

Supplementary Information for

DNA residence time is a regulatory factor of transcription repression

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Supplementary Text

Normalization of mRNA quantities

We experimentally determined for untreated cells (E) and cells treated with Dexamethasone (D) the mean number of *SGK1* mRNA molecules per cell (mean *SGK1* mRNA), R_{cell}^E and R_{cell}^D , the average number of *SGK1* mRNA molecules within a transcription burst of an allele (burst size), B_{gen}^E and B_{gen}^D , and the probability of cells to exhibit one or two active alleles (burst frequency), P_{cell}^E and P_{cell}^D .

To account for differences in cellular background between TALE-TF constructs, we normalized R_{cell}^D , B_{gen}^D and P_{cell}^D with respect to untreated cells:

$$R_{cell} = \frac{R_{cell}^D}{R_{cell}^E} < R_{cell}^E > \quad (1)$$

$$B_{gen} = \frac{B_{gen}^D}{B_{gen}^E} < B_{gen}^E > \quad (2)$$

$$P_{cell} = 1 - \frac{1 - P_{cell}^D}{1 - P_{cell}^E} < 1 - P_{cell}^E > \quad (3)$$

where $<\cdot>$ denotes the average over all constructs and the detour via the probability of a cell to exhibit no active allele, $1 - P_{cell}$ was taken to avoid division by small numbers.

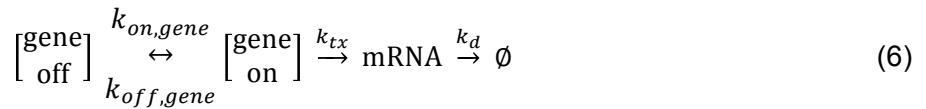
We next transformed cellular quantities into quantities per allele, by assuming that both genes act independent of each other:

$$R_{gen} = \frac{R_{cell}}{2} \quad (4)$$

$$P_{gen} = 1 - \sqrt{1 - P_{cell}} \quad (5)$$

Model for TALE-mediated repression

In the two-state model of gene transcription, the gene cycles between a transcriptionally silent 'off'-state and a transcriptionally active 'on'-state with the rate constant of gene activation, $k_{on,gene}$, and the off-rate constant, $k_{off,gene}$. In the 'on'-state, mRNA is transcribed with the rate constant of gene transcription, k_{tx} , and subsequently degraded with the degradation rate constant k_d (46):



The rate constants of the two-state model can be calculated from the mRNA quantities per allele:

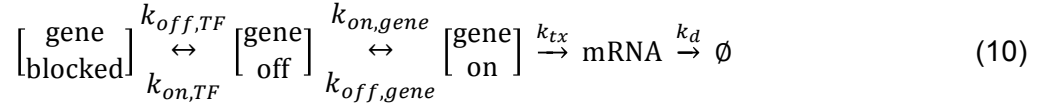
$$k_{on,gene} = \frac{R_{gen}}{B_{gen} (1 - P_{gen})} k_d \quad (7)$$

$$k_{off,gene} = \frac{R_{gen}}{B_{gen} P_{gen}} k_d \quad (8)$$

$$k_{tx} = \frac{R_{gen}}{P_{gen}} k_d \quad (9)$$

We account for competitive inhibition of the endogenous activating factor GR by TALE-TF constructs by extending the two-state model with a blocked state. The blocked state is populated

and depopulated upon binding and release of TALE-TF with the TF on-rate constant $k_{on,TF}$ and the TF off-rate constant $k_{off,TF}$:



Be $[B]$ the concentration of genes in the blocked state, $[Off]$ the concentration of genes in the 'off'-state and $[On]$ the concentration of genes in the 'on'-state. We find

$\frac{[Off]}{[B]} = \frac{k_{off,TF}}{k_{on,TF}}$ and $\frac{[On]}{[Off]} = \frac{k_{on}}{k_{off}}$. Thus, an effective equilibrium constant for the 'on'-state of the gene in the two-state approximation (equation (6)) is given by

$$K_{eff}^{on} = \frac{[On]}{[B] + [Off]} = \frac{k_{on,gene}}{k_{off,gene}} \left(\frac{k_{off,TF}}{k_{off,TF} + k_{on,TF}} \right) \quad (11)$$

The effective equilibrium constant can be converted to the effective on-rate constant of the gene in the two-state approximation (equation (6)) $k_{on,eff} = k_{on,gene} \left(1 - \frac{k_{on,TF}}{k_{on,TF} + k_{off,TF}} \right)$, or, equivalently,

$$k_{on,eff} = k_{on,gene} \left(1 - \frac{\tau_{DNA}}{\tau_{DNA} + \tau_{npl}} \right) \quad (12)$$

where $\tau_{DNA} = 1/k_{off,TF}$ is the DNA residence time of the TF and $\tau_{npl} = 1/k_{on,TF}$ is the time the TF is not bound to DNA.

Equation (12) reveals a linear dependency of the measured effective on-rate constant on the percentage of time that TALE-TF constructs are bound to DNA.

We modelled the measured effective transcription rate constant to be given by a similar dependency:

$$k_{tx,eff} = k_{tx} \left(1 - \frac{\tau_{DNA}}{\tau_{DNA} + \tau_{npl}} \right) \quad (13)$$

We fitted equation (12) to the measured effective on-rate constant as function of TALE-TF DNA residence time or equations (12) and (13) globally with common τ_{npl} to the measured effective on-rate constant and transcription rate constant as functions of TALE-TF DNA residence time using a nonlinear least-squares fitting procedure in Igor Pro (v.6.37, WaveMetrics). For the fits, cells without TALE-TF construct (control U2-OS wild type cells) were treated as having zero residence time.

Supplementary Methods

Cloning of TALE-TF constructs

Tale-TFs were assembled using the Golden Gate TALEN and TAL Effector Kit2.0 (Addgene kit # 1000000024). In a first step, two arrays corresponding to repeat modules 1–10 and 11-(n-1) were assembled in separate intermediate vectors, pFUS_A and pFUS_B(n-1), where n is the final number of repeat domains. We verified correct assembly by sequencing. In a second step, we assembled the two intermediate arrays and the nth repeat into the final expression vector and again confirmed correct assembly by sequencing. To create the final expression vector, we inserted the TALE coding region and C-terminal regulatory domain of plasmid pcDNA3.1-GoldenGate-VP64 (Addgene # 47389) into the pICE plasmid (Addgene # 46960), using the primers ggcaagcttatggactacaaagaccatgacg_for and ccagatctattcaagaagcgtagtcgg_rev and HindIII and XbaI restriction sites. We further removed Esp3I and SacII sites present in the pICE plasmid by site directed mutagenesis (QuickChange, Stratagene) to enable subsequent cloning with these enzymes. We next inserted a HaloTag PCR product using HindIII and SacII restriction sites into the pICE plasmid to enable N-terminal HaloTag-TALE fusions. To create GRcXR transcription factors, we replaced the C-terminal SV40 NLS and the VP64 domain with amino acids 477-777 of hGR α . To create the XR transcription factor, we removed the VP64 domain.

To generate constructs containing less than 12 repeat domains, we mutated the vector pFUS_A to enable self-ligation of the 2 BsaI restriction sites (Q5 site-directed Mutagenesis kit NEB, primers ggtggtctctctatgcaagcaagcgc_for and ggtggaaagcgggcagtg_rev and aagcaagcgctcgaaacg_for and catagccaccacttggtc_rev). We next inserted all except the nth repeat domains into vectors pFUS_B. In a second step, we assembled the self-ligated pFUS_A, pFUS_B carrying n-1 repeat domains and the last repeat vector containing the nth repeat into the destination vector.

The target sequences of all TALE-TFs are summarized in Table S1.

ChIP for TALE-TF

We performed ChIP for the TALE-TF with the HaloChIP system (Promega). We grew cells in 100 mm plates and treated them with 1 μ M Dex for at least 3 hours to achieve nuclear localization of GRcXR. Crosslinking was done for 10 min at a final formaldehyde concentration of 1 %. After cytoplasmic lysis, we lysed nuclei and sonicated (Diagenode Bioruptor) the samples to an average DNA fragment size between 500 and 1500 bp. Since shearing conditions could not be kept constant for all cell lines in parallel, this preparatory step differed slightly between constructs. Subsequently we incubated the sheared chromatin for 2 h with the HaloLink Resin, washed 4 times for 10 min with H₂O, followed by 4 washes with High Salt Wash Buffer. Subsequently we released DNA and analyzed it with standard PCR using the primers cccctcccttcgcttggt_for and ggaagaagtacaatctgcatttact_rev (1) to amplify a section of the *SGK1* promoter region including the

binding site of GRcXR and the primers aagaaggctgcagaattgtgtta_for and gggaaccatcctaatacagg_rev (2) for amplifying a section of the *TUBB2b* promoter region.

qPCR

We extracted total RNA using the RNeasy Plus Mini Kit (Qiagen) and converted it to cDNA with the SuperScript Vilo cDNA synthesis kit (Thermo Fischer) using 1.5 µg total RNA as starting material. We used *ActB* as endogenous control. Quantitative PCR was performed with the StepOnePlus Real-Time PCR system using the TaqMan Fast Advanced Master Mix (Thermo Fisher) and gene specific TaqMan probes for *SGK1* (HS00985033_g1, Thermo Fisher) and for *ActB* (HS01060665_g1, Thermo Fisher), *GILZ* (HS00608272_m1; Thermo Fisher), *PER1* (HS00242988_m1; Thermo Fisher), *IRF8* (HS00175238_m1; Thermo Fisher) and for *LAD1* (HS00194326_m1; Thermo Fisher). We performed control reactions with no cDNA template and with non-reverse-transcribed RNA. We calculated fold changes in transcription with the formula $2^{-(\Delta\Delta Ct)}$, where $\Delta\Delta Ct$ is $\Delta Ct(\text{sample cell}) - \Delta Ct(\text{wild type U2-OS cell treated with ethanol})$, ΔCt is $Ct(\text{gene}) - Ct(\text{ActB})$ and Ct is the cycle at which the threshold is crossed. Experiments were performed in triplicates on at least three biological replicates.

Western Blot analysis of GR expression

We lysed cells in RIPA buffer and determined the protein concentration by absorbance at 280 nm. We separated 100 µg of total protein by SDS-PAGE and transferred them to a nitrocellulose membrane (0.45 µm pore size). Antibodies used were anti-GR (purified mouse anti-glucocorticoid receptor clone 41, # 611226 BD Bioscience) at 1:2500 and anti- α tubulin (monoclonal anti- α tubulin, clone DM1A, # T9026 Sigma-Aldrich) at 1:2500. Detection was achieved with a secondary anti-mouse IgG (AP linked, # 7056 Cell Signaling Technology) at 1:5000 via chromogenic staining.

Microscopy of smRNA-FISH

The microscope was assembled around an inverted microscope body (Axio Observer, Zeiss) equipped with a CSU10 scan head (Yokogawa), an oil immersion objective (UPlanSApo 60x/1.35 NA, Olympus) and an EMCCD camera (DV-887, Andor). TMR was excited with 532 nm laser light, FITC with 473 nm laser light and Quasar 670 with 635 nm laser light.

Single molecule fluorescence microscopy

The fluorescence microscope was custom built around a commercial microscope body (Nikon, TiE). A 638 nm laser (Toptica, IBEAM-SMART-640-S, 150 mW) and a 561 nm laser (Cobolt Jive 300 mW) were collimated, combined using a dichroic mirror and controlled by an AOTF (AA Optoelectronics, AOTFnC-400.650-TN). We adjusted their beam diameter with a pinhole, focused them on the back focal plane of a high-NA objective (100x 1.45 Nikon Plan Apo) and displaced them to achieve inclined illumination. We performed bright field illumination with a far red LED (660 nm CoolLed, pE-100, 25W). The fluorescence light was filtered by a dichroic mirror (AHF, F73-

866), an emission filter (AHF, F72-866) and a notch filter (AHF, F40-072) before being detected by an EMCCD camera (Andor, iXon Ultra DU 897U). We controlled the setup using NIS Elements software (Nikon) and a NIDAQ data acquisition card (National Instruments).

Data analysis

Data analysis was performed in MATLAB following the procedures published in(3). In brief, we detected single molecules by a threshold of 3 to 4 standard deviations above background levels and determined their positions with a 2D-Gaussian curve. We considered a fluorescent molecule remaining for two successive frames within 0.5 to 1.5 pixels, dependent on the time-lapse time, as bound. Single molecule tracks separated by one frame in which the molecule was not detected were combined.

To extract DNA residence times of TALE-TFs, we implemented a global fitting approach to histograms of fluorescent ‘on’ times as described in(3) in Python 2.7.9. In brief, we used a Levenberg-Marquardt least squares algorithm to fit a double-exponential distribution to the data:

$$f_T(t) = A \cdot \left[B \cdot \left(k_b \cdot \left(\frac{\tau_{on}}{\tau_{tl}} \right) + k_1 \right) \cdot \exp \left(- \left[k_b \cdot \left(\frac{\tau_{on}}{\tau_{tl}} \right) + k_1 \right] \cdot t \right) + (1 - B) \cdot \left(k_b \cdot \left(\frac{\tau_{on}}{\tau_{tl}} \right) + k_2 \right) \right. \\ \left. \cdot \exp \left(- \left[k_b \cdot \left(\frac{\tau_{on}}{\tau_{tl}} \right) + k_2 \right] \cdot t \right) \right] \quad (14)$$

Parameters optimized were the bleaching rate k_b , the off-rates k_1 and k_2 , the fraction of long and short bound molecules B and $(1 - B)$ and the overall amplitude A . The parameters τ_{on} and τ_{tl} were preset and not subject to optimization. The stopping criterion was set to a relative change of 10^{-7} in the sum of error squares. Reduced χ^2 -values were compared between a double- and a single-exponential model.

Simulation of mRNA distributions

To reconstruct the mRNA distributions using the measured parameters for k_{on} , k_{off} and k_{tx} , we performed a Gillespie-simulation of the two-state model, using that the three-state model converges to the two-state model if the transitions between the blocked state and the off state of the gene are much faster than the transitions between the off state and the on state of the gene. We next optimized the simulated mRNA distributions to better fit the measured mRNA distributions by varying the burst size $B = k_{tx}/k_{off}$ and minimizing the norm

$$d = \sum_i |m_i - s_i|$$

where m_i is a data point and s_i a simulated point and i runs over the bins of the mRNA histogram. The best match was determined by taking the smallest d and yielded the correction factor for the burst size as ratio between optimized and measured burst size.

ChIP-qPCR

We cultivated cells for at least 3 days in DMEM medium without phenol red and with 10 % of charcoal-stripped FBS to achieve uninduced conditions for GR and SGK1 and induced cells with 1 μ M Dex for 3 hours. Thereafter, we performed ChIP as described in (2) with anti-GR (Proteintech). We analyzed the recovered and purified DNA by qPCR with Power SYBR Green PCR Master Mix (Applied Biosystems) in a 7900HT Fast Real Time PCR System (Applied Biosystems). We used the primers cccctcccttcgcttggt_for and ggaagaagtacaatctgcatttcact_rev (1) to amplify a section of the *SGK1* promoter region including the binding site of GRcXR. Results of ChIP pulldowns were normalized to the amount of input DNA(4). Quantitative PCR reactions were performed in triplicates on at least two biological replicates.

Supplementary Figures

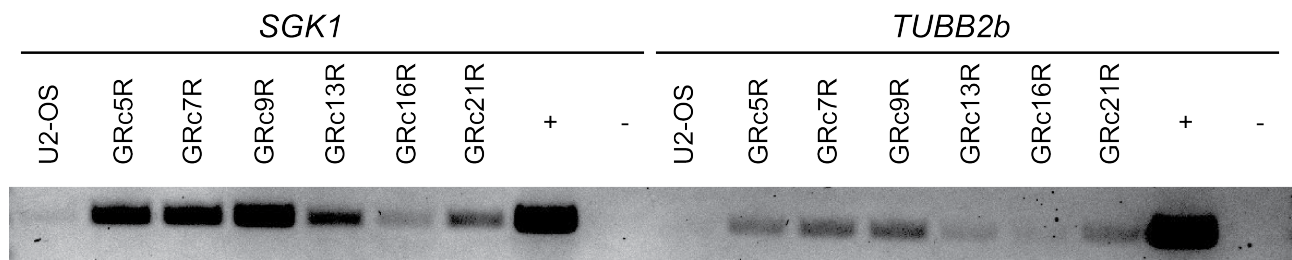


Figure S1. ChIP-PCR measurement of GRcXR binding to DNA. PCRs for *SGK1* and *TUBB2b* were both performed on the same condition-specific input DNA. Thus, the signals of both genes can be compared for each construct, but not between constructs. ChIP performed on wild type U2-OS cells not expressing GRcXR (U2-OS), U2-OS cell lysate (+) and water (-) were used as controls. All GRcXR constructs are efficiently recruited to their target sequence *SGK1*. Binding to *SGK1* is strongly preferred over binding to the non-target sequence *TUBB2b*.

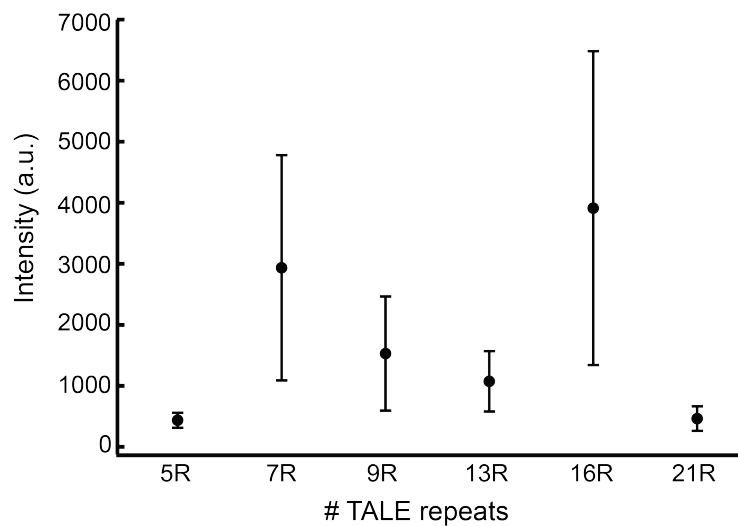


Figure S2. Concentration of GRcXR in U2-OS cells stably expressing the constructs. GRcXR concentrations were determined as the mean fluorescence intensity of labelled HaloTag inside the nucleus of Dex treated cells (n = 100 cells in each condition). Error bars denote s.d.

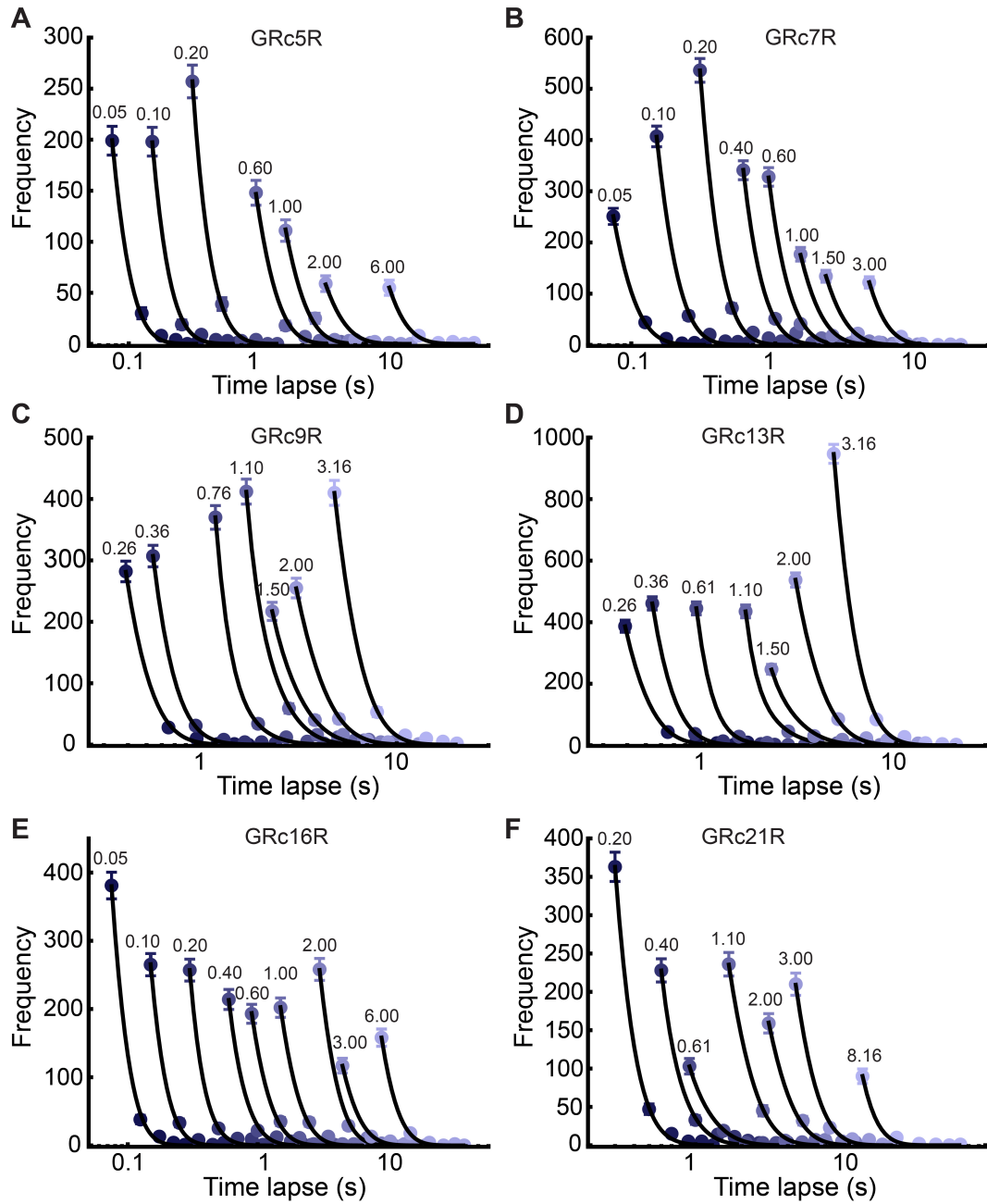


Figure S3. Determination of DNA residence times of GRcXR. Histograms of fluorescent 'on' times of GRcXR at different time-lapse conditions were globally fit by an exponential decay model with two off-rate constants (equation (14) in Supplementary Information). Numbers indicate the time-lapse time in s. Error bars denote s.d. **(A)** GRc5R ($n = 245$ (0.05 s); $n = 236$ (0.10 s); $n = 307$ (0.20 s); $n = 183$ (0.60 s); $n = 144$ (1.00 s); $n = 67$ (2.00 s); $n = 67$ (6.00 s)). Reduced χ^2 -values: 0.72 (double-) and 0.81 (single-exponential). **(B)** GRc7R ($n = 322$ (0.05 s); $n = 512$ (0.10 s); $n = 674$ (0.20 s); $n = 441$ (0.40 s); $n = 408$ (0.60 s); $n = 221$ (1.00 s); $n = 196$ (1.50 s); $n = 142$ (3.00 s)). Reduced χ^2 -values: 0.64(double-) and 0.68 (single-exponential). **(C)** GRc9R ($n = 331$ (0.26 s); $n = 367$ (0.36 s); $n = 431$ (0.76 s); $n = 534$ (1.10 s); $n = 291$ (1.50 s); $n = 317$ (2.00 s); $n = 506$ (3.16 s)). Reduced χ^2 -values: 0.9 (double-) and 1.76 (single-exponential). **(D)** GRc13R ($n = 467$ (0.26 s); $n = 536$ (0.36 s); $n = 487$ (0.61 s); $n = 512$ (1.10 s); $n = 314$ (1.50 s); $n = 702$ (2.00 s); $n = 1079$ (3.16 s)). Reduced χ^2 -values: 0.64(double-) and 0.68 (single-exponential). **(E)** GRc16R ($n = 381$ (0.05 s); $n = 271$ (0.10 s); $n = 261$ (0.20 s); $n = 211$ (0.40 s); $n = 201$ (0.60 s); $n = 201$ (1.00 s); $n = 261$ (2.00 s); $n = 161$ (3.00 s); $n = 161$ (6.00 s)). Reduced χ^2 -values: 0.64(double-) and 0.68 (single-exponential). **(F)** GRc21R ($n = 371$ (0.20 s); $n = 231$ (0.40 s); $n = 101$ (0.61 s); $n = 241$ (1.10 s); $n = 161$ (2.00 s); $n = 211$ (3.00 s); $n = 91$ (8.16 s)). Reduced χ^2 -values: 0.64(double-) and 0.68 (single-exponential).

(3.16 s)). Reduced χ^2 –values: 1.95 (double-) and 3.79 (single-exponential). **(E)** GRc16R ($n = 442$ (0.05 s); $n = 313$ (0.10 s); $n = 315$ (0.20 s); $n = 260$ (0.40 s); $n = 248$ (0.60 s); $n = 255$ (1.00 s); $n = 306$ (2.00 s); $n = 142$ (3.00 s); $n = 183$ (6.00 s)). Reduced χ^2 –values: 1.47 (double-) and 1.79 (single-exponential). **(F)** GRc21R ($n = 441$ (0.20 s); $n = 306$ (0.40 s); $n = 143$ (0.61 s); $n = 313$ (1.10 s); $n = 214$ (2.00 s); $n = 254$ (3.00 s); $n = 105$ (8.16 s)). Reduced χ^2 –values: 0.70 (double-) and 0.99 (single-exponential). n = number of molecules. For all TALE-TFs, a double-exponential model was preferred over a single-exponential model.

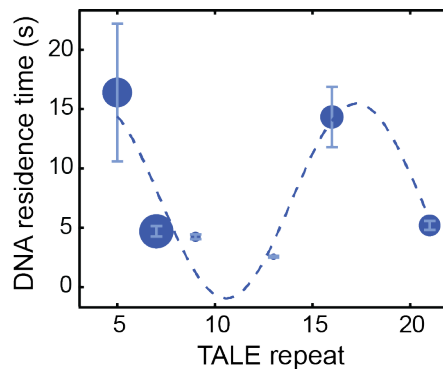


Figure S4. DNA residence times of GRcXR. GRcXR constructs are ordered by an increasing number of repeat domains. The dashed line is a sinusoidal function as guide to the eye. Error bars denote s.d.

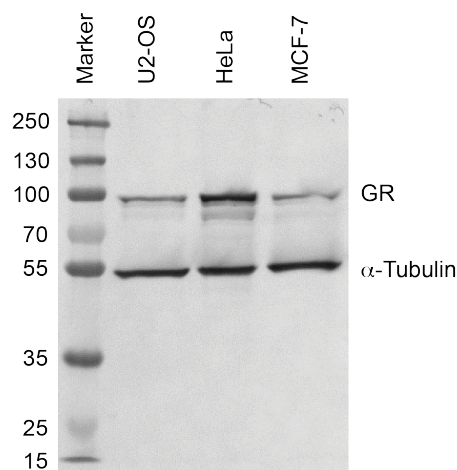


Figure S5. Western Blot of GR expression. Comparison of GR expression between U2-OS cells (lane 2), HeLa cells (lane 3) and MCF-7 cells (lane 4). Lane 1: PageRuler Plus Prestained Protein Ladder (ThermoFisher).

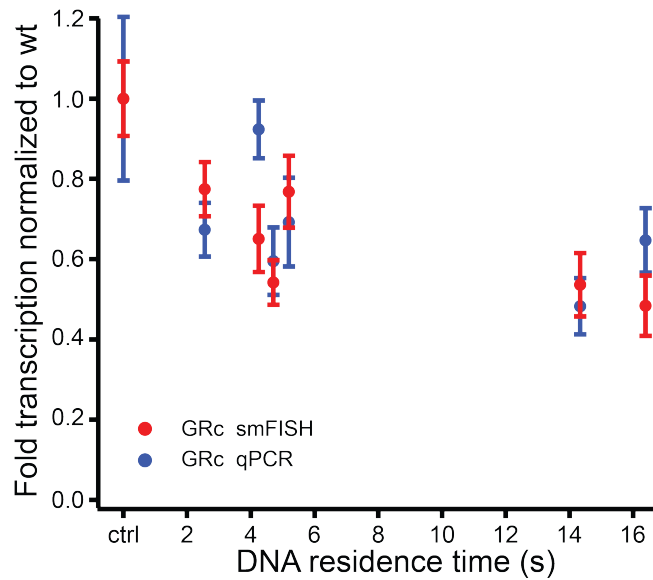


Figure S6. Comparison of fold transcription of *SGK1* as function of GRcXR DNA residence time between smFISH and qPCR experiments for wild type U2-OS cells (ctrl) and U2-OS cells expressing GRcXR. The fold change in transcription is normalized to the value in wild type U2-OS cells. High *SGK1* transcription at ~ 4 s and ~ 16 s in qPCR experiments might be due to a fraction of cells that lost GRcXR expression and thus do not repress *SGK1* transcription. In smFISH experiments, expression of GRcXR in individual cells could be directly controlled via fluorescence measurements. Error bars denote s.e.m. (smFISH) or s.d. (qPCR).

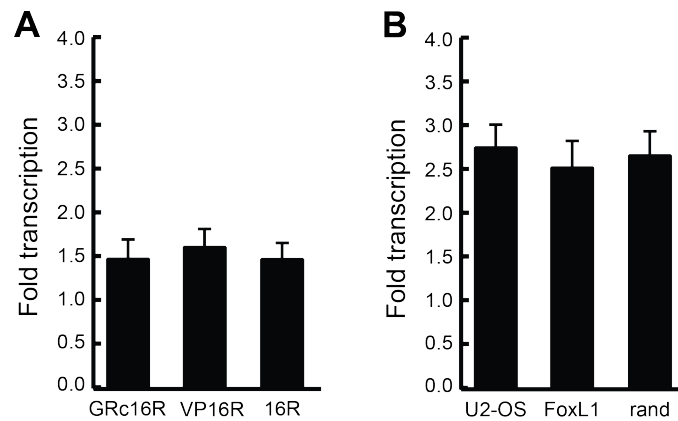


Figure S7. Comparison of GRcXR mediated *SGK1* repression with control constructs. Fold transcription was calculated as ratio between the mean number of mRNA molecules determined by smFISH upon Dex induction and in absence of Dex. **(A)** Positive control of *SGK1* repression by GRcXR: fold transcription of cells expressing GRc16R (regulatory domain AF-2, 270/211 cells), VP16R (regulatory domain VP64, 172/170 cells) and 16R (no regulatory domain, 129/155 cells). Error bars denote s.e.m. **(B)** Negative control: fold transcription of wild type U2-OS cells (U2-OS, 308/304 cells), cells expressing a TALE-TF targeting either the *FoxL1* promoter region (FoxL1, 134/158 cells) or a random sequence (rand, 279/263 cells). Error bars denote s.e.m.

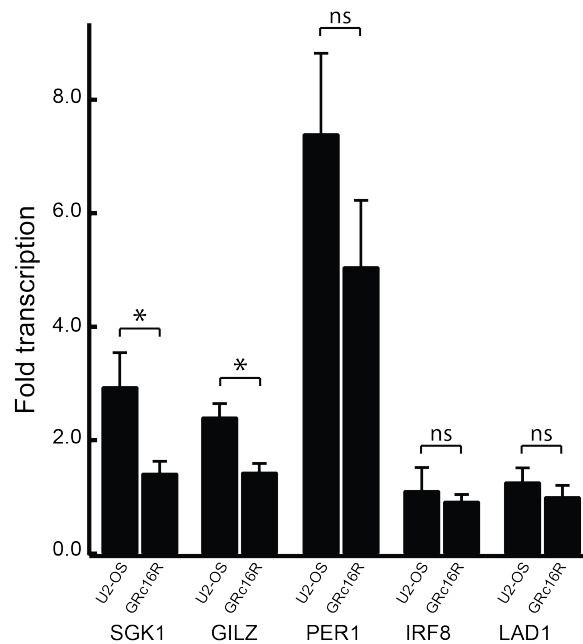


Figure S8. Effect of GRc16R on repression of GR genes containing the consensus GR half-site. Fold change of transcription of several GR genes from qPCR experiments (triplicates on at least 3 biological replicates) in wild type U2-OS cells and cells expressing GRc16R. GRc16R targets a GRE in the promoter region of *SGK1*, which is partially homologous to GREs of the other genes. *IRF8* and *LAD1* were not significantly activated in our experimental conditions. (*) indicates a significant difference according to Student's t-Test, two-tailed, $p < 0.05$. (ns) not significant. Error bars denote s.e.m.

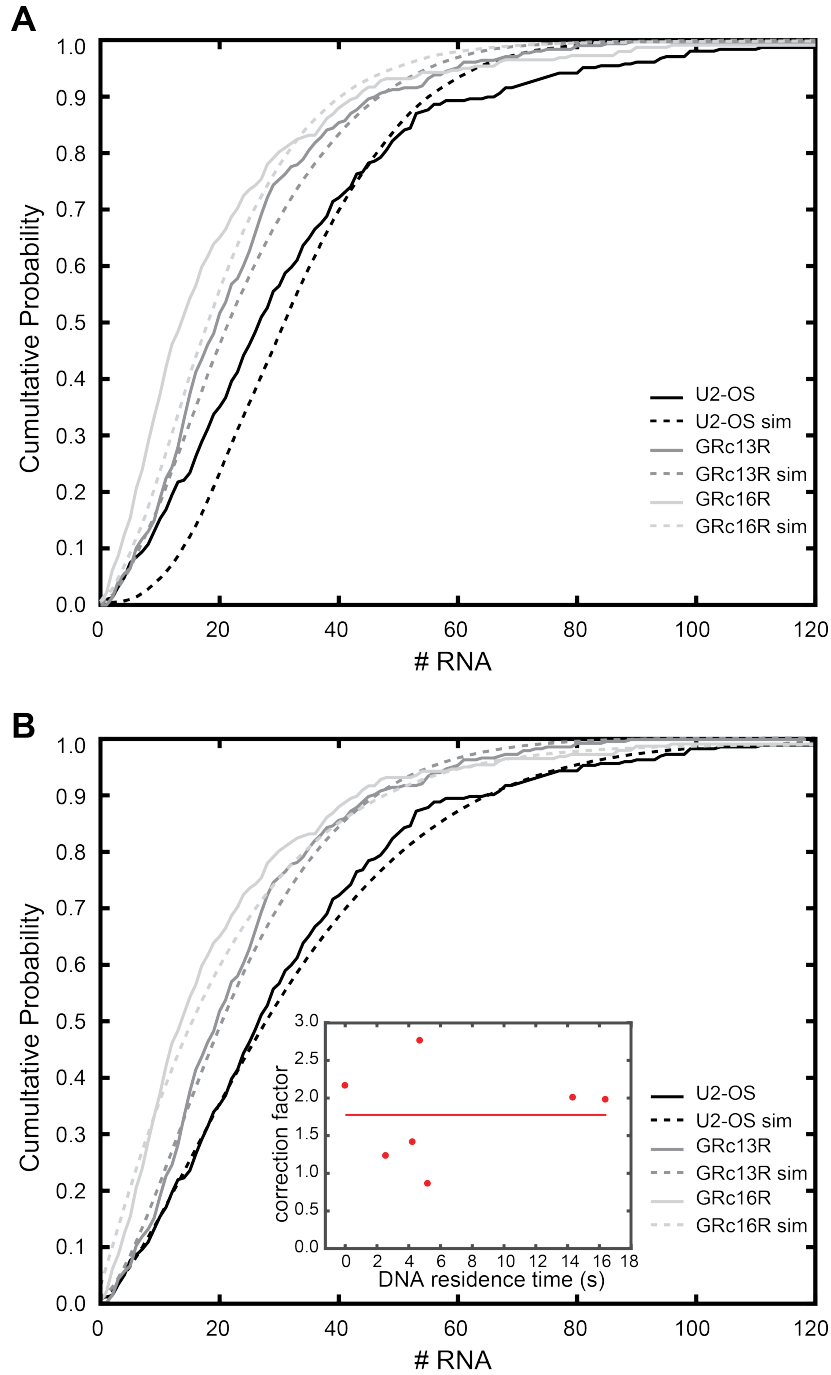


Figure S9. Comparison between measured and simulated mRNA distributions. **(A)** mRNA distributions of wild type U2-OS cells and cells expressing GRc13R or GRc16R simulated using the two-state model and the mean number of *SGK1* transcripts, the burst frequency and the burst size from smFISH measurements. **(B)** mRNA distributions of wild type U2-OS cells and cells expressing GRc13R or GRc16R simulated using the two-state model and the mean number of *SGK1* transcripts and the burst frequency from smFISH measurements, and optimized for the burst size by varying this parameter. *Inset:* correction factor (optimized divided by measured burst size) for all GRcXR. The red line indicates the mean correction factor.

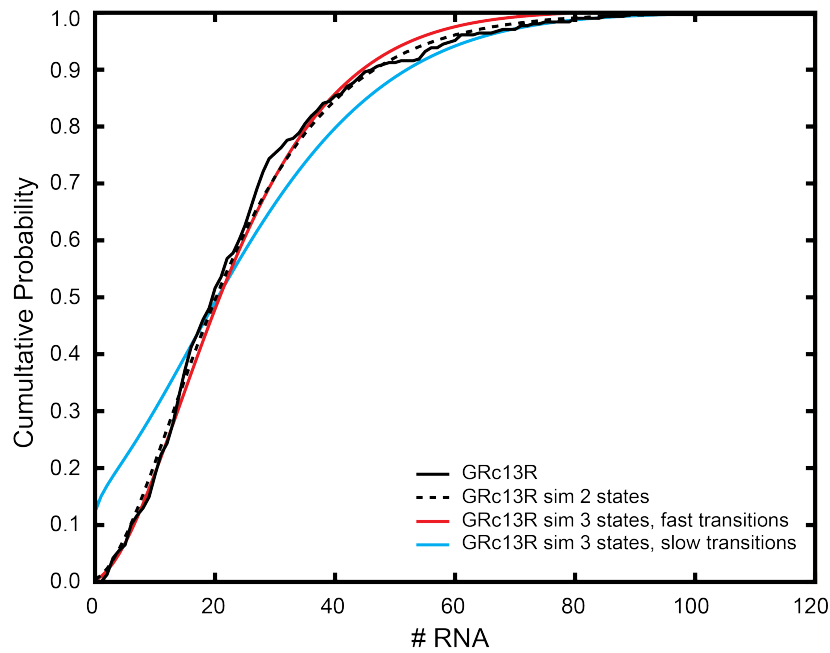


Figure S10. Comparison of mRNA distributions simulated using the two-state model (2 states) and the extended two-state model (3 states). mRNA distributions of cells expressing GRC13R were simulated using the mean number of *SGK1* transcripts and the burst frequency from smFISH measurements, and the optimized burst size (Supplementary Figure S8) based on the two-state model (dashed black line), based on the extended two-state model with fast transitions between blocked and off state of the gene (red line) and based on the extended two-state model with slow transitions between blocked and off state of the gene (blue line). The measured mRNA distribution of GRC13R cells is shown for comparison (black line). The two-state model and the extended two-state model converge independently of the transition parameters between blocked state and off state of the gene if these transitions are much faster than transitions between the off state and the on state of the gene. Consistently, the two-state model and the extended two-state model with fast transitions yield virtually indistinguishable distributions, while the extended two-state model with slow transitions does not well describe the data.

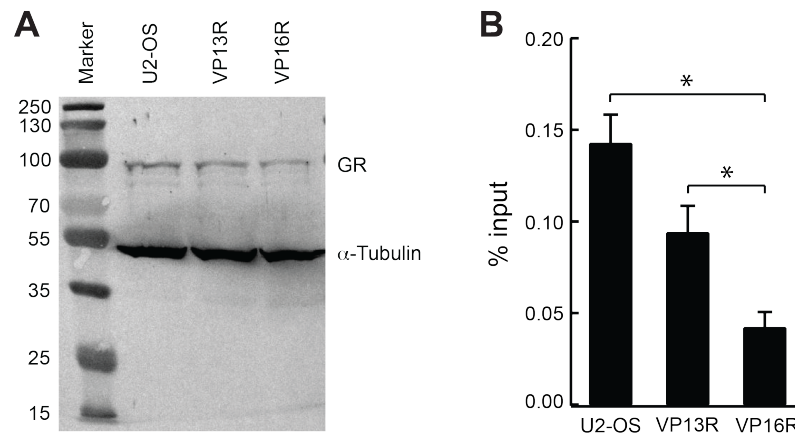


Figure S11. VPXR competes with endogenous GR in binding to *SGK1*. **(A)** Western Blot of GR expression. Comparison of GR expression between wildtype U2-OS cells (lane 2), U2-OS cells expressing VP13R (lane 3) and VP16R (lane 4). Lane 1: PageRuler Plus Prestained Protein Ladder (ThermoFisher). **(B)** ChIP-qPCR measurements of GR occupancy at the *SGK1* promoter in wild type U2-OS cells (triplicates of 2 biological replicates) and in cells expressing VP13R (triplicates of 3 biological replicates) and VP16R (triplicates of 3 biological replicates). The data is normalised to the amount of input DNA. (*) indicates a significant difference according to Student's t-Test, two-tailed, $p < 0.05$. According to this test the difference between U2-OS control and VP13R was not significant and represents a trend. Error bars denote s.d.

Supplementary Tables

Table S1. Target sequences of TALE-TF.

Target gene	TALE-TF	Target sequence
<i>SGK1</i>	GRc5R	TGAGA
<i>SGK1</i>	GRc7R	TGAGAAC
<i>SGK1</i>	GRc9R	TGAGAACAT
<i>SGK1</i>	GRc13R	TCTCTGAGAACAT
<i>SGK1</i>	GRc16R / VP16R / 16R	TAATCTCTGAGAACAT
<i>SGK1</i>	GRc21R	TGAAGTAATCTCTGAGAACAT
<i>FoxL1</i>	GRc16R	TGCTTACAGCGTCAAG
<i>random</i>	GRc15R	TATCAGTGATAGAGA

Table S2. DNA residence times of GRcXR.

TALE-TF	DNA residence time (s)*		Transient fraction*
GRc5R	0.10 ± 0.01	16.39 ± 5.81	0.72 ± 0.04
GRc7R	0.25 ± 0.01	4.70 ± 0.44	0.62 ± 0.03
GRc9R	0.28 ± 0.01	4.24 ± 0.21	0.97 ± 0.01
GRc13R	0.26 ± 0.01	2.56 ± 0.08	0.99 ± 0.01
GRc16R	0.14 ± 0.01	14.33 ± 2.54	0.83 ± 0.03
GRc21R	0.18 ± 0.01	5.20 ± 0.37	0.85 ± 0.01

* Errors represent s.d.

Supplementary Movies

Movie S1. GRc16R labeled with HaloTag-TMR ligand, expressed in a U2-OS cell in the presence of 1 μ M Dexamethasone. TMR was excited with a 561 nm laser. The movie shows 100 frames taken with 50 ms camera integration time at 5 Hz.

References

1. Hebbar, P.B. and Archer, T.K. (2007) Chromatin-dependent cooperativity between site-specific transcription factors in vivo. *Journal of Biological Chemistry*, **282**, 8284–8291.
2. Barish, G.D., Yu, R.T., Karunasiri, M., Ocampo, C.B., Dixon, J., Benner, C., Dent, A.L., Tangirala, R.K. and Evans, R.M. (2010) Bcl-6 and NF- κ B cistromes mediate opposing regulation of the innate immune response. *Genes & Development*, **24**, 2760–2765.
3. Gebhardt, J.C.M., Suter, D.M., Roy, R., Zhao, Z.W., Chapman, A.R., Basu, S., Maniatis, T. and Xie, X.S. (2013) Single-molecule imaging of transcription factor binding to DNA in live mammalian cells. *Nature Methods*, **10**, 421–426.
4. Voss, T.C., Schiltz, R.L., Sung, M.-H., Yen, P.M., Stamatoyannopoulos, J.A., Biddie, S.C., Johnson, T.A., Miranda, T.B., John, S. and Hager, G.L. (2011) Dynamic Exchange at Regulatory Elements during Chromatin Remodeling Underlies Assisted Loading Mechanism. *Cell*, 10.1016/j.cell.2011.07.006.