -C) - e^{-(k_c + k_d)t} {}_2F_1(1, K + 1, K + 2, -C \cdot e^{k_c t}) \right] + \frac{A_2 k_r}{k_d} (1 - e^{-k_d t})\end{aligned}$$

with the abbreviations $C = \frac{A_{1,0}}{1 - A_{1,0} - A_2}$, $K = \frac{k_d}{(1 - A_2)k_a}$ and $k_c = (1 - A_2)k_a$.

In these equations, $A_{1,0}$ is the initial population in state A_1 , A_2 is the (invariant) population in state A_2 , and ${}_2F_1(a, b, c, x)$ denotes the Gaussian hypergeometric function. The fit parameters corresponding to the fit functions shown in **Fig. 4A** are listed in **Supplementary Table S4**. The dissipation rate k_d was set to be equal for untreated and SAHA-treated cells.

Sequential model for transcriptional activation. Curves for the relative RNA levels in **Fig. 3B** were fitted with a sequential model involving n sequential reaction steps to transition from the inactive state ($I = S_0$) to the active state (A) according to the scheme $I \rightarrow S_1 \rightarrow S_2 \rightarrow \dots \rightarrow S_{n-1} \rightarrow A$. Here, S_i represent intermediate states, in which no RNA is produced. For simplicity, the same rate constant k_a was chosen for each transition. The differential equations for the individual states (I, S_i, A) and the RNA level (R) read

$$\begin{aligned}\frac{dI(t)}{dt} &= -k_a \times I(t) \\ \frac{dS_i(t)}{dt} &= k_a \times (S_{i-1}(t) - S_i(t)) \quad (0 < i < n) \\ \frac{dA(t)}{dt} &= k_a \times S_{n-1}(t) \\ \frac{dR(t)}{dt} &= k_r \times A(t) - k_d \times R(t)\end{aligned}$$

The solutions for $A(0) = A_0$ and $I(0) = 1 - A_0$ are given by

$$\begin{aligned}I(t) &= (1 - A_0) e^{-k_a t} \\ S_i(t) &= \frac{(1 - A_0) (k_a t)^i}{i!} e^{-k_a t} \\ A(t) &= 1 - (1 - A_0) \frac{G(n, k_a t)}{(n - 1)!} \\ R(t) &= \frac{(1 - A_0) k_r}{k_d (n - 1)!} \left[\frac{k_a (k_a t)^{n-1}}{k_a - k_d} \left(e^{-k_a t} + \frac{(n - 1) e^{-k_d t}}{((k_a - k_d) t)^{n-1}} G(n - 1, (k_a - k_d) t) \right) \right. \\ &\quad \left. + (n - 1)! \left(1 - \left(\frac{k_a}{k_a - k_d} \right)^{n-1} e^{-k_d t} \right) - G(n, k_a t) \right] + \frac{A_0 k_r}{k_d} (1 - e^{-k_d t})\end{aligned}$$

Here, A_0 is the initial population in the active state A , and $G(x, y)$ is the (upper) incomplete Gamma function. The fit parameters corresponding to the fit functions in **Fig. 4A** are listed in **Supplementary Table S4**. The decay rate k_d was set to be equal for untreated and SAHA-treated cells.

Sequential model with additional recruitment step. The scheme for the sequential activation model above was extended to explicitly include an additional recruitment step for the transcriptional activator with rate k_{rec} . In particular, an unbound inactive state (U) and an unbound active state (U_A) were considered, with transitions from U_I to I and U_A to A that occur with rate k_{rec} . Thus, the reaction schemes read $U_I \xrightarrow{k_{\text{rec}}} I \rightarrow S_1 \rightarrow S_2 \rightarrow \dots \rightarrow S_{n-1} \rightarrow A$ and $U_A \xrightarrow{k_{\text{rec}}} A$. In this case, the differential equations for the individual states (U_A , U_I , I , S_i , A) and the RNA level (R) read

$$\begin{aligned}\frac{dU_1(t)}{dt} &= -k_{\text{rec}} \times U_1(t) \\ \frac{dI(t)}{dt} &= k_a \times (U_1(t) - I(t)) \\ \frac{dS_i(t)}{dt} &= k_a \times (S_{i-1}(t) - S_i(t)) \quad (0 < i < n) \\ \frac{dA(t)}{dt} &= k_a \times S_{n-1}(t) + k_{\text{rec}} \times U_A(t) \\ \frac{dR(t)}{dt} &= k_r \times A(t) - k_d \times R(t)\end{aligned}$$

The solutions for $U_A(0) = A_0$ and $U_1(0) = 1 - A_0$ are given by

$$\begin{aligned}U_1(t) &= (1 - A_0) e^{-k_{\text{rec}} t} \\ I(t) &= \frac{(1 - A_0) k_a e^{-k_a t}}{k_{\text{rec}} - k_a} \left(1 - e^{-(k_{\text{rec}} - k_a) t}\right) \\ S_i(t) &= (1 - A_0) \frac{k_{\text{rec}} k_a^i}{(k_a - k_{\text{rec}})^{i+1}} \left(1 - \frac{G(i+1, (k_a - k_{\text{rec}}) t)}{i!}\right) e^{-k_{\text{rec}} t} \\ A(t) &= A_0 \left(1 - e^{-k_{\text{rec}} t}\right) \\ &\quad + (1 - A_0) \left(1 - \left(\frac{k_a}{k_a - k_{\text{rec}}}\right)^n \left(1 - \frac{G(n, (k_a - k_{\text{rec}}) t)}{(n-1)!}\right) e^{-k_{\text{rec}} t} - \frac{(k_a t)^{n-1}}{(n-1)!} e^{-k_a t} - \frac{G(n-1, k_a t)}{(n-2)!}\right) \\ R(t) &= \frac{A_0 k_r}{k_d (k_d - k_{\text{rec}})} \left(k_d \left(1 - e^{-k_{\text{rec}} t}\right) - k_{\text{rec}} \left(1 - e^{-k_d t}\right)\right) \\ &\quad + \frac{(1 - A_0) k_r}{k_d} \left(1 - \frac{G(n-1, k_a t)}{(n-2)!} + \frac{k_d e^{-k_{\text{rec}} t}}{k_d - k_{\text{rec}}} \left(\frac{k_a}{k_a - k_{\text{rec}}}\right)^n \left(\frac{G(n, (k_a - k_{\text{rec}}) t)}{(n-1)!} - 1\right)\right. \\ &\quad \left. + \frac{k_d e^{-k_d t}}{k_a} \left(\frac{k_a}{k_a - k_d}\right)^n \left(\frac{k_a k_{\text{rec}}}{k_d (k_d - k_{\text{rec}})} + \left(\frac{k_a}{k_d} - 1\right) \frac{G(n-1, (k_a - k_d) t)}{(n-2)!}\right)\right. \\ &\quad \left. + \left(1 - \frac{k_a}{k_d - k_{\text{rec}}}\right) \frac{G(n, (k_a - k_d) t)}{(n-1)!}\right)\end{aligned}$$

Here, $G(x,y)$ is the (upper) incomplete Gamma function. Resulting curves for different recruitment rates k_{rec} are shown in **Fig. 4B**.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.R., F.E., K.R.; Methodology: A.R., F.E., J.T., K.R.; Validation: A.R.; Formal Analysis: A.R., F.E., J.T.; Investigation: A.R., F.E., J.T., S.S.; Resources: A.R., F.E., J.T., K.R.; Writing – original draft preparation: A.R., F.E., K.R.; Writing – review & editing: A.R., F.E., K.R.; Visualization: A.R., F.E., K.R.; Supervision: K.R.; Project administration: K.R.; Funding acquisition: K.R.

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Data availability

RNA sequencing data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO accession number GSE103868.

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Figures

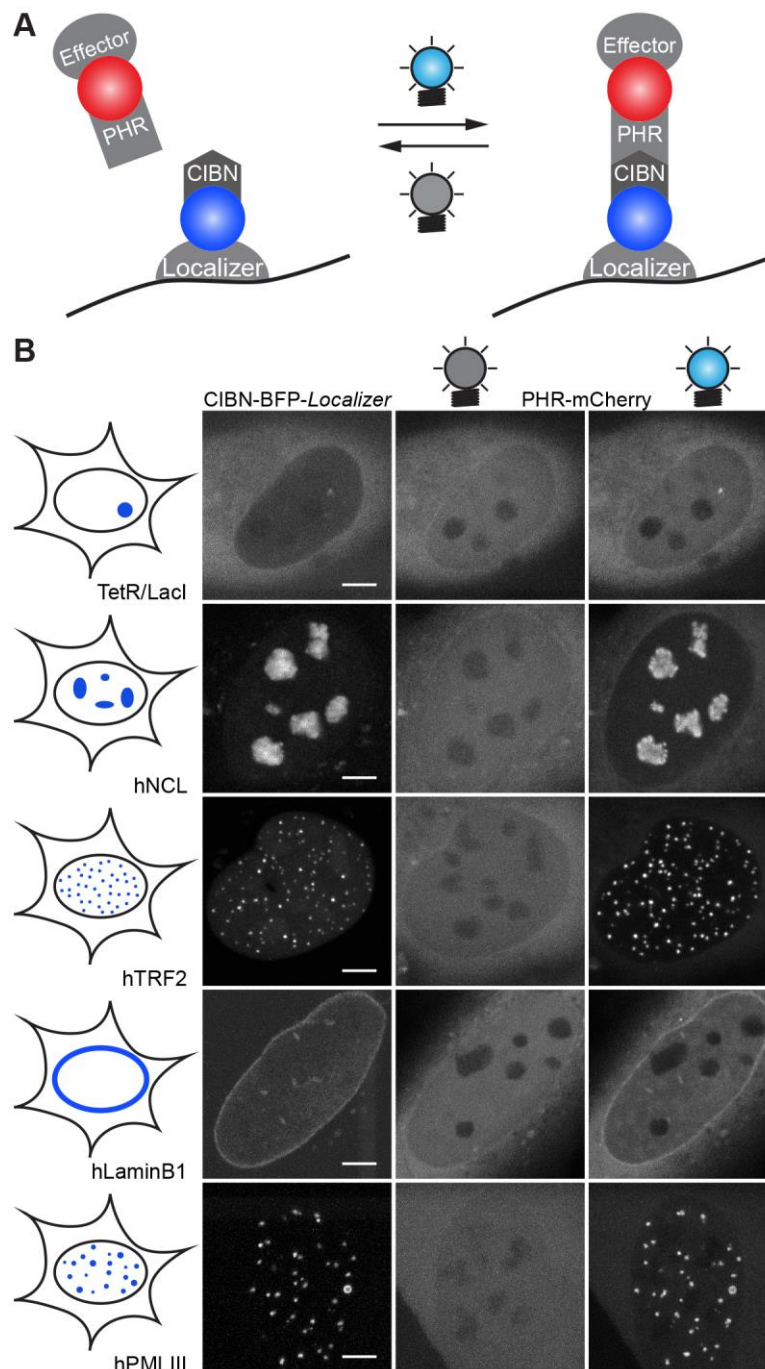


Fig. 1. Blue Light-Induced Chromatin Recruitment (BLInCR). (A) BLInCR is based on the interaction of the protein domains CIBN and PHR upon illumination with blue light. It allows binding of PHR-tagged effectors to CIBN-marked sites. The term 'Localizer' refers to a protein with a specific subnuclear localization with some examples shown in panel B. Red and blue circles represent fluorophores used for visualization. Only red and far-red

fluorophores can be visualized without triggering recruitment. **(B)** BLInCR enables recruitment to different genomic loci: *tetO* arrays, nucleoli, telomeres, the nuclear lamina or PML bodies. PHR-mCherry (a mock effector) was homogeneously distributed throughout the cell before exposure (center) and relocated to sites marked by CIBN-BFP-tagged localizers (left) upon blue light exposure (right). For each 'localizer', 10 to 20 cells were imaged. Scale bars: 5 μ m.

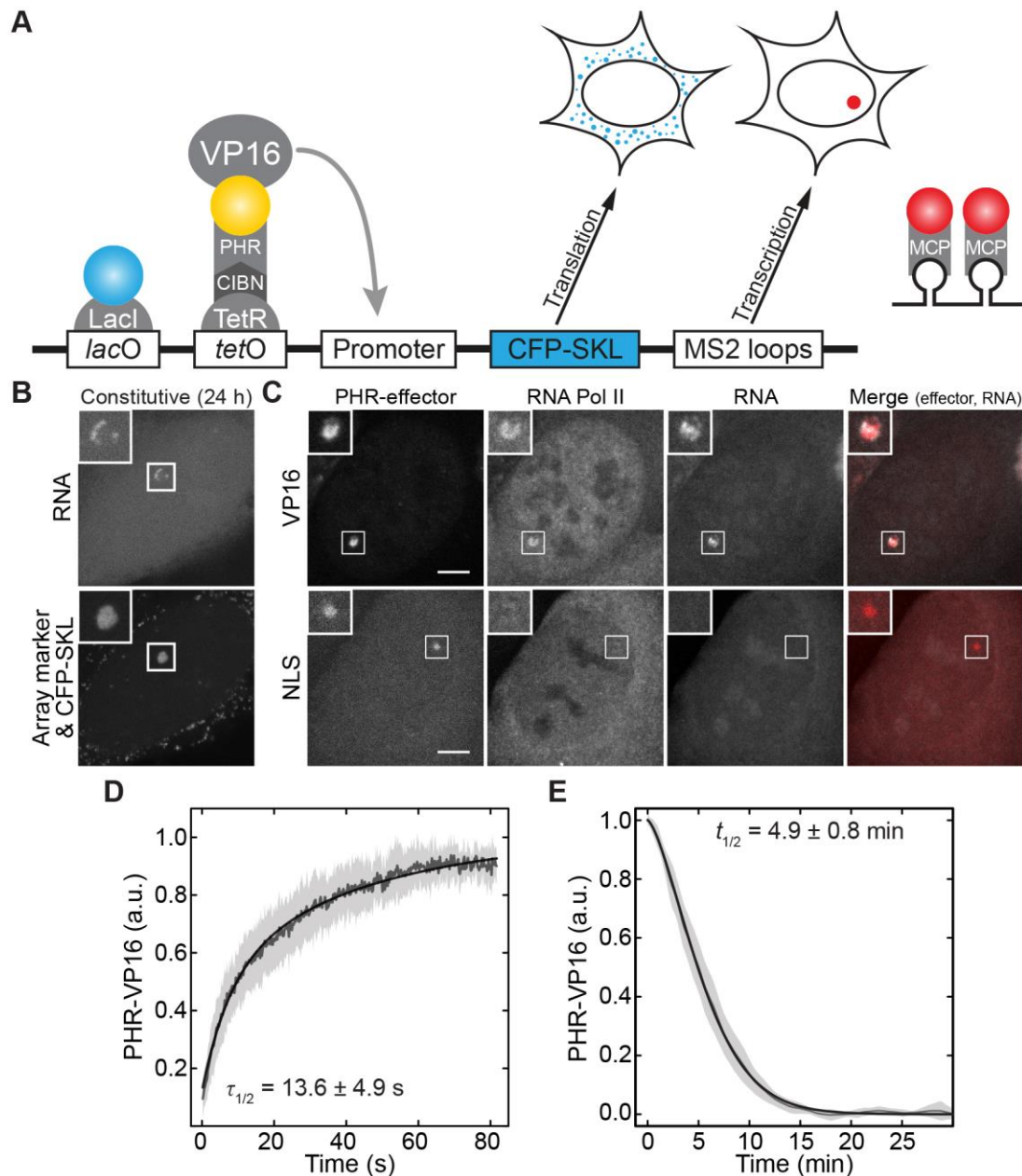


Fig. 2. Dynamic transcription control by BLInCR. (A) Schematic representation of the reporter system used to detect transcription in real-time (Janicki et al., 2004). Transcription of the reporter array can be monitored via cytoplasmic CFP-SKL (localizing to peroxisomes) or direct monitoring of MS2 loop transcripts with fluorescently tagged MS2 coat protein. Red, yellow and blue circles represent fluorophores used for visualization. (B) Constitutive recruitment of the transcriptional activator VP16 induced production of RNA (top, mKate2-MCP) and the encoded CFP-SKL protein (bottom), which localizes to cytoplasmic peroxisomes. CFP-LacI was included as a marker for the array (bottom). (C) Constitutive recruitment of VP16 induces recruitment of RNA polymerase II to the array. RNA Pol II was labeled with GFP, RNA via tagRFP-MCP, and the (mock) effector with the far-red

fluorophore iRFP713. For each condition, 12 images were recorded. Scale bars: 5 μm . **(D)** Association kinetics of PHR-VP16 with CIBN-TetR tethered to the *tetO* reporter array. Averaged experimental data (dark gray lines), fits (black lines) and standard deviations (light gray areas) are shown ($n=20$). The CIBN construct was tagged with tagRFP-T to locate the array without triggering PHR binding. The PHR construct was labeled with YFP for simultaneous recruitment and visualization. **(E)** Dissociation kinetics of the PHR-VP16 and CIBN-TetR complex ($n=13$). Plot coloring as in panel *D*. The PHR construct was tagged with mCherry to visualize its dissociation without triggering its interaction with CIBN.

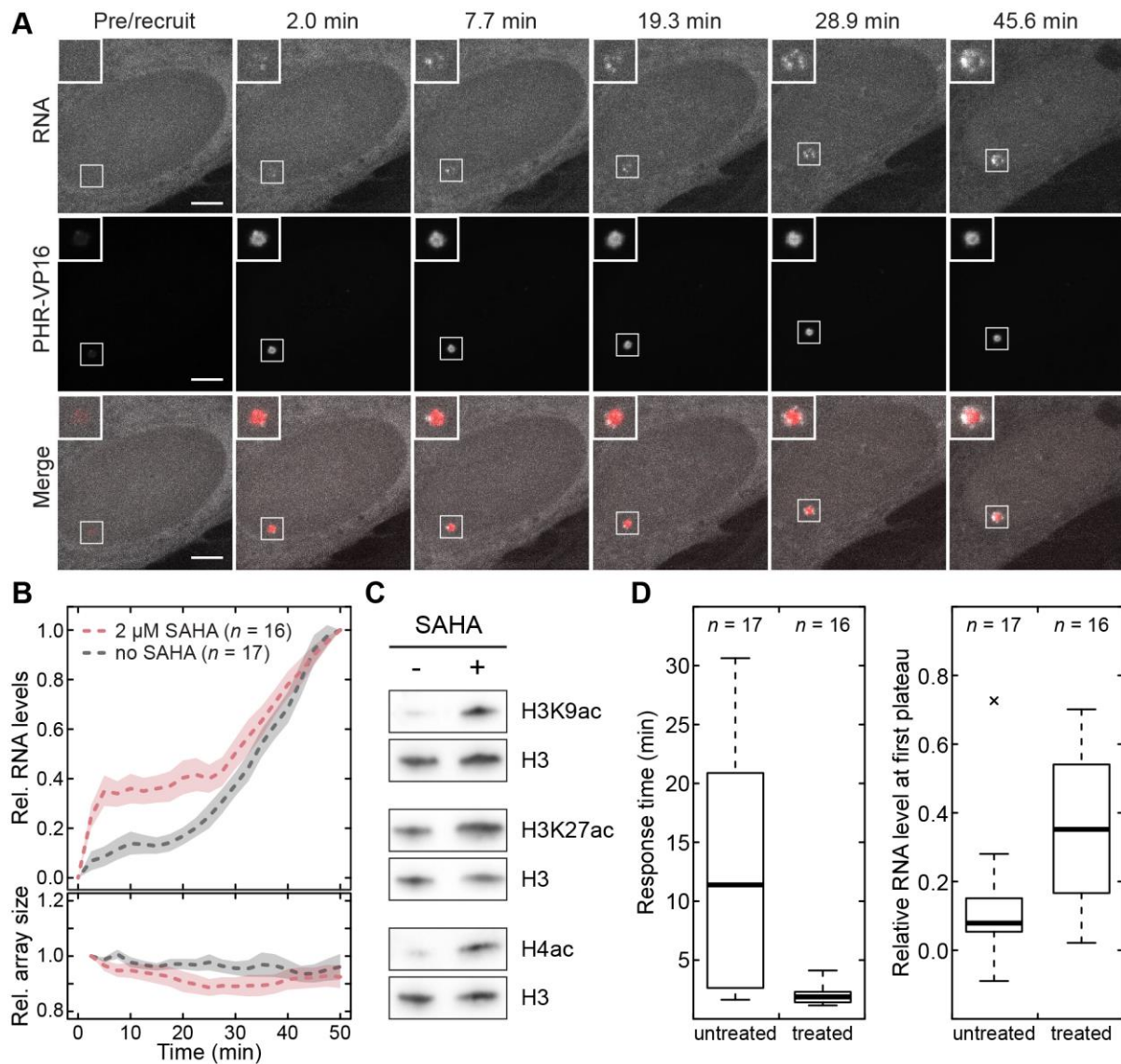


Fig. 3. BLInCR resolves the early kinetics of the transcription activation process. (A) Time series of VP16-induced transcription. Top: RNA production visualized by labeled MS2 coat protein (mKate2-MCP). Center: PHR-VP16 (labeled with YFP) was continuously present at the array during imaging. Bottom: Merged images. Scale bars: 5 μ m. (B) Top: BLInCR-induced transcription occurred with an early and a late activation phase. The early response was more pronounced in cells pretreated with SAHA overnight (red). Depicted are experimental averages (dashed lines) and standard errors of the mean (shaded areas). Bottom: Relative size of the reporter array over time. (C) SAHA treatment at 2 μ M concentration for 24 hours led to an increase of preexisting histone acetylation marks. (D) Cell-to-cell heterogeneity of response times (top) and relative RNA levels (bottom) in treated and untreated cells. Response times correspond to the time points at which first

transcripts were detectable. Relative RNA levels were measured at the point of inflection, i.e., at the first plateau between early and late transcription.

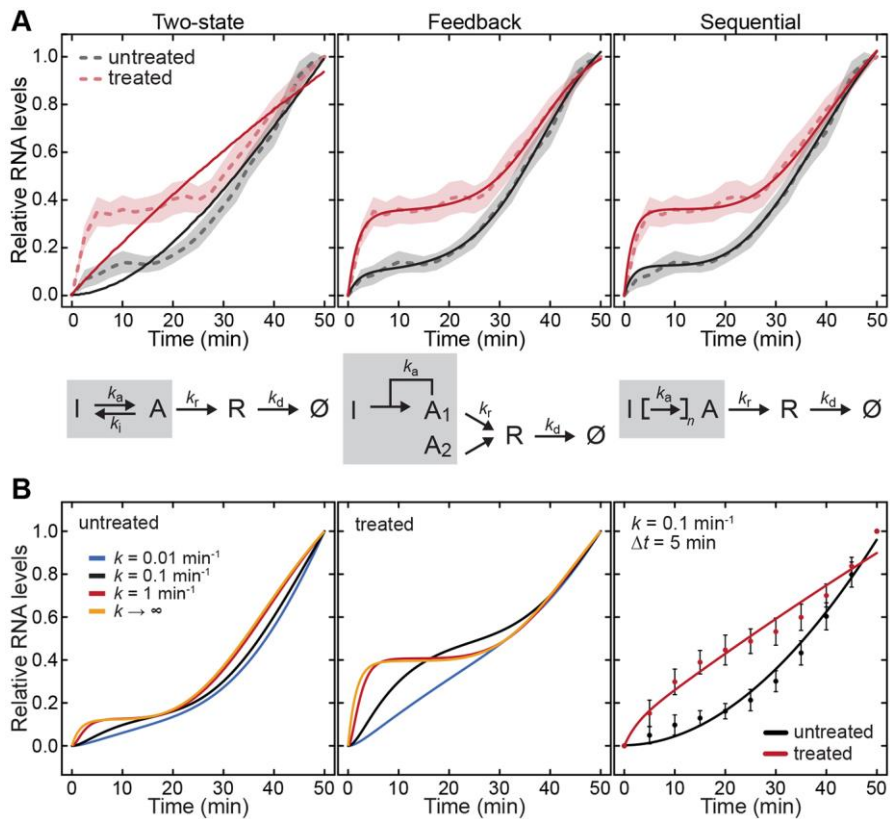


Fig. 4. Kinetic models for transcription activation. (A) The experimentally determined activation kinetics were fitted with three different kinetic models: a two-state model, a model including positive feedback, and a sequential activation model (from left to right). The biphasic activation kinetics were reproduced with both the feedback model and the sequential model but not with the two-state model. The parameters k_a , k_i , k_r and k_d denote the rates for activation, silencing, RNA transcription and dissipation of RNA transcripts from the array, respectively. A , A_1 and A_2 are active states, I is the inactive state; R and \emptyset denote RNA transcription and dissipation, respectively. Fit results are given in **Supplementary Table S4**. (B) Influence of recruitment speed on the resulting activation kinetics. The sequential model including an additional initial recruitment step with rate k was used as a proxy for the expected experimental data. Slow recruitment ($k \leq 0.1 \text{ min}^{-1}$) in combination with moderately fast RNA detection would yield data points that could also be fitted with the two-state model (right plot panel with error bars reflecting the experimentally measured ones).

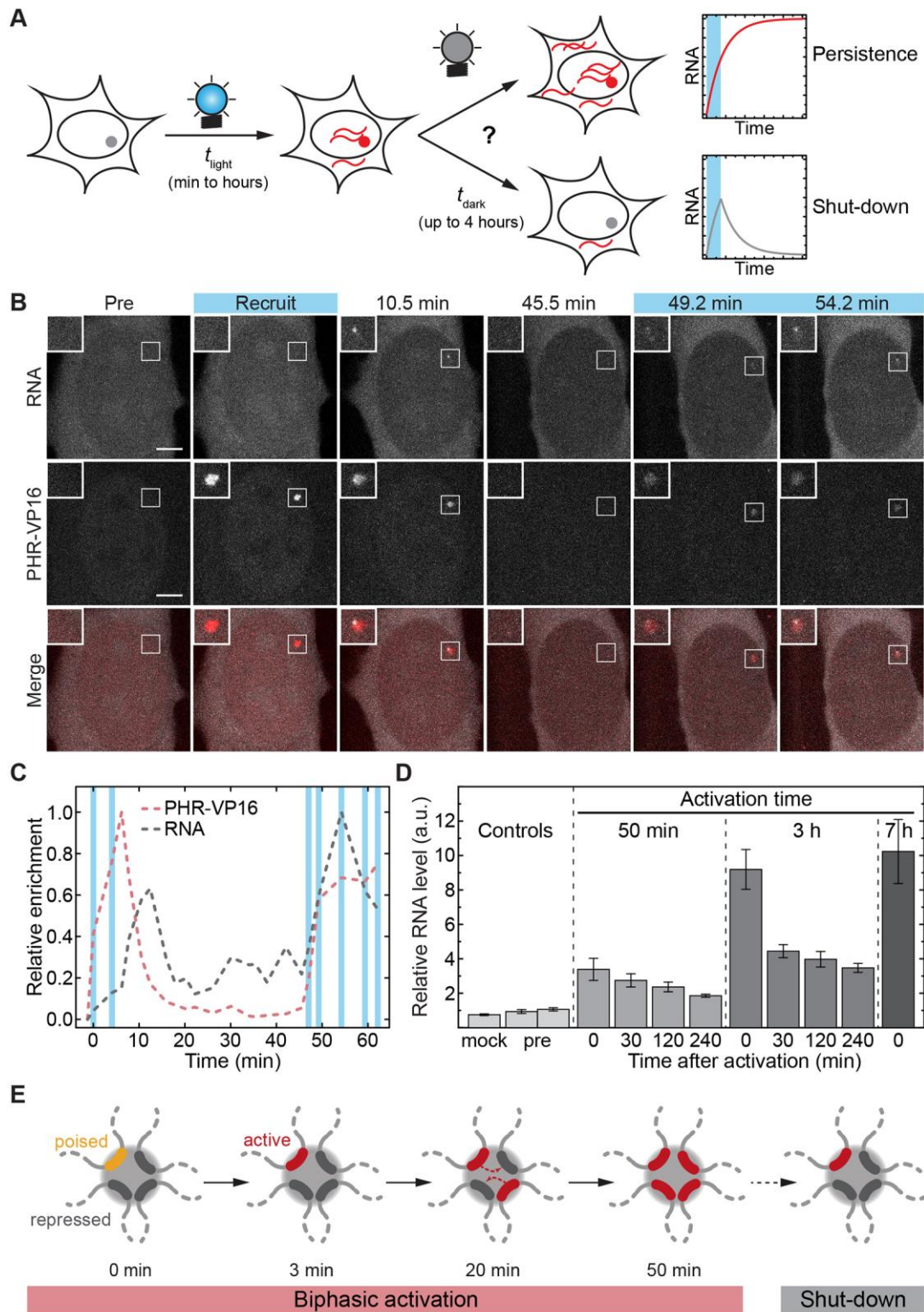
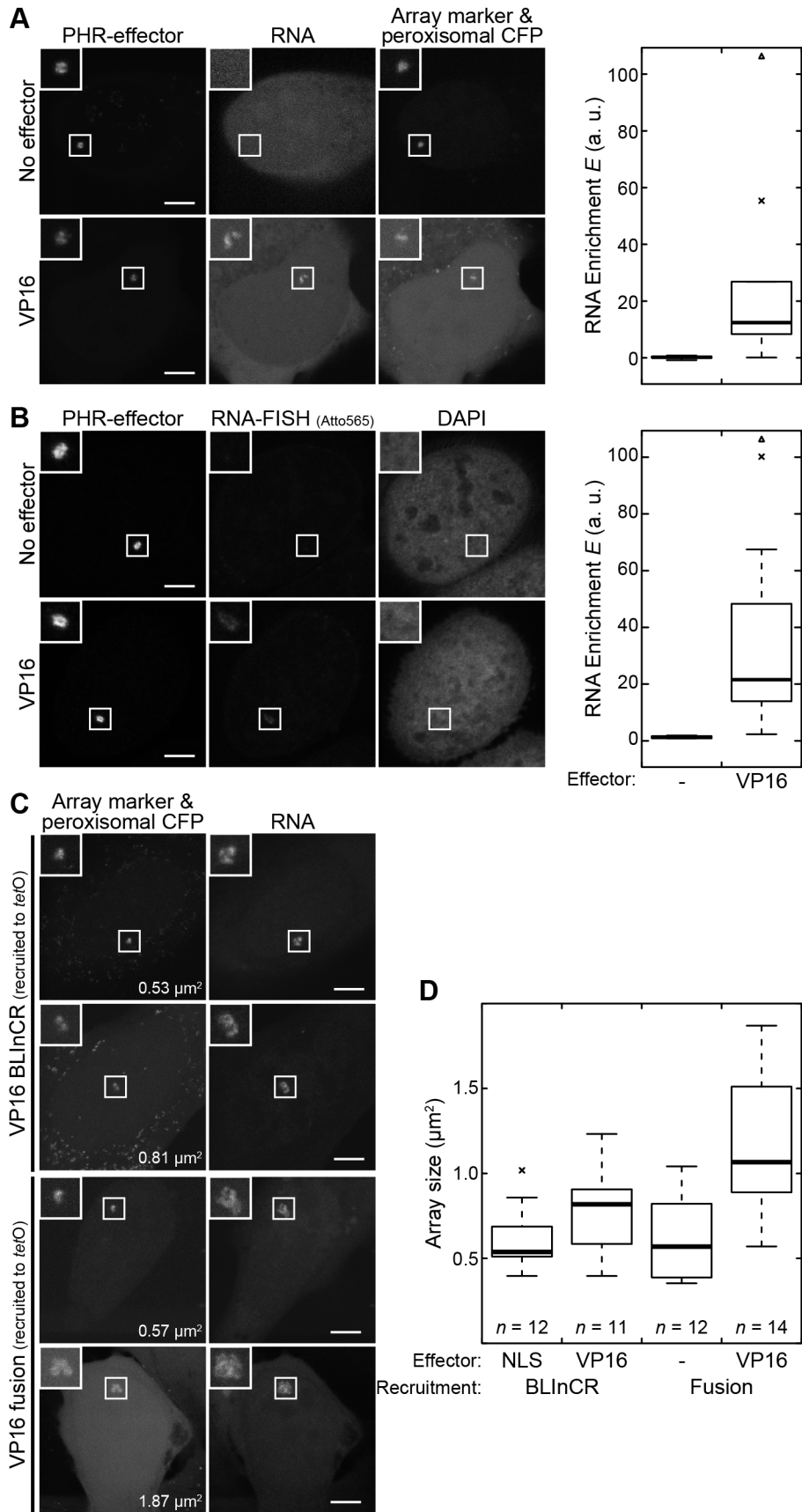


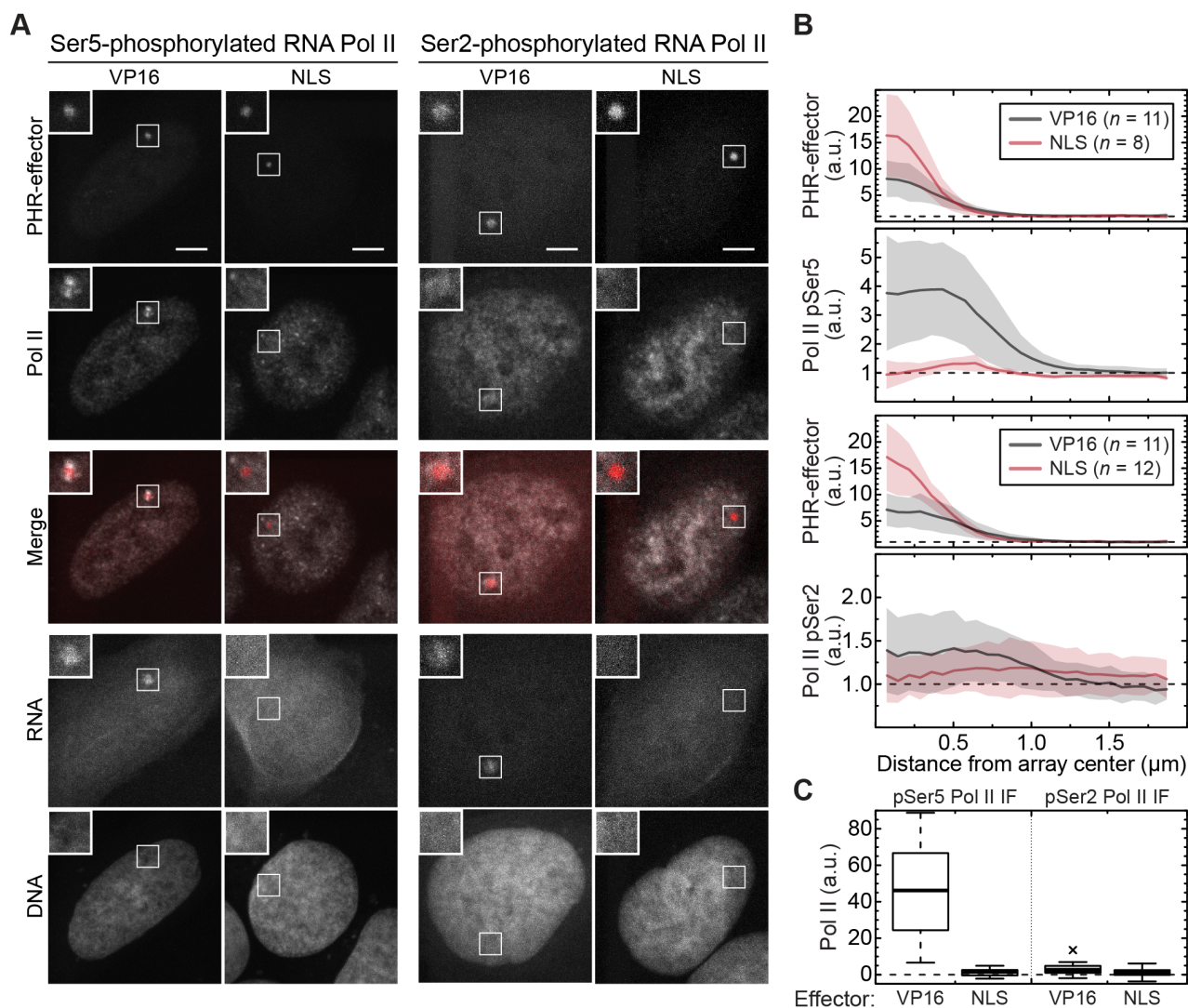
Fig. 5. Persistence of the activated state. (A) Scheme for the presence or absence of memory. If the activated state persists, the reporter array remains associated with nascent reporter RNA and the total amount of cellular reporter RNA increases after light has been switched off (top). In the alternative model, the nascent reporter RNA dissociates from the array and the total amount of cellular reporter RNA decreases when the initial activator is

lost from the promoter (bottom). **(B)** Transcription response to pulsed recruitment of VP16. Top, RNA; bottom, PHR-VP16. The pulsed RNA accumulation following activator binding and dissociation indicates that the active state did not persist in the absence of VP16. PHR-VP16 was labeled with far-red iRFP713 and RNA was visualized with tagRFP-MCP, so that both can be visualized without triggering PHR-VP16 recruitment. One exemplary cell out of 11 is displayed. Scale bars: 5 μm . **(C)** Quantification of the integrated signal at the array in panel *B*. Shaded areas represent blue light pulses. **(D)** Time course of total reporter RNA levels in the sample after induction. Cells were illuminated with blue light for 50 minutes, 3 hours or 7 hours and the amount of reporter RNA was quantitated by RT-qPCR at the indicated time points. As controls RNA levels for mock transfected sample (PHR without VP16) and the samples before light induction are shown. RNA levels decayed after light had been switched off. Note that in this assay the total amount of cellular reporter RNA is measured as opposed to the quantification of RNA levels at the reporter array in the fluorescence microscopy based analysis. **(E)** Model for the biphasic transcriptional activation kinetics and the reversibility of the activated state observed here using BLInCR. A fraction of poised promoters is initially present and activated within ~ 3 min. Maximum RNA accumulation at the array requires about 50 min. After loss of the activator, RNA production decays on the minute time scale.

Supplementary Figures

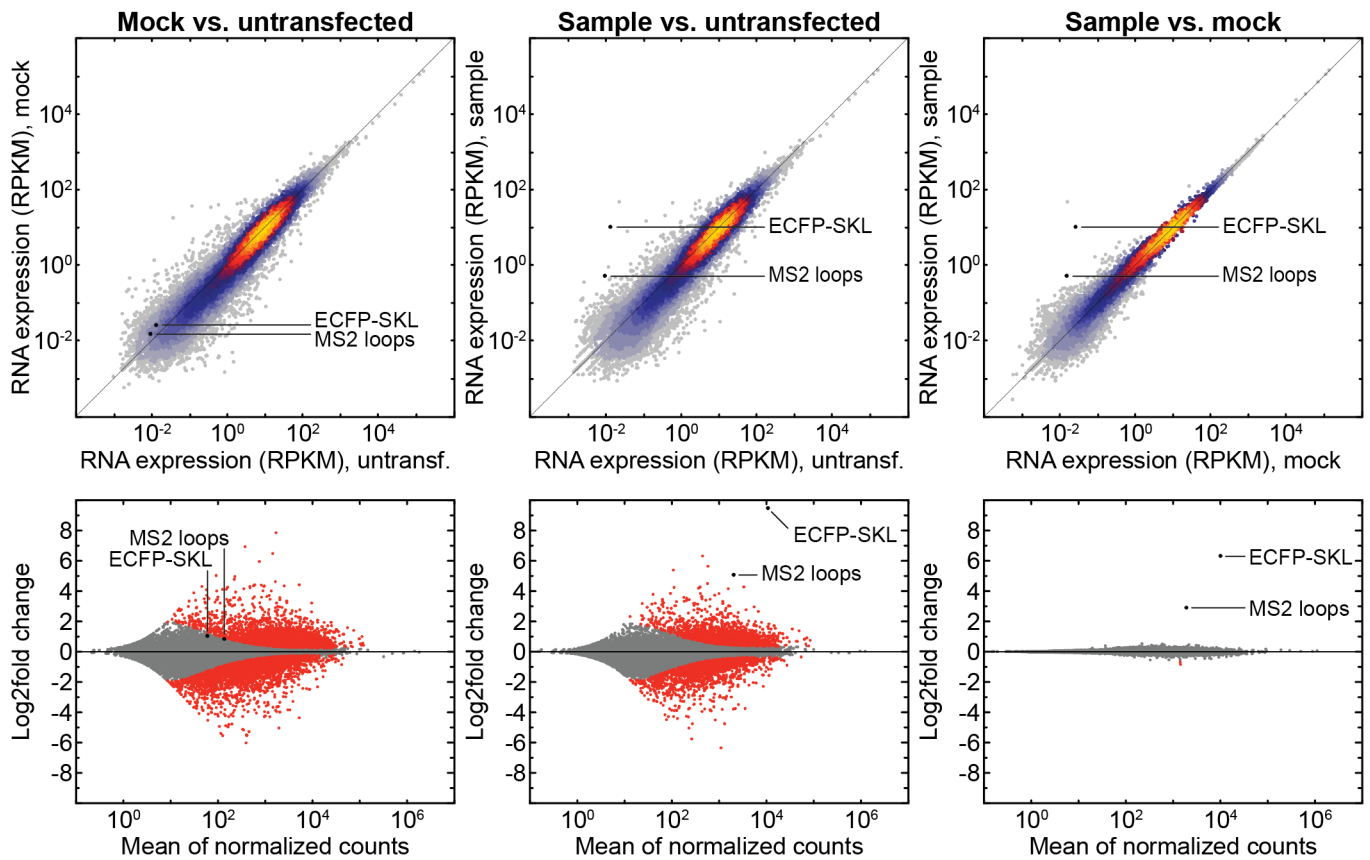


Supplementary Figure S1. Continuous light-dependent recruitment of PHR-YFP-VP16 to reporter array. Cells were continuously illuminated overnight with a blue LED, leading to recruitment of PHR constructs (via CIBN-TetR) to the *tetO* repeats of the reporter array. In the control reaction (“no effector”) transfection was with PHR-YFP instead of PHR-YFP-VP16. **(A)** Maximum intensity projection CLSM images of YFP-labeled effectors, RNA (visualized via mKate2-MCP), an array marker (CFP-LacI) and peroxisomal CFP. Quantification of RNA enrichment is shown on the right ($n=9$ for each condition). **(B)** Same as in panel A, but RNA stained by RNA-FISH ($n = 6$ and $n = 11$ for the control without effector and VP16, respectively). The amount of RNA detected at the array after VP16 recruitment showed a similar distribution for different RNA detection methods (compare quantification for panels A and B) and varied considerably across different cells. **(C)** Exemplary maximum intensity projection CLSM images visualizing the array through the independent marker CFP-LacI. VP16 was either recruited via BLInCR (by transfecting PHR-YFP-VP16 & CIBN-TetR) or with a fusion construct (GFP-TetR-VP16). All cells transfected with a VP16 construct showed mKate2-MCP enrichment, confirming that these cells were transcriptionally active. **(D)** Quantification of array sizes with enriched VP16 (shown in panel C) compared to negative controls without VP16 (i.e., PHR-YFP-NLS for BLInCR and TetR-GFP as a fusion construct). On average, cells expressing TetR-VP16 fusion constructs had somewhat larger arrays compared to cells with constitutive optogenetic recruitment of VP16. Notably, some of the activated cells in the latter population had condensed arrays that were comparable to those in the negative control PHR-YFP-NLS. Scale bars: 5 μm .

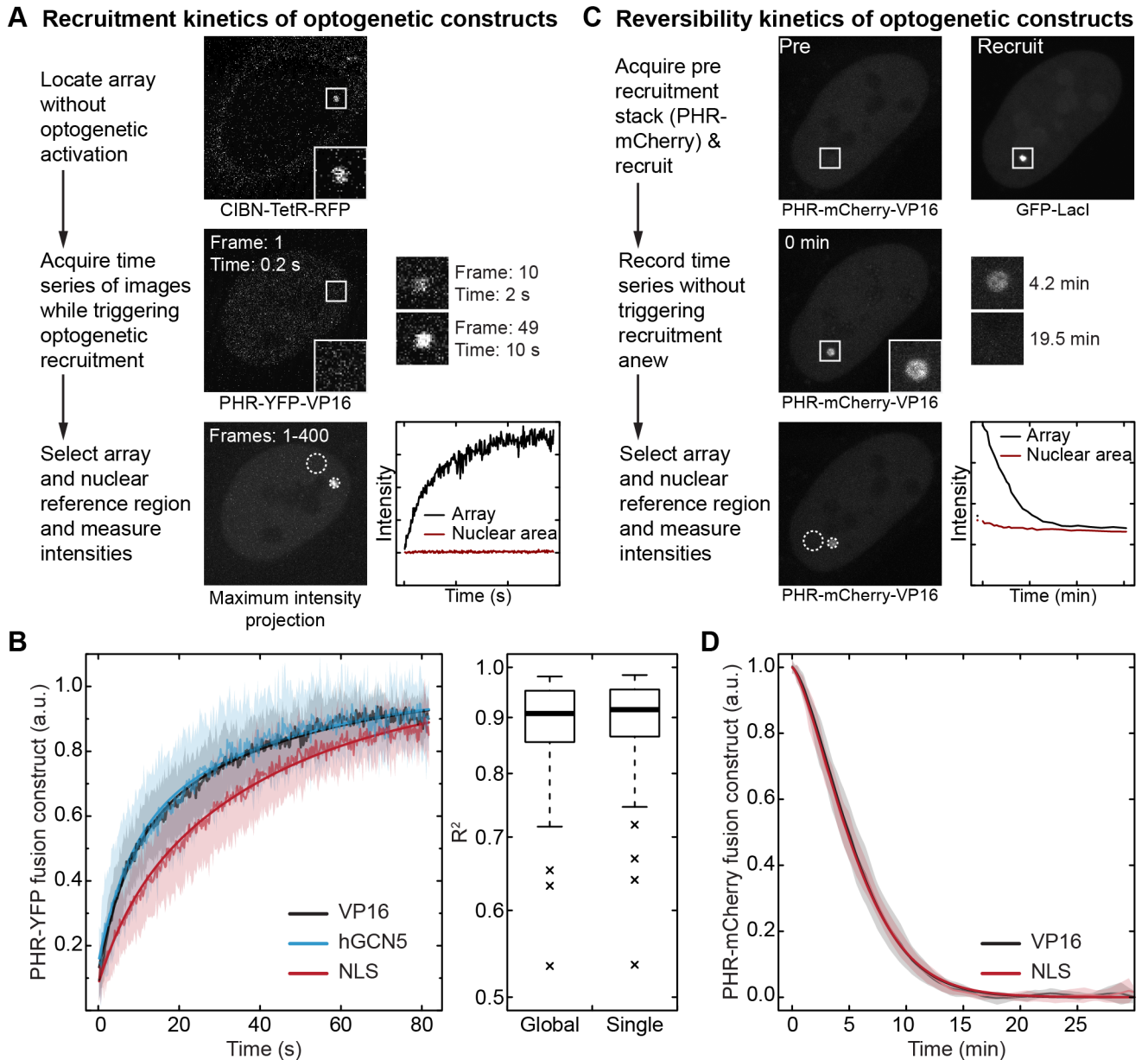


Supplementary Figure S2. Immunostaining of RNA polymerase II phosphorylated at serine 2 or 5. Cells were transfected with PHR-YFP-effector constructs, CIBN-TetR and mKate2-MCP to monitor RNA production. The respective pol II species were visualized with the primary antibodies and the Alexa647-labeled secondary antibody listed in the Methods section. DNA was counterstained with DAPI. **(A)** Exemplary maximum intensity projections of CLSM images showing that neither the initiating serine 5-phosphorylated (Ser5p) pol II nor the elongating serine 2-phosphorylated (Ser2p) pol II could be detected in non-activated control cells. Ser5p was clearly enriched in activated cells, whereas Ser2p was only slightly accumulated at the array. The absence of pol II Ser2p signal in most cells might be due to the small size of the transgene of ~3 kb. Reportedly, Ser2p tends to occur downstream of Ser5p and is only observed beyond 3 kb for some genes (Descostes et al. 2014, eLife 3, e02105). Scale bars: 5 μm . **(B)** Average DAPI-normalized radial intensity profiles around the array. Quantifications of transfected cells was done as described in panel A. **(C)** Total accumulated pol II signal calculated from the radial profiles ($\sum_{r=0}^{r=\text{FWHM}} 2\pi r I(r)$),

where r is the distance from the array center, FWHM the full width at half maximum calculated from the effector profiles and $I(r)$ the intensity at distance r from the radial profiles in panel B).

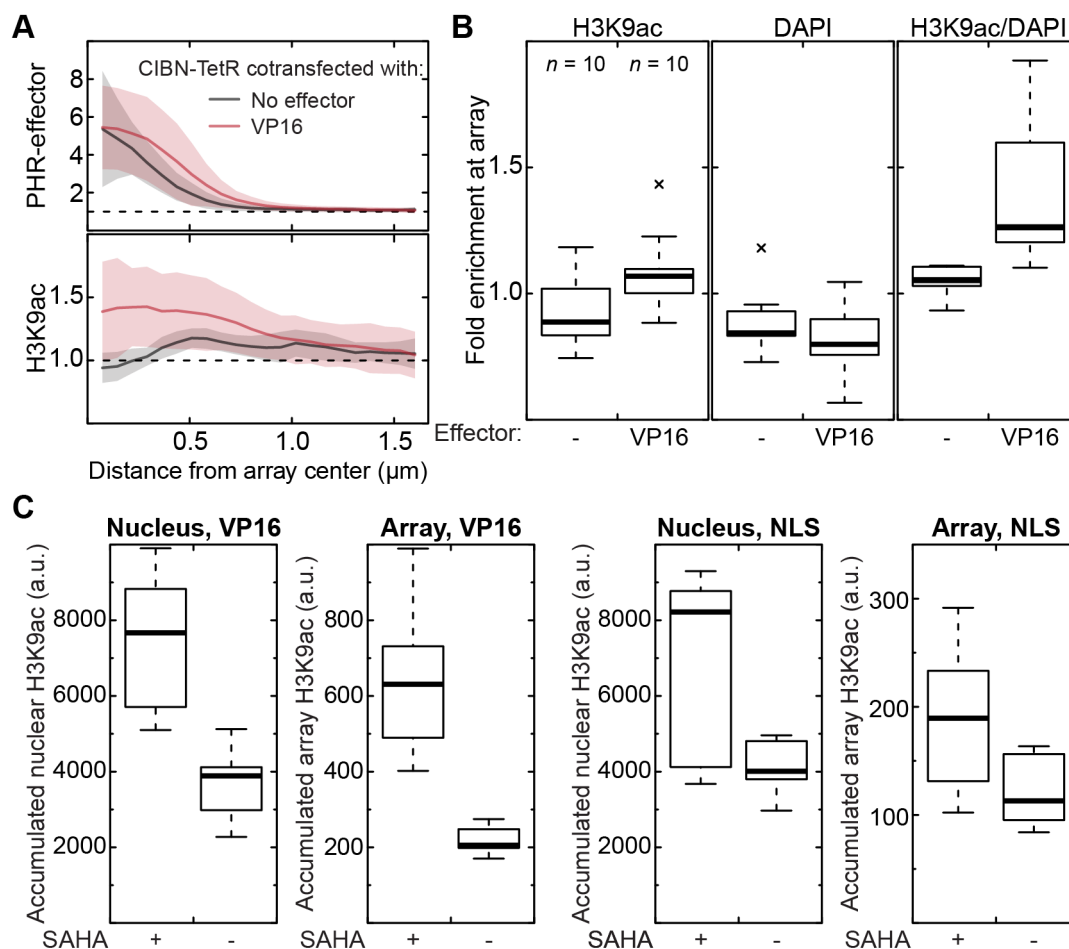


Supplementary Figure S3. Gene expression in U2OS 2-6-3 cells transfected with different constructs. Cells were transfected with YFP and TetR-RFP (mock) or PHR-YFP-VP16, CIBN-TetR and mCherry-MCP (sample). The upper set of plots shows RPKM values for annotated genes in mock vs. untransfected (left), sample vs. untransfected (center) and sample vs. mock (right) cells. The lower set of plots are MA plots derived from the differential gene expression analysis with DESeq2 for the respective combination. When comparing mock or sample vs. untransfected cells, hundreds of genes were differentially expressed, indicating that either expression of TetR or the transfection procedure itself causes substantial changes in gene expression. However, when comparing sample to mock-transfected cells, only the reporter elements (MS2 loops and ECFP-SKL) showed significant differential expression ($p_{\text{adj}} < 0.001$) and log₂-fold changes > 1 .



Supplementary Figure S4. Recruitment and reversibility with BLInCR. (A) The typical workflow of a BLInCR experiment is illustrated for cells transfected with CIBN-TetR-tagRFP-T (localizer) and PHR-YFP-VP16 (effector). First, the cellular structure bound by the localizer (here: *tetO* array) was located in the tagRFP-T channel (excitation with yellow-green light), which did not trigger optogenetic recruitment. Next, a YFP time series was recorded while triggering recruitment of the effector to the localizer (excitation with blue light). Finally, the structure that was targeted by the localizer (here: *tetO* array) and a nuclear reference region were selected in the maximum intensity projection of the time series and the mean intensities at those regions were measured for each image. (B) Recruitment kinetics of PHR-YFP-fused effector proteins (left). Experimental means (solid lines, light), standard deviations (shaded areas) and fits (solid lines, dark) are shown. A global fit using the same two rate constants for all curves (and all constructs) yielded similar fit qualities as fits of each single curve with individual rate constants (right). In particular, the cumulative residual

sum of squares was only 8.5% larger for the global fit. The fit parameters for the global fit and the number of cells for each construct are listed in **Supplementary Table S2**. **(C)** A typical workflow for a BLInCR reversibility experiment is illustrated for cells transfected with CIBN-TetR (localizer), GFP-LacI (marker) and PHR-mCherry-VP16 (effector). A stack of images was recorded in the mCherry channel (excitation with yellow-orange light) prior to recruitment. The array was visualized by recording two stacks in the marker channel with blue light excitation for 38 s, thereby triggering effector recruitment to the localizer. The dissociation of the effector from the targeted structure was monitored by recording image stacks every 30-120 s without triggering the PHR switch again (excitation with yellow-orange light). Finally, the targeted structure and a nuclear reference region were selected in the maximum intensity projection of the time series and the mean intensities at those regions were measured for each image. **(D)** Plot of experimental means (solid lines, light), standard deviations (shaded areas) and fits of average curves (solid lines, dark). Averages of fit parameters obtained from fitting the single curves and the number of cells for each construct are listed in **Supplementary Table S3**.



Supplementary Figure S5. Local histone acetylation levels.

(A) Average radial intensity profiles from maximum projections of CLSM images. Cells were transfected with CIBN-TetR and a YFP-labeled PHR-effector construct. The effector constructs (top) and the H3K9ac staining (IF with Alexa568-labeled secondary antibody) are shown. When VP16 was absent, there was a drop in H3K9ac levels compared to the surrounding chromatin, which was not present when VP16 was recruited (bottom). Conversely, there was a slight drop in DNA density when VP16 was present, possibly indicating array decondensation (see **Supplementary Fig. S1C** and panel B). (B) Fold enrichment of H3K9ac and DNA density (DAPI) at the array compared to a nuclear reference region. The DAPI-normalized H3K9ac fold enrichment is depicted on the right. It increased when VP16 was present, indicating that the array became acetylated when activated. The fold enrichments were taken from the center of the radial profiles (shown in panel A for H3K9ac). (C) Absolute fluorescence intensity of H3K9ac IF staining in the entire nucleus and at the array under different conditions. Cells were grown in a removable 4-well culture insert, which allows for culturing cells under different conditions on the same slide. Cells were transfected with CIBN-TetR for recruitment, mKate2-MCP to assess transcriptional activity, and either PHR-YFP-NLS or PHR-YFP-VP16. In addition, cells were either treated with 2 μM SAHA or left untreated as a control

(VP16, $n = 12/12$, NLS, $n = 6/8$ for treated/untreated cells, respectively). H3K9ac was stained using IF with the primary and secondary Alexa633-labeled antibodies listed in the Methods section. IF stainings were done on the same slide (after removing the culture insert) and images were recorded on the same day with identical imaging settings so that absolute intensities can be directly compared. As expected, SAHA led to increased nuclear and array H3K9ac levels both in activated and non-activated cells. Intensity levels at the array were calculated as in **Supplementary Fig. 2C** and are normalized to DAPI density.

Supplementary Tables

Supplementary Table S1. List of BLInCR constructs.

CIBN constructs	PHR constructs	Others
CIBN-tagBFP-TetR	PHR-mCherry	tagBFP-LacI
CIBN-YFP-TetR	PHR-iRFP713	CFP-LacI
CIBN-tagBFP-hLaminB1	PHR-YFP	GFP-LacI
CIBN-YFP-hLaminB1	PHR-mCherry-NLS	RFP-LacI
CIBN-tagBFP-hTRF1	PHR-YFP-NLS	TetR-GFP
CIBN-YFP-hTRF1	PHR-mCherry-VP16	TetR-YFP
CIBN-tagBFP-hTRF2	PHR-YFP-VP16	TetR-mRFP
CIBN-YFP-hTRF2	PHR-iRFP713-VP16	GFP-MCP
CIBN-tagBFP-hNCL	PHR-mCherry-hPMLIII	tagRFP-MCP
CIBN-YFP-hNCL	PHR-YFP-hPMLIII	mKate2-MCP
CIBN-tagBFP-hPMLIII	PHR-YFP-hGCN5	mCherry-MCP
CIBN-YFP-hPMLIII	PHR-YFP-hGCN5-NLS	tagBFP-TetR-VP16
CIBN-TetR-tagRFP-T	PHR-YFP-hGCN5mut	GFP-TetR-VP16
CIBN-LacI-tagRFP-T	PHR-YFP-hHP1 β	tagBFP-LacI-VP16
CIBN-hTRF1-tagRFP-T	PHR-mCherry-hHP1 β	GFP-LacI-VP16
CIBN-hTRF2-tagRFP-T		
CIBN-TetR		
CIBN-LacI		
CIBN-YFP		
CIBN-mCherry		

For developing BLInCR, constructs with different autofluorescent protein domain fusions were constructed and tested. The constructs used for the experiments described in the main and supplementary figures are indicated in bold. All constructs have been deposited at the Addgene plasmid repository.

Supplementary Table S2. Summary of kinetic parameters for optogenetic recruitment.

	PHR-YFP-VP16	PHR-YFP-hGCN5	PHR-YFP-NLS
Number of cells n	18	18	19
k_1 (s ⁻¹), fast reaction ^a		0.129 ± 0.003	
b , fraction fast ^b	0.40 ± 0.16	0.39 ± 0.23	0.19 ± 0.20
k_2 (s ⁻¹), slow reaction ^a		0.023 ± 0.001	
c , fraction slow ^b	0.48 ± 0.17	0.47 ± 0.25	0.73 ± 0.21
$\tau_{1/2}$ (s) ^b	13.6 ± 4.9	14.2 ± 7.1	22.2 ± 7.7

Recruitment curves were fitted with a model describing two parallel first-order reactions ($E(t) = a - b \cdot \exp(-k_1 \cdot t) - c \cdot \exp(-k_2 \cdot t)$). The recruitment rates k_1 and k_2 were fitted globally while the plateau value a and the fractions b and c were fitted individually for each cell to account for varying transfection efficiencies and differences between the recruited constructs. The plateau value a was used to normalize the curves.

^a The fit parameters of the globally fitted rates and their standard fit errors are listed.

^b The characteristic recruitment time $\tau_{1/2}$ for reaching half maximal levels and the contributions of the fast and slow reactions were computed as averages and standard deviations of all single values.

Supplementary Table S3. Summary of kinetic parameters for dissociation after optogenetic recruitment.

	PHR-mCherry-VP16	PHR-mCherry-NLS
Number of cells n	13	12
k (min ⁻¹)	0.07 ± 0.04	0.08 ± 0.03
m	1.53 ± 0.20	1.47 ± 0.22
$t_{1/2}$ (min)	4.9 ± 0.8	4.8 ± 0.6

Single reversibility curves were fitted with an exponential model comprising a time-dependent reaction rate ($E(t) = a \cdot \exp(-k \cdot t^m) + c$). Mean and standard deviation are shown.

Supplementary Table S4. Fit parameters for different models of BLInCR-induced transcription activation.

Two-state model			Feedback model			Sequential model		
	untreated	SAHA		untreated	SAHA		untreated	SAHA
A_0 (%)	0	7	$A_{1,0}$ (%)	0.6	0.1	A_0 (%)	9	27
k_a (min^{-1})	0.0042	0.0008	A_2 (%)	8	33	n	7	11
k_i (min^{-1})	0.011	0.002	k_a (min^{-1})	0.15	0.26	k_a (min^{-1})	0.16	0.23
k_r (min^{-1})	0.32	0.33	k_r (min^{-1})	0.61	0.54	k_r (min^{-1})	0.89	0.82
k_d (min^{-1})		0.017	k_d (min^{-1})		0.50	k_d (min^{-1})		0.62
Fit quality		0.9714	Fit quality		0.9992	Fit quality		0.9985

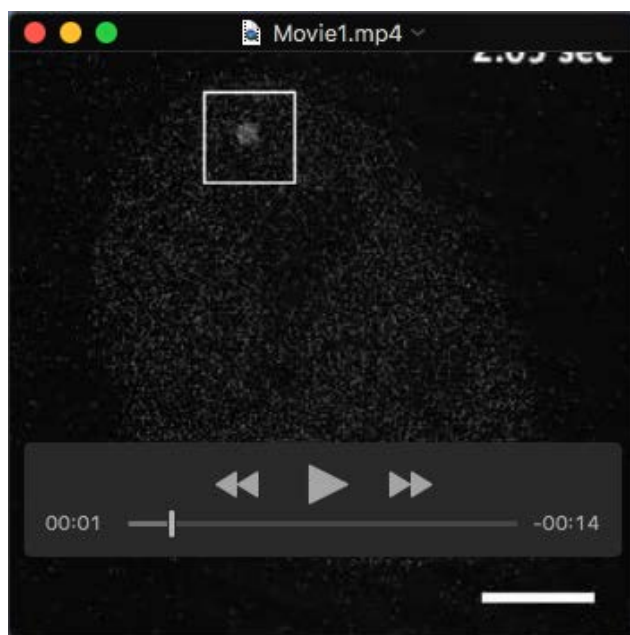
Differential equations and their solution are described in the Methods section. Averaged curves and fit curves are depicted in **Fig. 4A**. The fit quality corresponds to the adjusted coefficient of determination R^2 .

Supplementary Table S5. Antibodies used in this study.

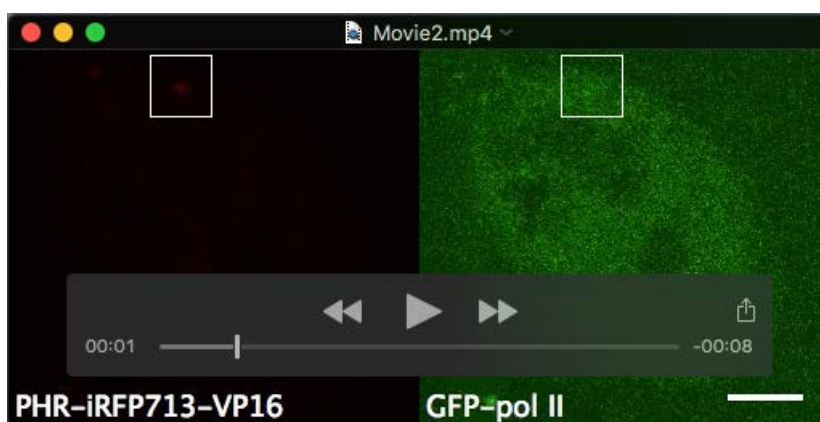
Target	Host	Dilution	Source
<i>Primary antibodies</i>			
H3K9ac	Rabbit	1:1000 (IF)	ActiveMotif #39917, lot 16111002 (Fig. S5A,B) and lot 23913004 (Fig. S5C)
		1:1000 (WB)	ActiveMotif #39137, lot: 09811002
H3K27ac	Rabbit	1:1000 (WB)	Abcam #4729, lot: CR238071-2
H4ac	Rabbit	1:2000 (WB)	Millipore #06-866, lot: DAM1416550
H3	Mouse	1:1000 (WB)	Cell Signaling Technology #14269, lot: 20
GFP	Rabbit	1:500 (IF)	Abcam #290, lot: GR135929-1
RNA pol II CTD phospho Ser2	Rat	1:100 (IF)	Clone 3E10, ActiveMotif #61083, lot: 20212002
RNA pol II CTD phospho Ser5	Rat	1:100 (IF)	Clone 3E8, ActiveMotif #61085, lot: 16513002
<i>Secondary antibodies</i>			
Rabbit IgG, HRP-linked	Goat	1:2000 (WB)	Cell Signaling Technology #7074, lot: 25
Mouse IgG, HRP-linked	Horse	1:2000 (WB)	Cell Signaling Technology #7076, lot: 27
Rabbit IgG, Alexa 488-linked	Goat	1:500 (IF)	Life Technologies #A11034, lot: 1141875
Rabbit IgG Alexa 568-linked	Goat	1:500 (IF)	Life Technologies #A11036, lot: 997761
Rabbit IgG Alexa 633-linked	Goat	1:500 (IF)	Life Technologies #A21071, lot: 51445A
Rat IgG Alexa 647-linked	Goat	1:500 (IF)	Life Technologies #A21247, lot: 1884217

IF: immunofluorescence, WB: western blot.

Supplementary Movies



Movie 1. Light-dependent recruitment of PHR-YFP-VP16 to tethered CIBN-TetR. Note that the brightness has been adjusted non-linearly (gamma = 0.65) for better visibility of the signals at the array and within the nucleoplasm. Speed: 10 fps (~2x). Scale bar: 5 μ m. The movie is provided as a separate file in mp4 format.



Movie 2. Co-recruitment of GFP-tagged RNA polymerase II. Note that the brightness has been adjusted non-linearly (gamma = 0.65) for better visibility of the signals at the array and within the nucleoplasm. PHR-VP16 was tagged with far-red iRFP713. Speed: 4 fps (~20x). Scale bar: 5 μ m. The movie is provided as a separate file in mp4 format.

Supplementary Online Information

Real-time observation of light-controlled transcription in living cells

Anne Rademacher, Fabian Erdel, Jorge Trojanowski, Sabrina Schumacher & Karsten Rippe

Content

Supplementary Figures

Figure S1. Continuous light-dependent recruitment of PHR-YFP-VP16 to reporter array.

Figure S2. Immunostaining of RNA polymerase II phosphorylated at serine 2 or 5.

Figure S3. Gene expression in U2OS 2-6-3 cells transfected with different constructs.

Figure S4. Recruitment and reversibility with BLInCR.

Figure S5. Local histone acetylation levels.

Supplementary Tables

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Table S3. Summary of kinetic parameters for dissociation after optogenetic recruitment.

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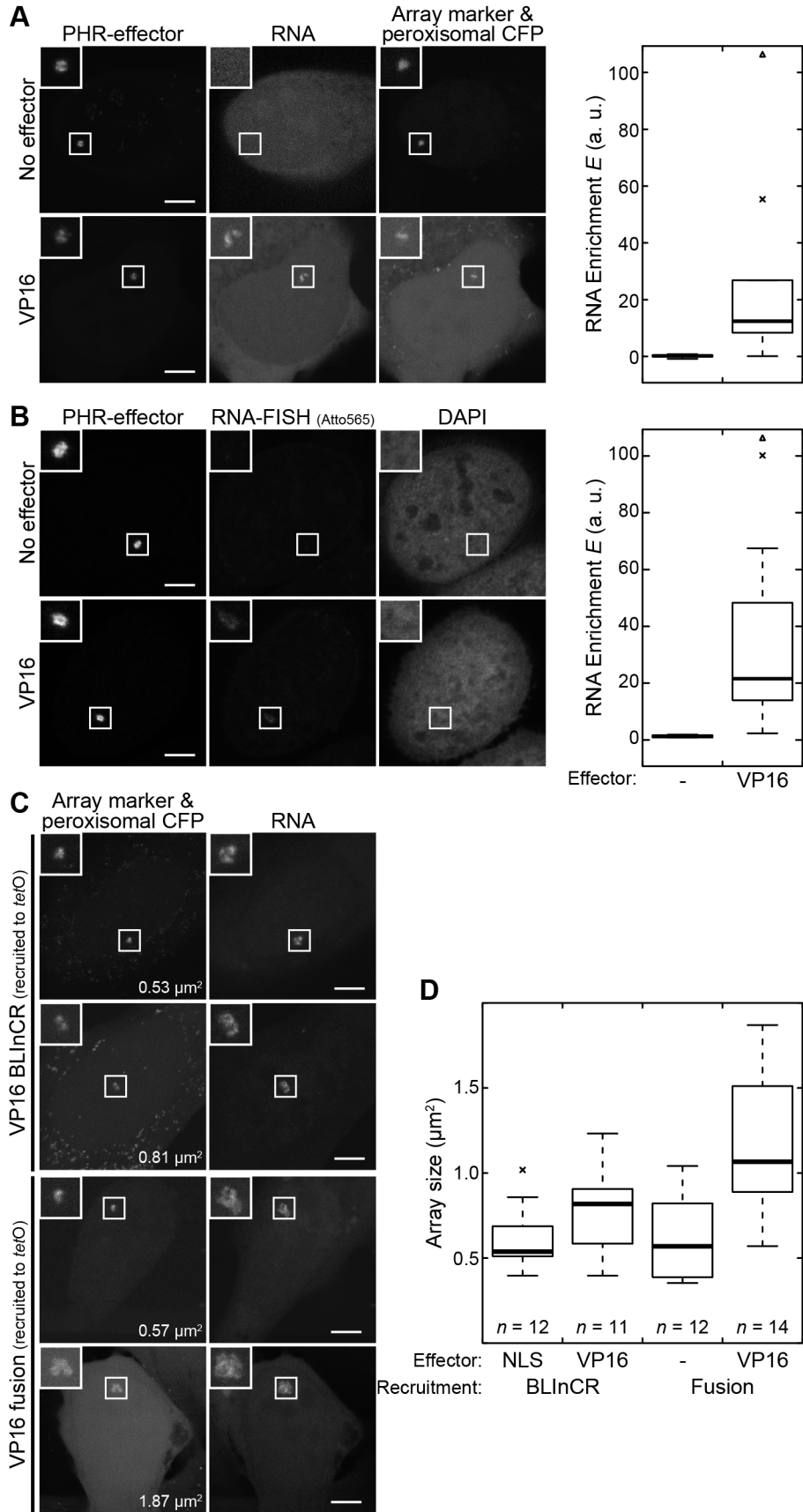
Table S5. Antibodies used in this study.

Supplementary Movies

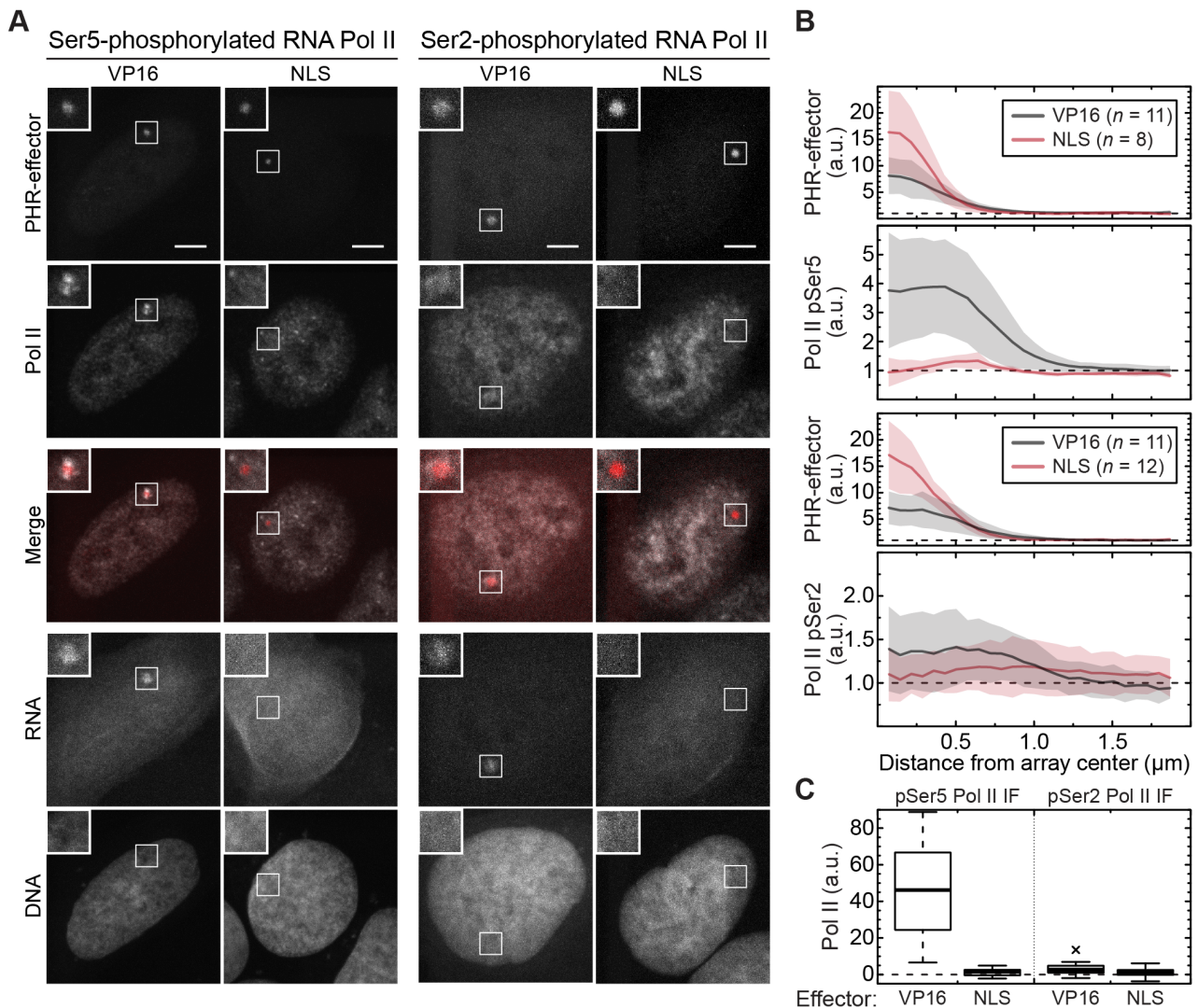
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Supplementary Figures

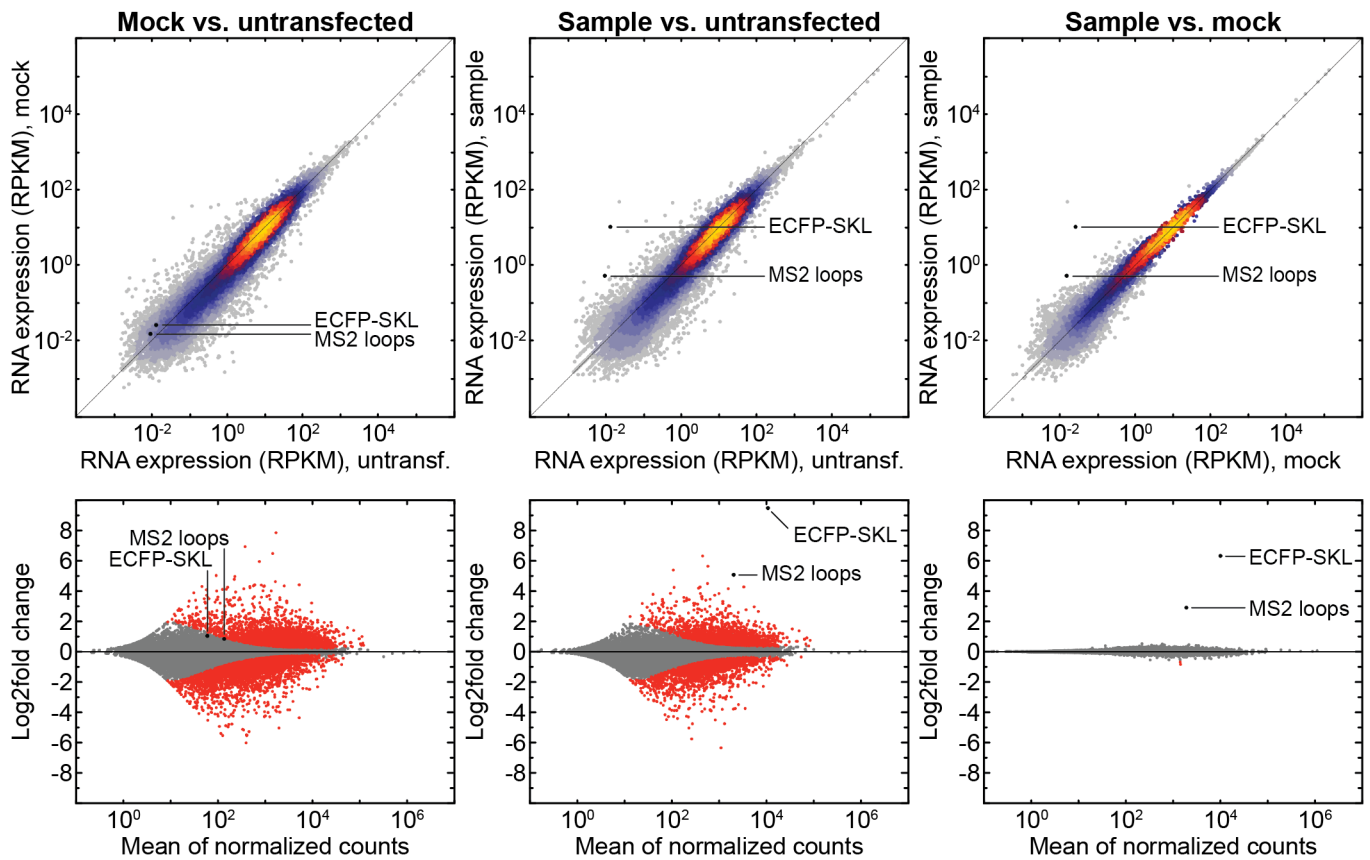


Supplementary Figure S1. Continuous light-dependent recruitment of PHR-YFP-VP16 to reporter array. Cells were continuously illuminated overnight with a blue LED, leading to recruitment of PHR constructs (via CIBN-TetR) to the *tetO* repeats of the reporter array. In the control reaction (“no effector”) transfection was with PHR-YFP instead of PHR-YFP-VP16. **(A)** Maximum intensity projection CLSM images of YFP-labeled effectors, RNA (visualized via mKate2-MCP), an array marker (CFP-LacI) and peroxisomal CFP. Quantification of RNA enrichment is shown on the right ($n=9$ for each condition). **(B)** Same as in panel A, but RNA stained by RNA-FISH ($n = 6$ and $n = 11$ for the control without effector and VP16, respectively). The amount of RNA detected at the array after VP16 recruitment showed a similar distribution for different RNA detection methods (compare quantification for panels A and B) and varied considerably across different cells. **(C)** Exemplary maximum intensity projection CLSM images visualizing the array through the independent marker CFP-LacI. VP16 was either recruited via BLInCR (by transfecting PHR-YFP-VP16 & CIBN-TetR) or with a fusion construct (GFP-TetR-VP16). All cells transfected with a VP16 construct showed mKate2-MCP enrichment, confirming that these cells were transcriptionally active. **(D)** Quantification of array sizes with enriched VP16 (shown in panel C) compared to negative controls without VP16 (i.e., PHR-YFP-NLS for BLInCR and TetR-GFP as a fusion construct). On average, cells expressing TetR-VP16 fusion constructs had somewhat larger arrays compared to cells with constitutive optogenetic recruitment of VP16. Notably, some of the activated cells in the latter population had condensed arrays that were comparable to those in the negative control PHR-YFP-NLS. Scale bars: 5 μm .

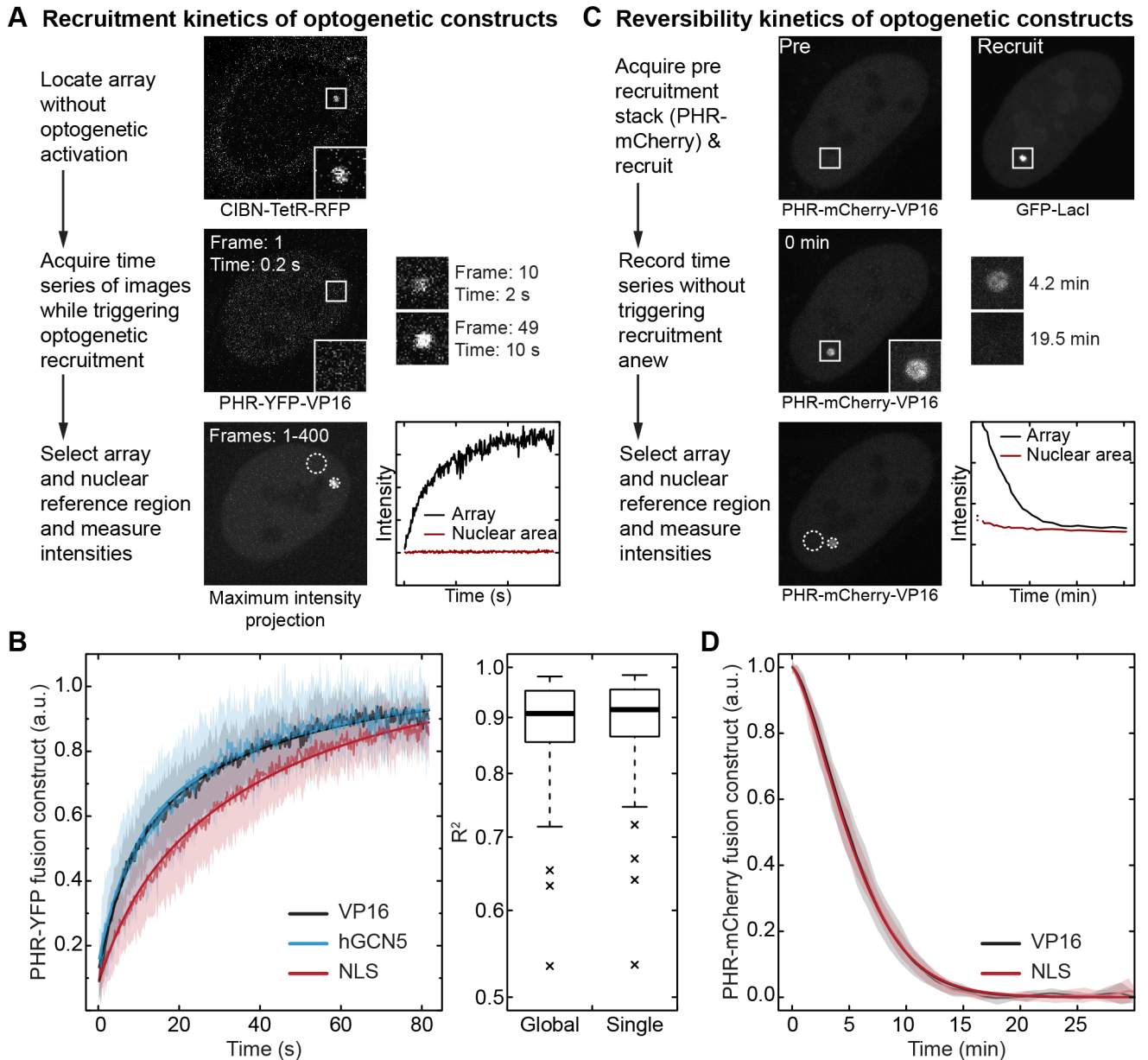


Supplementary Figure S2. Immunostaining of RNA polymerase II phosphorylated at serine 2 or 5. Cells were transfected with PHR-YFP-effector constructs, CIBN-TetR and mKate2-MCP to monitor RNA production. The respective pol II species were visualized with the primary antibodies and the Alexa647-labeled secondary antibody listed in the Methods section. DNA was counterstained with DAPI. **(A)** Exemplary maximum intensity projections of CLSM images showing that neither the initiating serine 5-phosphorylated (Ser5p) pol II nor the elongating serine 2-phosphorylated (Ser2p) pol II could be detected in non-activated control cells. Ser5p was clearly enriched in activated cells, whereas Ser2p was only slightly accumulated at the array. The absence of pol II Ser2p signal in most cells might be due to the small size of the transgene of ~3 kb. Reportedly, Ser2p tends to occur downstream of Ser5p and is only observed beyond 3 kb for some genes (Descostes et al. 2014, eLife 3, e02105). Scale bars: 5 μ m. **(B)** Average DAPI-normalized radial intensity profiles around the array. Quantifications of transfected cells was done as described in panel A. **(C)** Total accumulated pol II signal calculated from the radial profiles ($\sum_{r=0}^{r=FWHM} 2\pi r I(r)$),

where r is the distance from the array center, FWHM the full width at half maximum calculated from the effector profiles and $I(r)$ the intensity at distance r from the radial profiles in panel B).

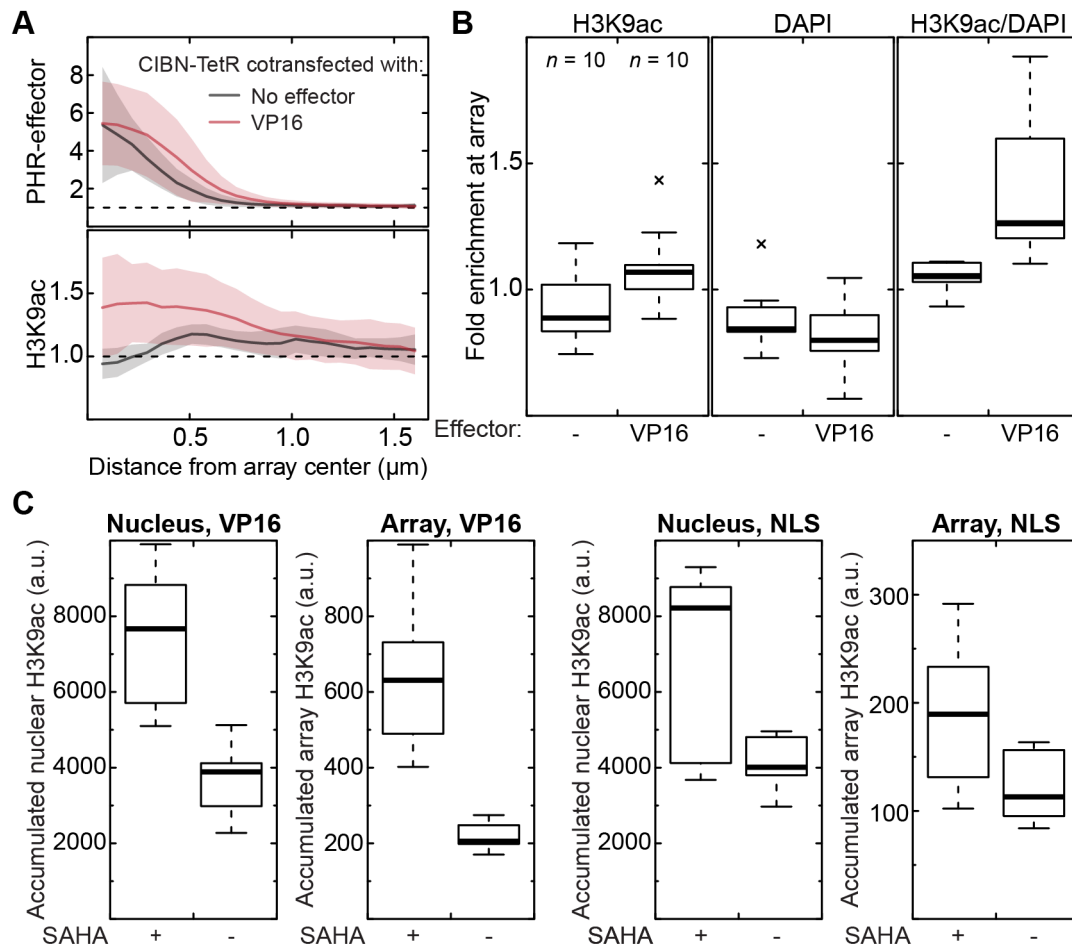


Supplementary Figure S3. Gene expression in U2OS 2-6-3 cells transfected with different constructs. Cells were transfected with YFP and TetR-RFP (mock) or PHR-YFP-VP16, CIBN-TetR and mCherry-MCP (sample). The upper set of plots shows RPKM values for annotated genes in mock vs. untransfected (left), sample vs. untransfected (center) and sample vs. mock (right) cells. The lower set of plots are MA plots derived from the differential gene expression analysis with DESeq2 for the respective combination. When comparing mock or sample vs. untransfected cells, hundreds of genes were differentially expressed, indicating that either expression of TetR or the transfection procedure itself causes substantial changes in gene expression. However, when comparing sample to mock-transfected cells, only the reporter elements (MS2 loops and ECFP-SKL) showed significant differential expression ($p_{\text{adj}} < 0.001$) and log2-fold changes > 1 .



Supplementary Figure S4. Recruitment and reversibility with BLInCR. (A) The typical workflow of a BLInCR experiment is illustrated for cells transfected with CIBN-TetR-tagRFP-T (localizer) and PHR-YFP-VP16 (effector). First, the cellular structure bound by the localizer (here: *tetO* array) was located in the tagRFP-T channel (excitation with yellow-green light), which did not trigger optogenetic recruitment. Next, a YFP time series was recorded while triggering recruitment of the effector to the localizer (excitation with blue light). Finally, the structure that was targeted by the localizer (here: *tetO* array) and a nuclear reference region were selected in the maximum intensity projection of the time series and the mean intensities at those regions were measured for each image. (B) Recruitment kinetics of PHR-YFP-fused effector proteins (left). Experimental means (solid lines, light), standard deviations (shaded areas) and fits (solid lines, dark) are shown. A global fit using the same two rate constants for all curves (and all constructs) yielded similar fit qualities as fits of each single curve with individual rate constants (right). In particular, the cumulative residual

sum of squares was only 8.5% larger for the global fit. The fit parameters for the global fit and the number of cells for each construct are listed in **Supplementary Table S2**. **(C)** A typical workflow for a BLInCR reversibility experiment is illustrated for cells transfected with CIBN-TetR (localizer), GFP-LacI (marker) and PHR-mCherry-VP16 (effector). A stack of images was recorded in the mCherry channel (excitation with yellow-orange light) prior to recruitment. The array was visualized by recording two stacks in the marker channel with blue light excitation for 38 s, thereby triggering effector recruitment to the localizer. The dissociation of the effector from the targeted structure was monitored by recording image stacks every 30-120 s without triggering the PHR switch again (excitation with yellow-orange light). Finally, the targeted structure and a nuclear reference region were selected in the maximum intensity projection of the time series and the mean intensities at those regions were measured for each image. **(D)** Plot of experimental means (solid lines, light), standard deviations (shaded areas) and fits of average curves (solid lines, dark). Averages of fit parameters obtained from fitting the single curves and the number of cells for each construct are listed in **Supplementary Table S3**.



Supplementary Figure S5. Local histone acetylation levels.

(A) Average radial intensity profiles from maximum projections of CLSM images. Cells were transfected with CIBN-TetR and a YFP-labeled PHR-effector construct. The effector constructs (top) and the H3K9ac staining (IF with Alexa568-labeled secondary antibody) are shown. When VP16 was absent, there was a drop in H3K9ac levels compared to the surrounding chromatin, which was not present when VP16 was recruited (bottom). Conversely, there was a slight drop in DNA density when VP16 was present, possibly indicating array decondensation (see **Supplementary Fig. S1C** and panel **B**). **(B)** Fold enrichment of H3K9ac and DNA density (DAPI) at the array compared to a nuclear reference region. The DAPI-normalized H3K9ac fold enrichment is depicted on the right. It increased when VP16 was present, indicating that the array became acetylated when activated. The fold enrichments were taken from the center of the radial profiles (shown in panel **A** for H3K9ac). **(C)** Absolute fluorescence intensity of H3K9ac IF staining in the entire nucleus and at the array under different conditions. Cells were grown in a removable 4-well culture insert, which allows for culturing cells under different conditions on the same slide. Cells were transfected with CIBN-TetR for recruitment, mKate2-MCP to assess transcriptional activity, and either PHR-YFP-NLS or PHR-YFP-VP16. In addition, cells were either treated with 2 μM SAHA or left untreated as a control

(VP16, $n = 12/12$, NLS, $n = 6/8$ for treated/untreated cells, respectively). H3K9ac was stained using IF with the primary and secondary Alexa633-labeled antibodies listed in the Methods section. IF stainings were done on the same slide (after removing the culture insert) and images were recorded on the same day with identical imaging settings so that absolute intensities can be directly compared. As expected, SAHA led to increased nuclear and array H3K9ac levels both in activated and non-activated cells. Intensity levels at the array were calculated as in **Supplementary Fig. 2C** and are normalized to DAPI density.

Supplementary Tables

Supplementary Table S1. List of BLInCR constructs.

CIBN constructs	PHR constructs	Others
CIBN-tagBFP-TetR	PHR-mCherry	tagBFP-LacI
CIBN-YFP-TetR	PHR-iRFP713	CFP-LacI
CIBN-tagBFP-hLaminB1	PHR-YFP	GFP-LacI
CIBN-YFP-hLaminB1	PHR-mCherry-NLS	RFP-LacI
CIBN-tagBFP-hTRF1	PHR-YFP-NLS	TetR-GFP
CIBN-YFP-hTRF1	PHR-mCherry-VP16	TetR-YFP
CIBN-tagBFP-hTRF2	PHR-YFP-VP16	TetR-mRFP
CIBN-YFP-hTRF2	PHR-iRFP713-VP16	GFP-MCP
CIBN-tagBFP-hNCL	PHR-mCherry-hPMLIII	tagRFP-MCP
CIBN-YFP-hNCL	PHR-YFP-hPMLIII	mKate2-MCP
CIBN-tagBFP-hPMLIII	PHR-YFP-hGCN5	mCherry-MCP
CIBN-YFP-hPMLIII	PHR-YFP-hGCN5-NLS	tagBFP-TetR-VP16
CIBN-TetR-tagRFP-T	PHR-YFP-hGCN5mut	GFP-TetR-VP16
CIBN-LacI-tagRFP-T	PHR-YFP-hHP1 β	tagBFP-LacI-VP16
CIBN-hTRF1-tagRFP-T	PHR-mCherry-hHP1 β	GFP-LacI-VP16
CIBN-hTRF2-tagRFP-T		
CIBN-TetR		
CIBN-LacI		
CIBN-YFP		
CIBN-mCherry		

For developing BLInCR, constructs with different autofluorescent protein domain fusions were constructed and tested. The constructs used for the experiments described in the main and supplementary figures are indicated in bold. All constructs have been deposited at the Addgene plasmid repository.

Supplementary Table S2. Summary of kinetic parameters for optogenetic recruitment.

	PHR-YFP-VP16	PHR-YFP-hGCN5	PHR-YFP-NLS
Number of cells n	18	18	19
k_1 (s ⁻¹), fast reaction ^a		0.129 ± 0.003	
b , fraction fast ^b	0.40 ± 0.16	0.39 ± 0.23	0.19 ± 0.20
k_2 (s ⁻¹), slow reaction ^a		0.023 ± 0.001	
c , fraction slow ^b	0.48 ± 0.17	0.47 ± 0.25	0.73 ± 0.21
$\tau_{1/2}$ (s) ^b	13.6 ± 4.9	14.2 ± 7.1	22.2 ± 7.7

Recruitment curves were fitted with a model describing two parallel first-order reactions ($E(t) = a - b \cdot \exp(-k_1 \cdot t) - c \cdot \exp(-k_2 \cdot t)$). The recruitment rates k_1 and k_2 were fitted globally while the plateau value a and the fractions b and c were fitted individually for each cell to account for varying transfection efficiencies and differences between the recruited constructs. The plateau value a was used to normalize the curves.

^a The fit parameters of the globally fitted rates and their standard fit errors are listed.

^b The characteristic recruitment time $\tau_{1/2}$ for reaching half maximal levels and the contributions of the fast and slow reactions were computed as averages and standard deviations of all single values.

Supplementary Table S3. Summary of kinetic parameters for dissociation after optogenetic recruitment.

	PHR-mCherry-VP16	PHR-mCherry-NLS
Number of cells n	13	12
k (min ⁻¹)	0.07 ± 0.04	0.08 ± 0.03
m	1.53 ± 0.20	1.47 ± 0.22
$t_{1/2}$ (min)	4.9 ± 0.8	4.8 ± 0.6

Single reversibility curves were fitted with an exponential model comprising a time-dependent reaction rate ($E(t) = a \cdot \exp(-k \cdot t^m) + c$). Mean and standard deviation are shown.

Supplementary Table S4. Fit parameters for different models of BLInCR-induced transcription activation.

Two-state model			Feedback model			Sequential model		
	untreated	SAHA		untreated	SAHA		untreated	SAHA
A_0 (%)	0	7	$A_{1,0}$ (%)	0.6	0.1	A_0 (%)	9	27
k_a (min^{-1})	0.0042	0.0008	A_2 (%)	8	33	n	7	11
k_i (min^{-1})	0.011	0.002	k_a (min^{-1})	0.15	0.26	k_a (min^{-1})	0.16	0.23
k_r (min^{-1})	0.32	0.33	k_r (min^{-1})	0.61	0.54	k_r (min^{-1})	0.89	0.82
k_d (min^{-1})		0.017	k_d (min^{-1})		0.50	k_d (min^{-1})		0.62
Fit quality		0.9714	Fit quality		0.9992	Fit quality		0.9985

Differential equations and their solution are described in the Methods section. Averaged curves and fit curves are depicted in **Fig. 4A**. The fit quality corresponds to the adjusted coefficient of determination R^2 .

Supplementary Table S5. Antibodies used in this study.

Target	Host	Dilution	Source
<i>Primary antibodies</i>			
H3K9ac	Rabbit	1:1000 (IF)	ActiveMotif #39917, lot 16111002 (Fig. S5A,B) and lot 23913004 (Fig. S5C)
		1:1000 (WB)	ActiveMotif #39137, lot: 09811002
H3K27ac	Rabbit	1:1000 (WB)	Abcam #4729, lot: CR238071-2
H4ac	Rabbit	1:2000 (WB)	Millipore #06-866, lot: DAM1416550
H3	Mouse	1:1000 (WB)	Cell Signaling Technology #14269, lot: 20
GFP	Rabbit	1:500 (IF)	Abcam #290, lot: GR135929-1
RNA pol II CTD phospho Ser2	Rat	1:100 (IF)	Clone 3E10, ActiveMotif #61083, lot: 20212002
RNA pol II CTD phospho Ser5	Rat	1:100 (IF)	Clone 3E8, ActiveMotif #61085, lot: 16513002
<i>Secondary antibodies</i>			
Rabbit IgG, HRP-linked	Goat	1:2000 (WB)	Cell Signaling Technology #7074, lot: 25
Mouse IgG, HRP-linked	Horse	1:2000 (WB)	Cell Signaling Technology #7076, lot: 27
Rabbit IgG, Alexa 488-linked	Goat	1:500 (IF)	Life Technologies #A11034, lot: 1141875
Rabbit IgG Alexa 568-linked	Goat	1:500 (IF)	Life Technologies #A11036, lot: 997761
Rabbit IgG Alexa 633-linked	Goat	1:500 (IF)	Life Technologies #A21071, lot: 51445A
Rat IgG Alexa 647-linked	Goat	1:500 (IF)	Life Technologies #A21247, lot: 1884217

IF: immunofluorescence, WB: western blot.

Supplementary Movies

Movie 1. Light-dependent recruitment of PHR-YFP-VP16 to tethered CIBN-TetR. Note that the brightness has been adjusted non-linearly ($\gamma = 0.65$) for better visibility of the signals at the array and within the nucleoplasm. Speed: 10 fps ($\sim 2x$). Scale bar: 5 μm . The movie is provided as a separate file in mp4 format.

Movie 2. Co-recruitment of GFP-tagged RNA polymerase II. Note that the brightness has been adjusted non-linearly ($\gamma = 0.65$) for better visibility of the signals at the array and within the nucleoplasm. PHR-VP16 was tagged with far-red iRFP713. Speed: 4 fps ($\sim 20x$). Scale bar: 5 μm . The movie is provided as a separate file in mp4 format.