Electronic supplementary information

for

Nongenetic Control of Receptor Signaling Dynamics with a DNA-based Optochemical Tool

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1. Supporting figures

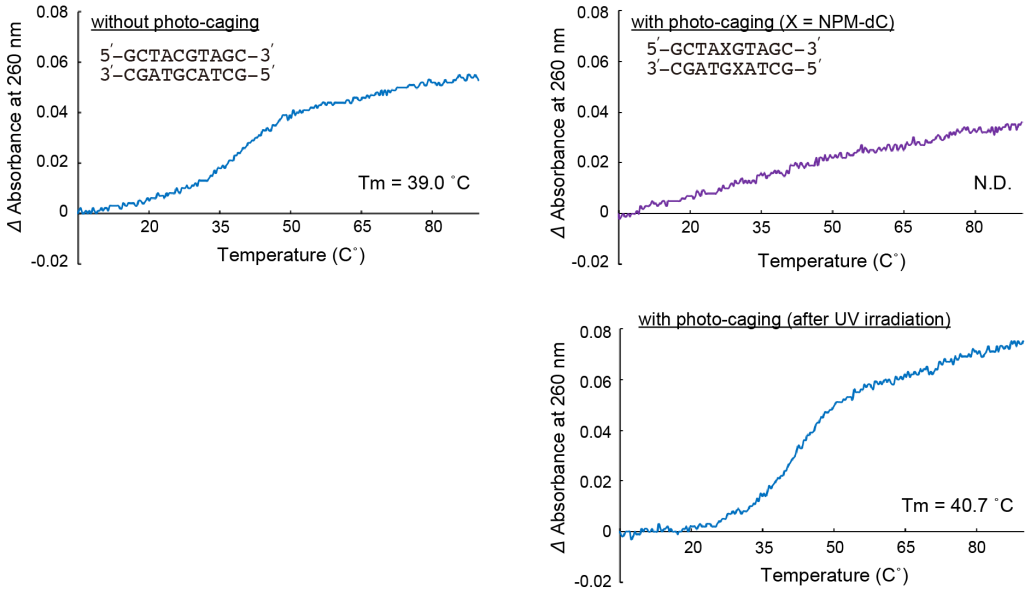

Fig. S1 Melting curves of the linker sequence of Apt (2.5 μM, top left) or cApt (2.5 μM, top right) in DPBS. The linker sequence of cApt was irradiated with 365 nm UV light (8 mW, 60 s) and used for the melting curve analysis (bottom, right).

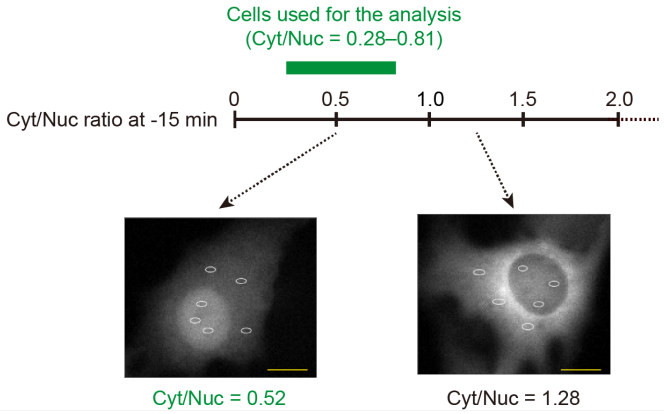

Fig. S2 Representative fluorescence images showing expression of Erk-KTR-Clover in HeLa cells. The cytoplasmic versus nuclear fluorescence of the Erk-KTR-Clover (Cyt/Nuc ratio) in each cell was calculated using average nuclear or cytoplasmic fluorescent intensities, determined from three randomly selected ROIs (white circles) in a background-subtracted image. To limit the effects of variability in basal Erk activity of each cell, the cells with Cyt/Nuc ratio between 0.28 and 0.81 at the beginning of time-lapse imaging (t = -15 min) were randomly selected and used for the analysis. Scale bars: 10 μm.

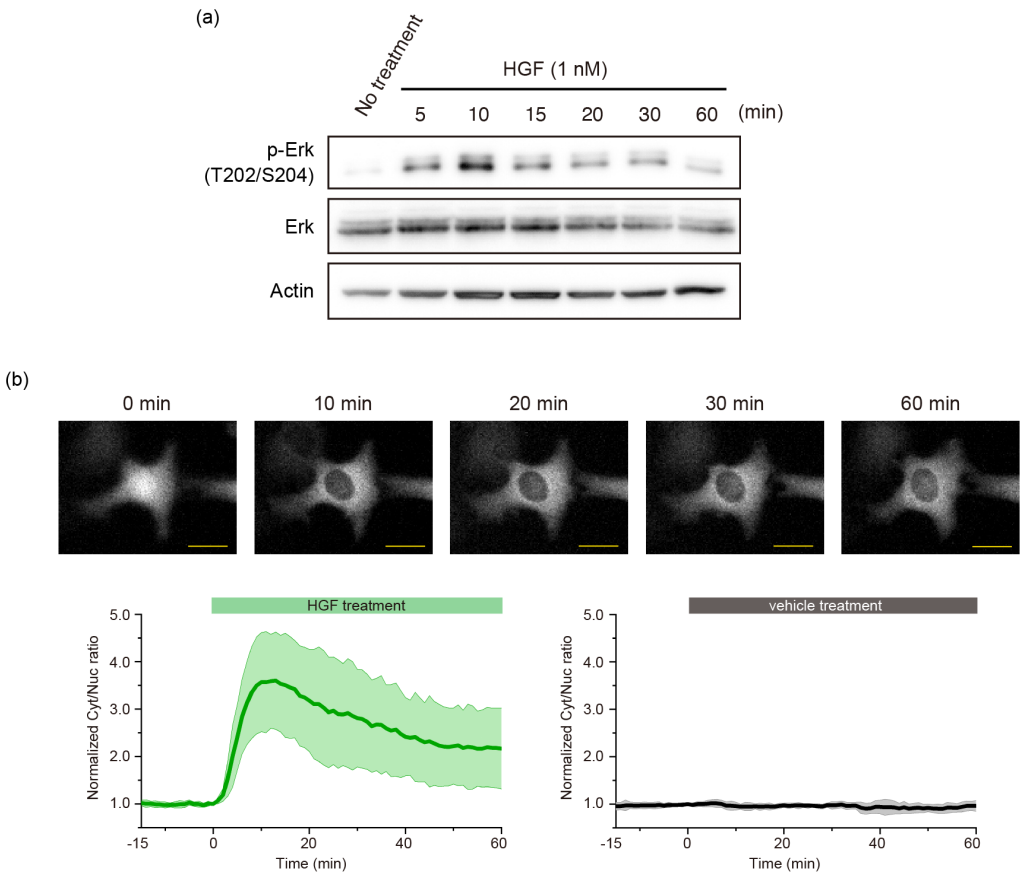

Fig. S3 (a) Immunoblotting analysis of Erk phosphorylation kinetics in HeLa cells, treated with HGF (1 nM). (b) Time-lapse imaging of translocation of Erk-KTR-Clover in HeLa cells, treated with HGF (1 nM) or vehicle control (DPBS) for 60 min. Scale bars: 20 μm. Data are expressed as average ratios of eight cells. The colored areas indicate SD.

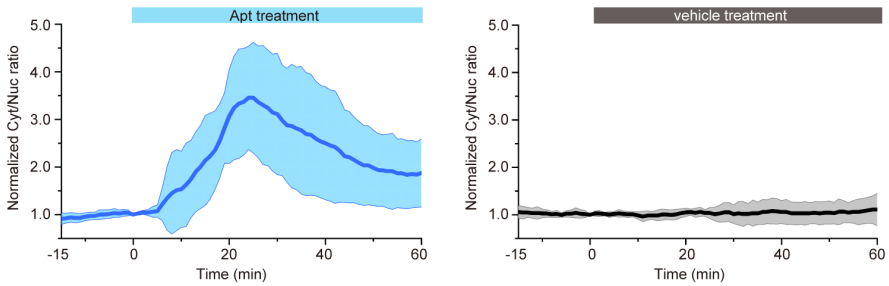

Fig. S4 Normalized ratio of the fluorescent intensity of Erk-KTR-Clover in the cytosol and nucleus. HeLa cells, expressing Erk-KTR-Clover, were treated with Apt (50 nM; monomer concentration) or vehicle control (DPBS) for 60 min. Data are expressed as average ratios of 10 cells. The colored areas indicate SD.

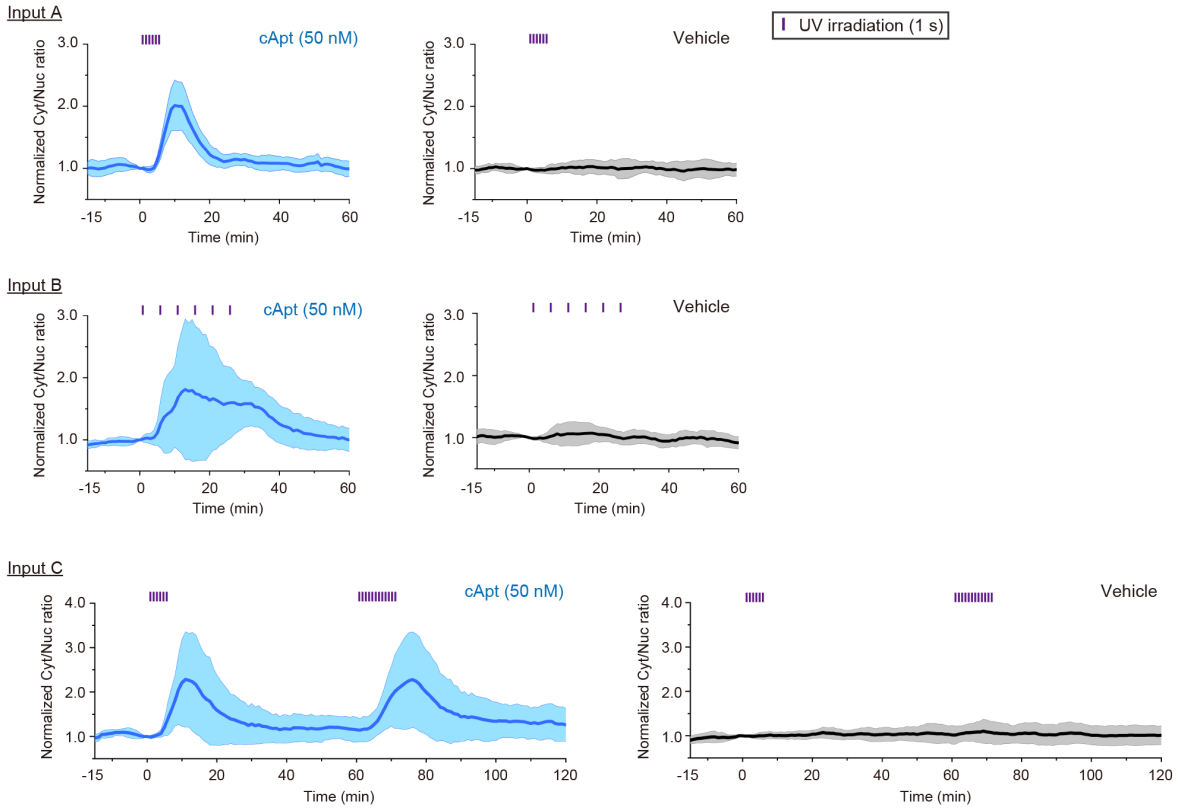

Fig. S5 Normalized ratio of the fluorescent intensity of Erk-KTR-Clover in the cytosol and nucleus. HeLa cells, expressing Erk-KTR-Clover, were incubated in the absence (gray) or presence (blue) of cApt (50 nM; monomer concentration), and a 365-nm peak UV light (0.2 W/cm^2) was irradiated according to the temporal pattern shown in Fig. 4a. Data are expressed as average ratios of 10 cells. The colored areas indicate SD.

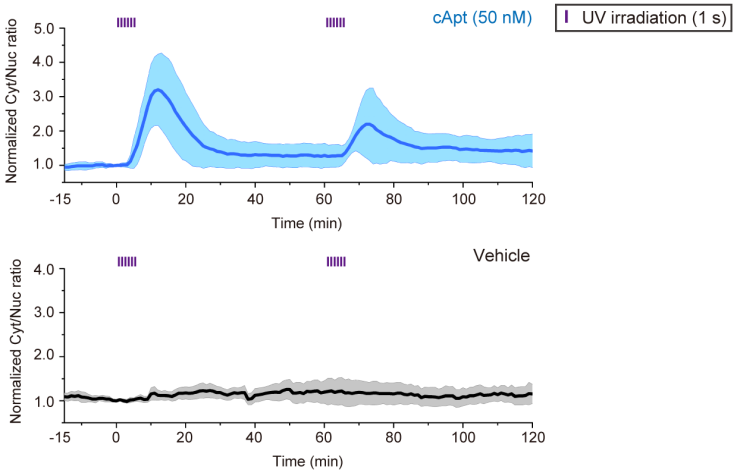

Fig. S6 Normalized ratio of the fluorescent intensity of Erk-KTR-Clover in the cytosol and nucleus. HeLa cells, expressing Erk-KTR-Clover, were incubated in the absence (gray, N = 6) or presence (blue, N = 10) of cApt (50 nM; monomer concentration), and a 365-nm peak UV light (0.2 W/cm^2) was irradiated. The data are expressed as average ratio and the colored areas indicate SD.

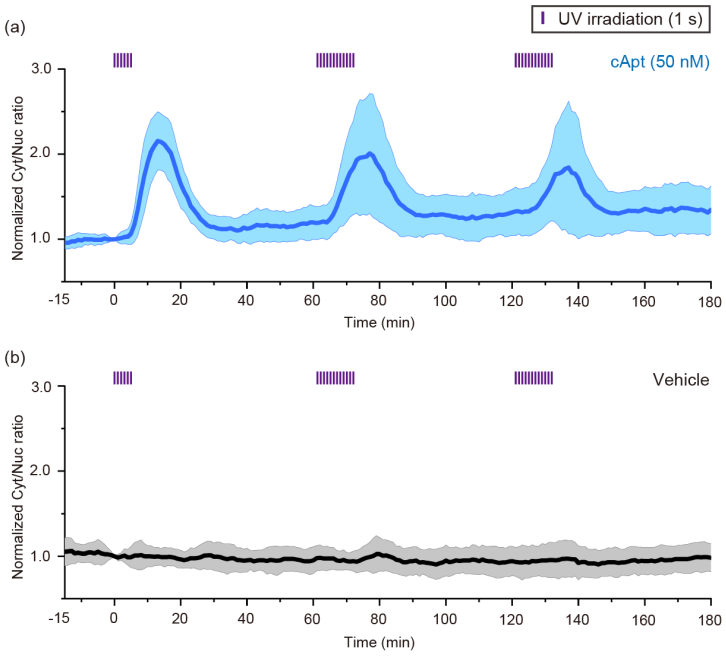

Fig. S7 Normalized ratio of the fluorescent intensity of Erk-KTR-Clover in the cytosol and nucleus. HeLa cells, expressing Erk-KTR-Clover, were incubated in the absence (gray) or presence (blue) of cApt (50 nM; monomer concentration), and repetitive UV irradiations were applied to the cells (purple bars). Data are expressed as average ratios of 10 cells. The colored areas indicate SD.

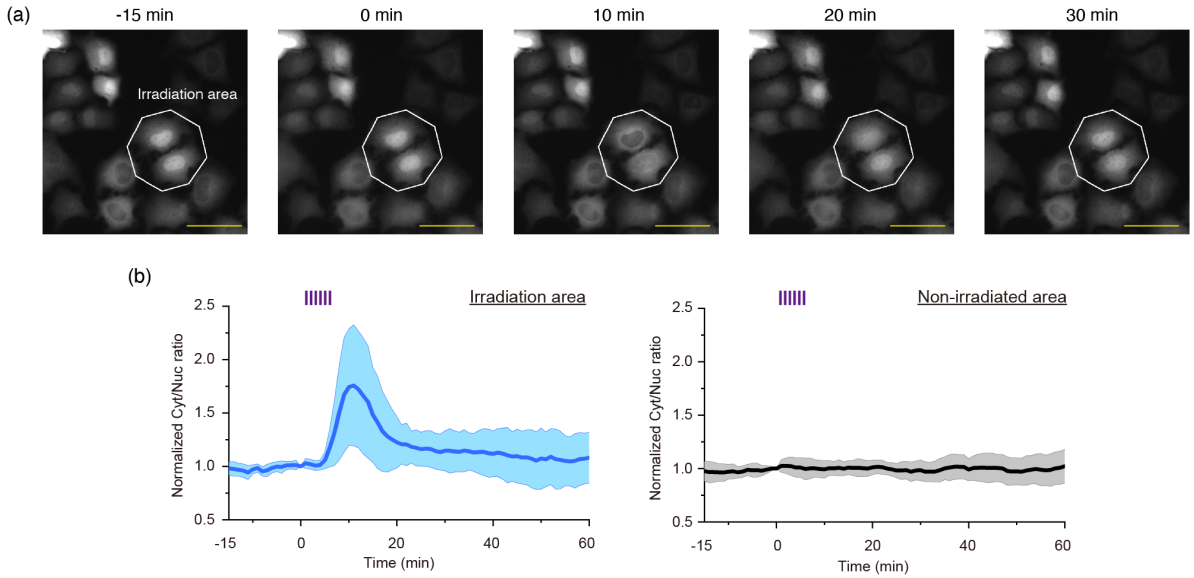

Fig. S8 (a) Representative time-lapse imaging showing translocation of Erk-KTR-Clover. The irradiation pattern A (Fig. 4a) was applied to the octagonal area (white) and the translocation of reporter in the cells, inside or outside of the irradiation area, was monitored. HeLa cells, expressing Erk-KTR-Clover, were incubated in the presence of cApt (50 nM; monomer concentration) Scale bars: 50 μm. (b) Normalized ratio of the fluorescent intensity in the cytosol and nucleus of the cells inside (blue, N = 7) or outside (gray, N = 16) of the irradiation area. The data are expressed as average ratio and the colored areas indicate SD.

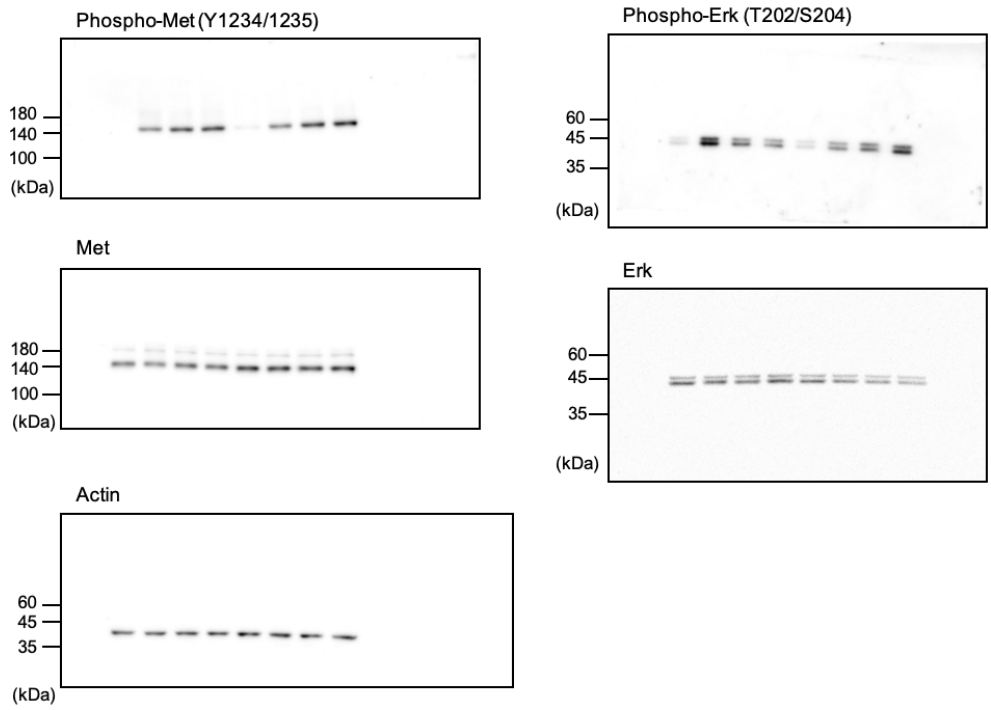

Fig. S9 Uncropped images of Western blotting shown in Fig. 3.

2. Methods

2-1 General information

Reagents were purchased from the standard suppliers and used without further purification. All oligonucleotides were purchased from Fasmac (Kanagawa, Japan) and Gene design (Osaka, Japan) and used without further purification. The cApt sequence was synthesized by Gene design, using 6-nitropiperonyl-α-methyl (NPM)-caged deoxycytidine (dC) phosphoramidite. The synthesized cApt was characterized by ESI-MS after HPLC purification (calcd: 18949.33, found: 18952.20). The NPM-caged dC phosphoramidite was synthesized according to the literature with a slight modification.[1] Refolding and annealing of DNA samples were performed with a thermal cycler. A xenon light source (MAX-301, Asahi Spectra, Tokyo, Japan) was used for uncaging experiments, shown in Fig. 3 and S1. Fluorescent images were obtained with an inverted microscope (IX-81, Olympus, Tokyo, Japan), equipped with a sCMOS camera (Zyla4.2, Andor technology, Belfast, Northern Ireland). The filter cube U-MWIB3 and U-MNU2 (Olympus, Tokyo, Japan) were used for the detection of Erk-KTR-Clover and uncaging of the caged-nucleotide, respectively. Immunoblotting images were obtained with a luminescence detection system (AE-9300H, ATTO, Tokyo, Japan). Absorbance measurements were performed with Infinite M200 pro (Tecan, Bezirk Meilen, Switzerland). UV melting measurement was performed with UV-VIS spectrometer (UV-1650PC, Shimadzu, Kyoto, Japan).

2-2 Cell culture

DU145 cells and HeLa cells were cultured in RPMI1640 supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic. All cells were maintained at 5% CO_2 in a humidified incubator at 37 °C.

2-3 Preparation of cell lysates

The cells were seeded onto 35 mm dishes and cultured overnight at 5% CO_2 in a humidified incubator at 37 °C. The cells were then starved for 24 h in RPMI1640 without FBS. After the starvation, the medium was replaced, and the cells were stimulated with an appropriate ligand for 15 min at 37 °C. The ligand solution was pre-warmed at 37˚C just before the addition to the medium. The UV light was irradiated to the ligand solution that was kept at 37˚C on the thermal cycler for an appropriate time. Next, the cells were

washed twice with DPBS and lysed with a lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mM AEBSF, 1 μg/mL leupeptin, 1% Triton X-100, and 10% glycerol). The cell lysates were centrifuged at 10,000–11,000 × g for 20 min and then the supernatants were recovered. The total protein concentration of each cell lysate was measured with the standard BCA assay after the dilution.

2-4 Immunoblotting assay

The cell lysates were separated by SDS-PAGE and then transferred to a PVDF membrane. The membrane was incubated with a primary antibody at 4 °C overnight, following the incubation with an appropriate secondary antibody at room temperature for 1 h. The primary antibodies for phospho-Met (3077), Met (8198), phospho-Erk1/2 (4370), Erk1/2 (4695, Fig. 3) and beta-actin (4967) were obtained from Cell Signaling Technology (Danvers, USA). The primary antibody for Erk1/2 (sc-514302, Fig. S3) was obtained from Santa Cruz Biotechnology (Dallas, USA). The Anti-Rabbit Immunoglobulins/HRP secondary antibody was obtained from Agilent Technologies (P0488, Santa Clara, USA). The Anti-Mouse Immunoglobulins/HRP secondary antibody (W4021) was obtained from Promega (Madison, USA). The membranes were probed using ImmunoStar LD (296-69901, FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan) or Immobilon Western Chemiluminescent HRP Substrate (WBKLS0500, Merck Millipore, Burlington, USA).

2-5 UV melting measurement

UV melting measurement was performed with UV-VIS spectrometer (UV-1650PC, Shimadzu, Kyoto, Japan) over a temperature of 5–90 °C (heat rate = 0.5 °C /min) using a quartz cuvette. DNA (2.5 μM) was dissolved in Dulbecco's phosphate-buffered saline and refolded with a thermal cycler (95 °C for 5 min, and then cooled to 4 °C) before measurements. The UV melting curve was obtained by subtracting the background absorbance at 320 nm from the absorbance at 260 nm. Two independent experiments were performed and the mean Tm values were indicated.

2-6 Preparation of Erk-KTR-Clover expressing cells

An episomal vector encoding Erk-KTR-Clover was constructed for time-lapse imaging of Erk-KTR-Clover translocation. The Erk-KTR-Clover sequence was amplified from pENTR-ERKKTR Clover plasmid (Addgene 59138) and inserted into pEBMulti-Puro vector. The sequence of the constructed vector was confirmed by Sanger sequencing. HeLa cells were seeded at 60 mm-plates and cultured in RPMI1640 supplemented with 10% FBS overnight. On the day of transfection, the cells were washed twice with DPBS and incubated with DNA/PEI mixture in RPMI1640 supplemented with 10% FBS overnight. To enrich Erk-KTR-Clover expressing cells, drug selection (1 mg/ml puromycin in RPMI1640 supplemented with 10% FBS) was started on the next day after the transfection. The cells were used for time-lapse imaging of Erk-KTR-Clover translocation at least 2 days after the drug selection.

2-7 Time-lapse imaging of Erk-KTR-Clover translocation

The Erk-KTR-Clover-expressing HeLa cells were seeded onto a chambered coverglass (8-wells, AGC Techno Glass, Tokyo, Japan) and cultured in RPMI1640 supplemented with 10% FBS. After the cells adhered to the coverglass, they were incubated in a starving medium (RPMI1640 without phenol red) overnight. On the day of experiment, the media was replaced and the chambered coverglass was mounted on a stage top incubator (INUG2F-IX3W, Tokai Hit, Shizuoka, Japan). The cells were incubated at 5% CO_2 in a humidified atmosphere at 37 °C during the experiment. For uncaging experiments, the cells were pre-incubated in the presence or absence of cApt (50 nM; monomer concentration) in the starving medium at least for 30 min. Time-lapse images were captured in 1 min intervals. The filter cubes U-MWIB3 and U-MNU2 (Olympus, Tokyo, Japan) were used for detection of Erk-KTR-Clover and for photo-deprotection of the caged-nucleotide, respectively. The Iris diaphragm was used for local UV irradiation. The location of UV irradiation at the sample plane was checked by observing Erk-KTR-Clover-expressing HeLa cells, cultured on the chambered coverglass in a high confluency. Both global and local irradiations were performed for the indicated duration while conducting time-lapse imaging. The cytoplasmic versus nuclear fluorescence of the Erk-KTR-Clover (Cyt/Nuc ratio) in each cell was calculated using average nuclear or cytoplasmic fluorescent intensities, determined from three randomly selected ROIs in a background-subtracted image. To limit the effects of variability in basal Erk activity of each cell, the cells with Cyt/Nuc

ratio between 0.28 and 0.81 at the beginning of time-lapse imaging (t = -15 min) were randomly selected and used for the analysis. The image analysis was conducted using Image J software (http://rsbweb.nih.gov/ij/).

3. Sequence data

Apt: CGA TCG ATG GAT GGT AGC TCG GTC GGG GTG GGT GGG TTG GCA ATC GAT CGG CTAC GTA GC

cApt: CGA TCG ATG GAT GGT AGC TCG GTC GGG GTG GGT GGG TTG GCA ATC GAT CGG CTAX GTA GC

(X = NPM-caged deoxycytidine. Hybridization linker sequences are highlighted in red)

4. Reference

1. J. M. Govan, R. Uprety, J. Hemphill, M. O. Lively and A. Deiters, *ACS Chem. Biol.,* **2012**, *7*, 1247.