Electronic supplementary information (ESI) for

A Self-Reporting Chemically Induced Protein Proximity System Based on a Malachite Green Derivative and the L5** Fluorogen Activating Protein

Guihua Zeng,[1] Yi Wang,[2] Marcel Bruchez,[2] and Fu-Sen Liang[1,a]

[1] Department of Chemistry and Chemical Biology, University of New Mexico, Albuquerque, NM87131, USA. [2] Department of Chemistry, Department of Biological Sciences, and Molecular Biosensors and Imaging Center, Carnegie Mellon University, Pittsburgh, PA15213, USA.

Supporting Figures

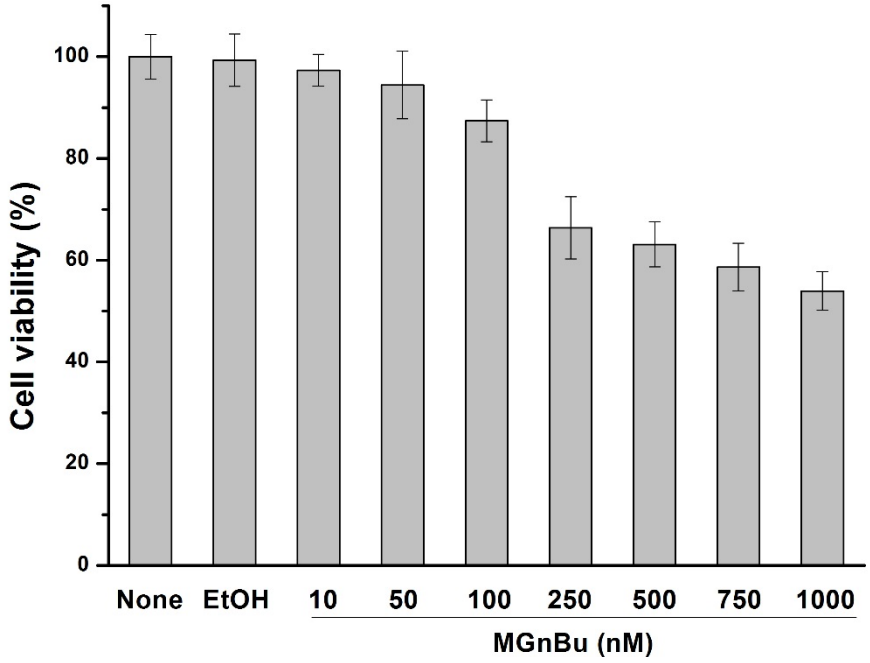

Figure S1. Cytotoxicity of MGnBu tested using MTT assays. HEK293T cells were plated in 96-well plate for 24 hour and then treated with either EtOH (0.5% v/v) or 10 to 1000 nM MGnBu, or non-treatment for another 24 h, The viability of cells was quantified by MTT assay. For MTT assay, the medium in each well was removed and replaced with 100 μL of fresh phenol free medium and treated with 10 μL of the 12 mM MTT stock solution for 4 hours at 37°C. Then removed 85 μL of the medium from each well and added 50 μL of DMSO. After 10 min incubation at 37°C, mixed each sample again and read the absorbance at 540 nM. The absorbance was normalized to non-treatment group.

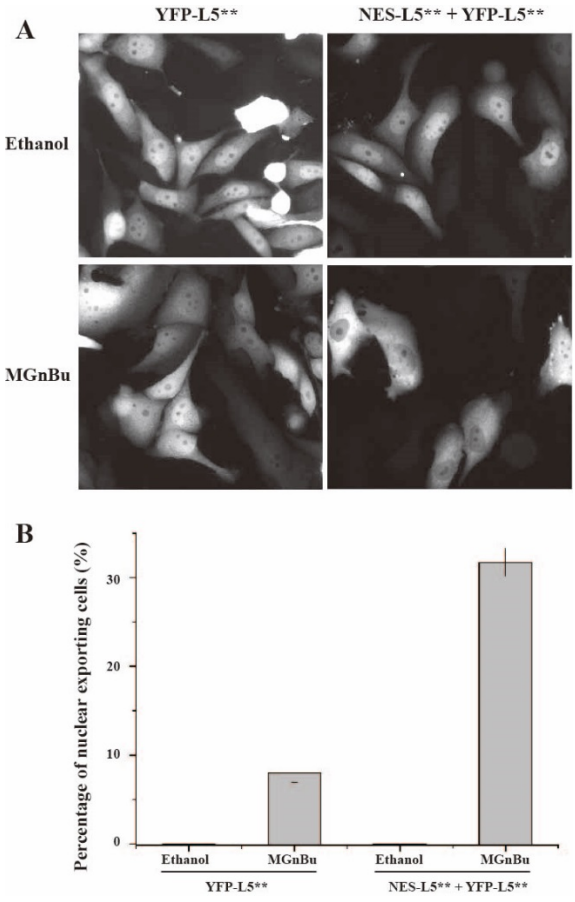

Figure S2. Analysis of nuclear exclusion of dimerized EYFP-L5.** (A) Representative images for CHO cells transfected with either SV40-EYFP-L5** only or SV40-EYFP-L5** plus SV40-NES-L5** for 24 h, followed by ethanol or 500 nM MGnBu treatment for another 24 h. (B) Quantified data for the conditions in (A).

Experimental Section

Mammalian cell culture and transfection

All cells were cultured in DMEM medium (Gibco) supplemented with 10% FBS, 2 mM GlutaMAX (life technologies), 100 U/ml penicillin (life technologies) and 100 μg/ml streptomycin (life technologies) at 37℃ in a humidified atmosphere containing 5% CO_2.

Fluorescence micro plate reader quantification and luciferase activity quantification. (1) For compounds screening and dosage dependent experiments, HEK293T cells were seeded in 24-well plate at 100,000/well for 24 h and then transfected with 0.4 μg of Actin-VP-L5**-ires-Gal-L5** & 0.2 μg of 5xUAS-luciferase and waited for another 24 h. Then, cells were treated with ethanol or 500 nM of MG or MGnBu, or treated with 0 nM to 1000 nM of MGnBu. After 24 h, cells were harvested and washed with PBS twice, and resulting cells were re-suspended in 100 μL of PBS and fluorescence was quantified by fluorescent plate reader (ex/em 640 nm/670 nm). Meanwhile, cells samples with the same conditions were harvested and quantified with the luciferase activity. (2) For experiments testing orthogonality with different CIP systems, HEK293T cells were seeded in 24-well plate at 100,000/well for 24 h and then transfected with 0.2 μg of 5XUAS-luciferase together with 0.4 μg of Actin-VP-L5**-ires-Gal-L5**, or SV40-VP-PYL-ires-Gal-ABI, or SV40-VP-GAI-ires-Gal-GID1, or SV40-VP-Frb-ires-Gal-3FKBP, for another 24 h. And then cells treated with ethanol, or 500 nM MGnBu, or 10 μM ABA, 100 μM GA-AM, 10 nM rap. After 24 h, cells were harvested and washed with PBS twice, and resulting cells were re-suspended in 100 μL of PBS and fluorescence was quantified by fluorescent plate reader. Meanwhile, cells samples with the same conditions were harvested and quantified the luciferase activity. All cellular experiments were conducted 4 to 5 independent times and each time with triplicate or quadruplicate.

Protein translocation. CHO were seeded over glass coverslips in 24-well plates at 50,000/well for 24 h. Then cells were transfected with 0.2 μg SV40-EYFP-L5** and 0.4 μg SV40-NES-L5** for another 24 h. Cell were treated with ethanol or with 500 nM of MGnBu for 2 h. After indicated time, cells were fixed with 4% paraformaldehyde and mounted on a glass slide with Vectashield (VWR) mounting media and images of cells were then taken by using a fluorescence microscope. For drug withdrawal experiments, after treatment with 500 nM of MGnBu for 2 h, cells were washed with warm and fresh medium for three times (5 mins between each wash) and then incubated with 15 min, 30 min, 1 h and 2 h. After indicated time, cells were fixed to make the cover slides and images were taken by using a fluorescence microscope. For all conditions, in the meanwhile of making the coverslips, cells samples with the same conditions were harvested and fluorescence was quantified by fluorescent plate reader (ex/em 640 nm/670 nm). All cellular experiments were conducted 4 to 5 independent times and each time with triplicate or quadruplicate.

Cloning and plasmid construction

All DNA fragments were amplified by PCR (Polymerase chain reaction) from other intermediate constructs with the enzyme of Phusion DNA Polymerase (New England Biolabs)

under S1000 thermal cycler with Dual 48/48 Fast Reaction Module (Bio-Rad). All the restriction enzymes used below are purchased from New England Biolabs. All the constructs with L5** were amplified using pcDNA3.1-KozATG-dL5-2XG4S-mCer3 (plasmid #73207 from Addgene) as the template.

Actin-VP-L5**-ires-Gal-L5** was derived from SV40-VP-PYL-ires-Gal-ABI by replacing PYL to L5** using AscI and BamHI sites (amplified by primers CCGAC AGGCG CGCCA CAGGC CGTCG TTACC CAAGA A and CCGAC AGGAT CCTCA AGCGT AATCT GGAAC ATCGT ATGGG TAGGA CAGAA CCGTC AGTTG TGT), replacing ABI to L5** using MluI and NotI sites (amplified by primers CCGAC AACGC GTCAG GCTGT GGTGA CTCAG GAG and CCGAC AGCGG CCGCT TCACT TGTCG TCATC GTCTT TGTAG TCGGA GAGGA CGGTC AGCTG GGT), and then replacing SV40 to Actin using SpeI and EcoRI sites (insert restricted from Actin-EGFP-PYL) [17].

SV40-NES-L5** was generated from SV40-VP-PYL-ires-Gal-ABI by replacing VP-PYL-ires-Gal-ABI to NES-L5** using EcoRI and NotI sites (amplified by primers CCGAC AGAAT TCGCC ACCAT GCTTC CTCCA CTAGA ACGTC TGACT CTGGA TGGAT CCCAG GCTGT GGTGA CTCAG GAG and CCGAC AGCGG CCGCT TCACT TGTCG TCATC GTCTT TGTAG TCGGA GAGGA CGGTC AGCTG GGT).

SV40-EYFP-L5** was generated from SV40-VP-PYL-ires-Gal-ABI by replacing ABI to L5** using MluI and NotI sites (amplified primers are the same as the ones when making Actin-VP-L5**-ires-Gal-L5**) and replacing VP-PYL-ires-Gal to EYFP using EcoRI and MluI sites (amplified by primers CCGAC AGAAT TCATG GTGAG CAAGG GCGAG GAGCT G and CCGAC AACGC GTCTT GTACA GCTCG TCCAT GCC, template YFP-GID1 from Addgene #37305).

SV40-VP-GAI-ires-Gal-GID1 was generated from SV40-VP-PYL-ires-Gal-ABI by replacing PYL to GAI using AcsI and BamHI sites (amplified by primers CCGAC AGGCG CGCCA GGATC TGGTG GAAAG AGAGA TCATC ATCAT CAT and CCGAC AGGAT CCTCA AGGAT TAAGG TCGGT GAGCA T) and replacing ABI to GID1 using MluI and NotI sites (amplified by primers CCGAC AACGC GTGGA TCTGG TGGAG CTGCG AGCGA TGAAG TTAAT and CCGAC AGCGG CCGCT CAACA TTCCG CGTTT ACAAA CGC). Templates were Actin-4XNLS-GID1 and Actin-GAI-ABI [17].

SV40-EYFP-ABI was generated from SV40-VP-PYL-ires-Gal-ABI by replacing VP-PYL-ires-Gal to EYFP using EcoRI and MluI sites (amplified by primers CCGAC AGAAT TCATG GTGAG CAAGG GCGAG GAGCT G and CCGAC AACGC GTCTT GTACA GCTCG TCCAT GCC).

Fluorescence microscopy

Zeiss Axio Observer. D1 outfitted with HBO 100 microscopy illumination system (excitation 470/40 and emission 525/50) was used for Ruffle formation experiments. Fluorescent channels in all experiments were adjusted to the same intensity ranges. Acquisition times ranged from 50 to 500 ms.

Live cell imaging experiment for CHO cells with unclear export experiments and its analysis.

CHO cells were seeded in 35 mm dish (MatTek: P35G-1.5-14-C) at 200K/well for 24 h and then 1.6 μg SV40-NES-L5** and 0.8 μg SV40-EYFP-L5** were transfected for another 24 h. After changing with fresh medium, cells were treated with ethanol or 500 nM MGnBu. Images were taken every 30 secs for duration of 1 h for the same area with Leica TCS SP8 Confocal microscopy. Images generated were analyzed for fluorescent intensity using Leica LAS-X software. Equal sized regions of interest were analyzed to get the EYFP fluorescence intensity ratio of the cytoplasm over nucleus for each time point, and then normalized to the initial ratio (as shown in **Figure** 3G). Fluorescence intensity under MGnBu Channel for the whole cells were quantified for each time point and then normalized to initial intensity (as shown in **Figure** 3F). The initial ratio or intensity were normalized to 1 for both conditions. Four cells for each condition were analyzed to get the average data.

Statistical analysis of cell population

To determine the translocation of EYFP upon the drug treatment, we measured the fluorescence intensity ratio of cytoplasm/nucleus for each analyzed cell in each condition, and defined the cells as unclear exporting when the ratio is larger than one. And percentage of the cells with EYFP unclear exporting was calculate by the number of unclear exporting cells over total EYFP positive cells number. Cell images were collected from 5 different areas (4 quarters and center) in each of the 3 independent experiments. For each condition, 110 to 200 cells were analyzed along the diagonal lines. Mean fluorescence of nucleus and cytoplasm were analyzed by using Image J and get the ratio of these two for each analyzed cell.