Supporting Information

Control of Protein Function through Optochemical Translocation

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Supporting Figures:

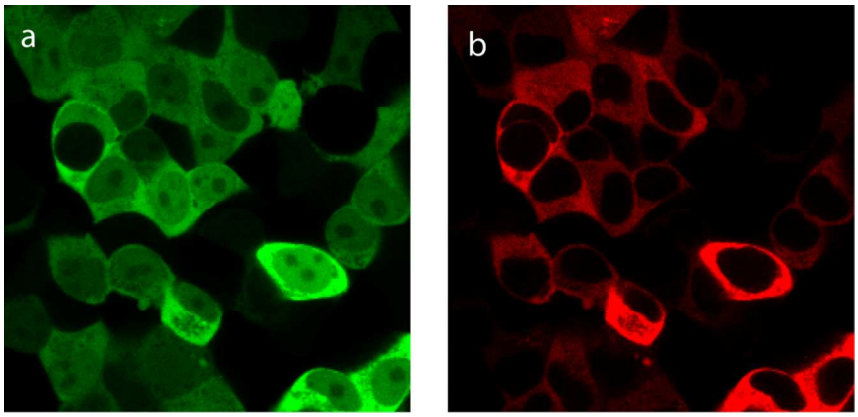


Fig. S1: Suppression efficiency. eGFP-OptoNLS-SATB1-mCherry is expressed in HEK293T cells. (a) eGFP can be found in nucleus and cytoplasm, since it is expressed as a fusion protein to SATB1 upon full amber suppression as well as upon unsuccessful suppression. The latter case results in expression of free eGFP, which is small enough to enter the nucleus via diffusion, and thus can be found in nucleus as well as in cytoplasm. The cytoplasmic eGFP signal is stronger than the nuclear signal, since it consists of free eGFP and eGFP fused to SATB1. The suppression efficiency, determined as the ratio of the difference of cytoplasmic and nuclear intensity versus cytoplasmic intensity, $(I_c-I_n)/I_c$, is about 14%. (b) mCherry is expressed only as a fusion to SATB1 upon full amber suppression and its signal shows the restriction of eGFP-OptoNLS-SATB1-mCherry to the cytoplasm.

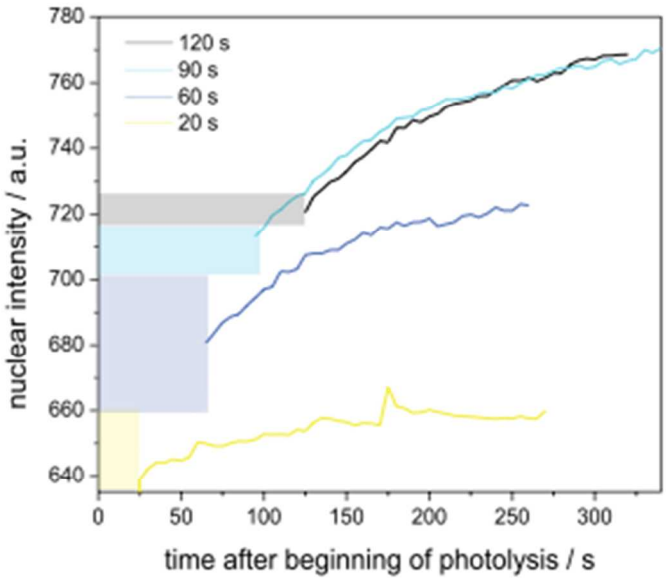


Fig. S2: FOXO3 import. Nuclear intensity of OptoNLS-FOXO3-mCherry versus time for different illumination times. With increasing illumination time, FOXO3 import rates into the nucleus are faster, the level of nuclear protein at saturation is higher and the time till saturation is longer. After about 5 min FOXO3 import has reached saturation for all illumination times. At 90s illumination time, a maximum of efficiency in nuclear entry seems to be reached. An increase of illumination time to 120s does not lead to an increase in import signal.

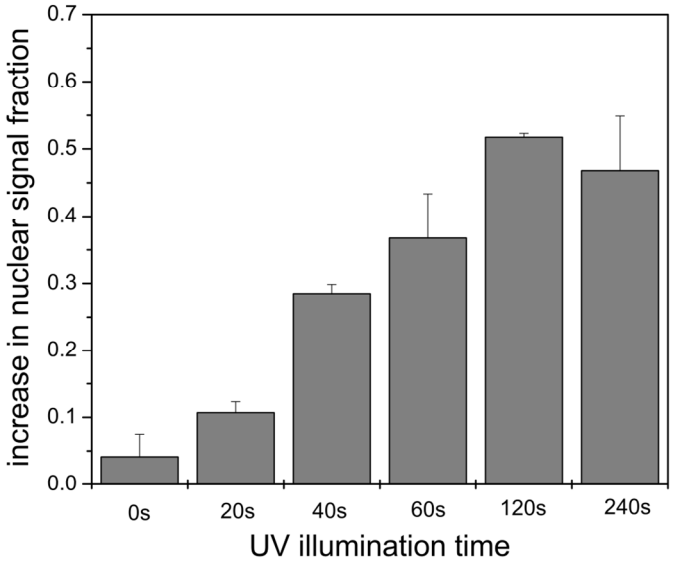


Fig. S3: Dose dependence of the optically gated nuclear import. Difference of nuclear intensity of eGFP-OptoNLS-SATB1-mCherry before illumination and 7 h after.

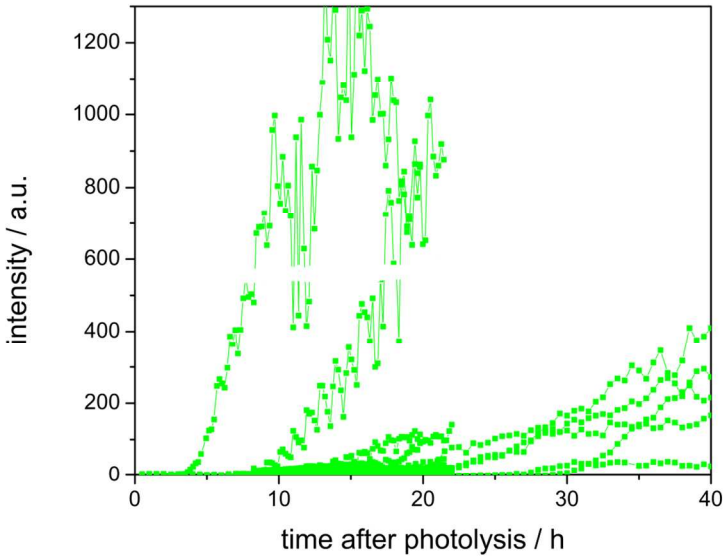
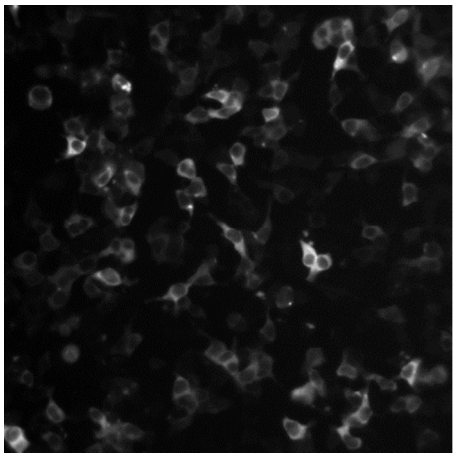
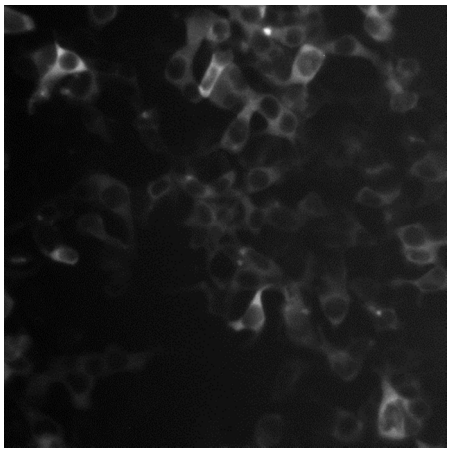


Fig. S4: Transcriptional activation of a FOXO3 driven GFP reporter upon optically triggered nuclear import of OptoNLS-FOXO3-mCherry. Intensity vs. time traces of GFP reporter signal of 6 experiments and 4 transfections.

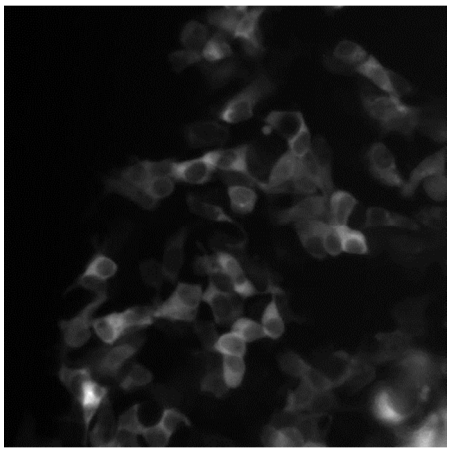
Supporting Movies:



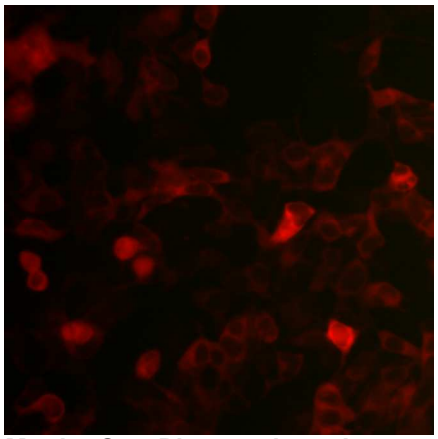
Movie S1: Photocontrolled OptoNLS-SATB1 import. OptoNLS-SATB1 was expressed in HEK293T cells. Right before the start of the movie, cells were illuminated with blue light for 60 s. The movie shows successful optically controlled SATB1-import in the mCherry channel. The movie covers 4.5 h and images were taken every 27 min.



Movie S2: Control experiment of photocontrolled OptoNLS-SATB1 import. OptoNLS-SATB1 was expressed in HEK293T cells. The movie shows the mCherry signal of cells from the same dish and experiment as Movie S2, just a different region, which was not illuminated with blue light before imaging. Images were taken at the same time and over the same period of time as those in Movie S1. This control shows no import and confirms that the import shown in Movie S1 was induced by UV illumination.



Movie S3: FOXO3 import. OptoNLS-FOXO3-mCherry was expressed in HEK293T cells. Cells were illuminated with blue light for 90 s right before imaging started. The movie shows successful optically triggered import of FOXO3 and runs over 4 min with an image every 5 s.



Movie S4: Photoactivated transcription. OptoNLS-FOXO3-mCherry and the FOXO3 driven GFP reporter were expressed in HEK293T cells. The red signal shows the mCherry signal of OptoNLS-FOXO3-mCherry and the green signal shows the GFP-reporter. Two frames were imaged before illumination with blue light. The illumination is marked by a red image. The images following illumination were acquired starting 1 h after illumination, at a rate of one image per 30 min, over a period of 41 h. The movie shows successful nuclear import of FOXO3 followed by GFP expression.

Supporting Text

To quantify the modulation depth of the OptoNLS system, we determined the nuclear signal fraction of the SATB1, OptoNLS-SATB1 and OptoNLS-FOXO3 constructs. The nuclear signal fraction quantifies the degree of nuclear exclusion or enrichment via the ratio of the nuclear intensity (I_n) over the total signal, $I_n / (I_n + I_c)$, where I_c is the cytoplasmic intensity and both I_n and I_c have been background corrected (see Supporting Methods below). A value of 0 corresponds to complete nuclear exclusion, a value of 0.5 to equilibration, and a value of 1 corresponds to complete nuclear localization. The loss of function SATB1 mutant, Lys29→Ala, has a nuclear signal fraction of 0.037 ± 0.037 in HEK293T cells, whereas the positive control (eGFP-SATB1) had a nuclear signal fraction of 0.950 ± 0.087, corresponding to a 25-fold enrichment of nuclear localization. Similarly, the OptoNLS-SATB1 had a nuclear signal fraction of 0.049 ± 0.026 before illumination and of 0.84 ± 0.15 seven hours post 120 UV-illumination. The OptoNLS-FOXO3 had a nuclear signal fraction of 0.06 ± 0.03 before and of 0.64 ± 0.16 after irradiation, resulting in a 10-fold enrichment of nuclear FOXO3.

Supporting Methods:

Calculation of nuclear signal fraction: The intensities in nucleus and cytoplasm for each cell were measured using ImageJ. Additionally, a mean background intensity resulting from areas without fluorescent cells was determined for each image. The mean background intensity was subtracted from the nucleus and cytoplasm intensities of each cell in the corresponding image to correct for background illumination. When the background corrected intensities were less than zero, they were set to zero, since negative intensities don't exist. The resulting background corrected intensities for nucleus I_n and cytoplasm I_c were used to calculate the nuclear signal fraction f according to:

$$f = \frac{I_n}{I_n + I_c}$$

Data given in the main text are averaged over 40-100 cells from at least 3 different trials of illumination and subsequent imaging and the error is the standard deviation.

Cloning. The PCKRS-PyltRNA plasmid was constructed from PCKRS and PyltRNA plasmids using NheI-MfeI and SpeI-EcoRI, respectively. The two fragments were then re-ligated. Mutants of Lys29 in the SATB1 NLS were generated with via QuikChange, introducing either the TAG or alanine codon. GFP-SATB1 was a gift from the T. Kowhi-Shigematsu lab. FOXO3 (Addgene plasmid 14937) was obtained from the J. Massagué lab. eGFP-OptoNLS-mCherry, OptoNLS-FOXO3-mCherry, OptoNLS-TEV-CFP-CFP and GFP-SATB1-mCherry with a TEV cleavage site in a Cumate expression vector were generated using the SLIC technique.

Transfection and tissue culture. Hek 293T cells were grown in DMEM with 10% FBS and plated on 35 mm dishes (MatTek corporation). Transfection was performed with Lipofectamine (Invitrogen) according to manufacturer's instructions using 4 µg of each plasmid required for the specific experiment. After transfection caged lysine was added yielding a final concentration of 2 mM. Cells were incubated for 24 h until imaging. For the TEV cleavage experiment, MCF10A cells stably selected for the Cumate inducible expression system (System Biosciences) were grown in growth medium and transfected using the neon electroporator (Invitrogen) with 3 µg of each plasmid. Cumate was added for the first 10 h to allow for SATB1 expression. After ~10 h, caged lysine was added yielding a final concentration of 2 mM to allow for TEV expression and Cumate was removed to stop SATB1 expression and avoid premature cleavage in the cytoplasm before import into the nucleus. Cells were then incubated for another ~6 h. Before imaging, in all experiments medium was replaced with DMEM containing no caged lysine. Nuclear import of the FOXO3 transcription factor was monitored with the Cignal FOXO GFP Reporter system (CCS-6022, www.sabiosciences.com) which consists of the GFP gene under the control of a minimal (m)CMV promoter and tandem repeats of the FOXO transcriptional response element.

Imaging and analysis. Confocal imaging was performed on an Axiovert 700 (Zeiss) using 488 and 555 nm lasers and a 63x oil immersion objective (NA = 1.4, Zeiss). Timelapse imaging and photolysis was performed on an inverted epifluorescence microscope (Olympus, IX81) at an intensity of about 50 µW in the back aperture of the objective. Photorelease was performed using a 20x objective (Olympus, AchN) and a DAPI filter (Ex: 350/50 nm, Chroma) and imaging was conducted with a GFP filter (Ex: 470/40 nm, Em: 525/50 nm, Chroma) and an mCherry filter (Ex: 560/40 nm, Em: 630/75 nm, Chroma). Image analysis was performed using ImageJ and Matlab.