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Supporting Information

A Highly Sensitive Fluorescent Light-up Probe for Real-Time Detection of Endogenous Protein Target and its Antagonism in Live Cells

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EXPERIMENTAL SECTION

Materials. TPECM (TPETPAFN) was synthesized according to the previous report.[1] Ponasterone (PonA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), penicillin-streptomycin solution, fetal bovine serum (FBS), trypsin-EDTA solution, methanol, trifluoroacetic acid, acetonitrile, dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) were purchased from Sigma-Aldrich. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, Breford, USA). Alkyne-functionalized peptide with a sequence of MPRFMDYWEGLS-Pra (alkyne) was customized from GL Biochem Ltd. Mdm2. DO-1 and 2A9 antibodies were kind gifts from Borivoj Vojtesek. Nutlin compounds (3a and 3b) were kindly provided by Roche.

Characterization. The UV-vis absorption spectra of samples were measured using a Shimadzu UV-1700 spectrophotometer. Their fluorescence spectra were measured using a fluorometer (LS-55, Perkin Elmer, USA). The interaction of probe with Mdm2 protein and drugs were conducted using a microplate reader (Tecan, infiniteM200) with a 384-well polypropylene plate. A 0.1% trifluoroacetic acid solution in H_2O and acetonitrile was used as the eluent for HPLC experiments (Agilent). Mass spectra were recorded on Agilent 5975 DIP-MS for EI and the AmaZon X LC-MS for ESI. Average particle size and size distribution of the NPs were determined by dynamic light scattering (DLS) with particle size analyzer (90 Plus, Brookhaven Instruments Co. USA) at a fixed angle of 90° at room temperature.

Synthesis of TPECM-12.1Pep probe. Alkyne-functionalized peptide MPRFMDYWEGLS-Pra (10 mg, 6.0 μmol) and TPECM (3.7 mg, 6.0 μmol) were dissolved in a mixture of dimethyl sulfoxide and water (v/v = 5/1, 1.0 mL). The click reaction was initiated by sequential addition of catalytic amounts of CuSO4 (1.92 mg, 12 μmol) and sodium ascorbate (4.8 mg, 24 μmol). The

reaction was continued under stirring at room temperature for another 24 h. The final product was purified by HPLC and lyophilized under vacuum to yield the probe (7.8 mg, 56.9% yield) as a light yellow powder. MALDI-TOF Calculated for $[2M+H]^+$: 4578.28, found: 4578.29.

Expression and purification of Mdm2. Hexahistidine-Mdm2 recombinant protein (residues 18-125) was purified as previously described.[3] Briefly, cDNA encoding residues 18-125 of Mdm2 was cloned into pET19b (Novagen), transformed into *E.coli* BL21 (DE3) and induced with 1mM IPTG. Bacterial cells were then disrupted and first purified using a Ni-nitrilotriacetic acid (NTA) column (eluted with a 1M imidazole gradient), followed by cation exchange chromatography (eluted using a 1M NaCl gradient).

Cell culture. H1299-p53 Ecdysone-Inducible (EI) cells (kind gifts from Paul Neilsen) are stably integrated with an inducible cassette allowing strong, constitutive p53 expression, through the use of ponasterone A, in the naturally p53-null background of H1299 cells.[3] DKO cells are $p53^{-/-}Mdm2^{-/-}$ mouse embryonic fibroblasts kindly provided by Gigi Lozano.[4] T22 reporter cells carry a stably integrated, p53 responsive, RGCΔfosLacZ reporter gene. For the reporter assay, T22 cells were seeded overnight onto a 96-well culture dish before a 20-hour treatment of respective p53-Mdm2 inhibitors the following day. After which, β-galactosidase activity was measured using the Dual-Light luminescent assay kit (Life Technologies), following manufacturer's protocol.[5] H1299-EI, DKO and T22-reporter cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified environment containing 5% CO_2.

Western blot

Total cell lysates for western blot analysis were first obtained by harvesting cells using 1x LDS solution. Cells were sonicated and heat denatured (3 minutes at 95°C) before electrophoretically

separated on a Bolt 10% Bis-Tris gel (Life Technologies) and transferred onto a nitrocellulose membrane using the iBlot® transfer system (Life Technologies).[6] Protein-immobilized membranes were then blocked in 5% milk (w/v, PBS/0.1% Tween-20) before a 1-hour incubation in the respective primary antibodies (5% milk/PBST), washed (3x PBST), and further incubated for 30 minutes in anti-mouse secondary antibodies before final washes (5x PBST). Chemiluminescence substrate solution (Clarity™ ECL, Biorad) was then added onto the immuno-blotted membranes, exposed onto medical grade x-ray films (Agfa) and developed (Medical X-ray processor, Carestream Health).

Cellular imaging. H1299-EI cells were cultured in chamber (μ-Slide 8 well, Ibidi) at 37 °C. After 80% confluence, the medium was removed and the adherent cells were washed three times with DMEM. Pon-A (2 $\mu g \cdot mL^{-1}$) in DMEM medium were then added to the chamber to induce p53 expression. After 24 hours of Pon-A treatment, the cells were washed three times with DMEM and TPECM-12.1Pep (2 μM) was added into the chamber. After 4h incubation, the cells were directly imaged by CLSM (Zeiss LSM 410, Jena, Germany) with imaging software (Fluoview FV1000). H1299-EI cells without Pon-A induction, DKO cells and H1299-EI cells carrying mutant p53 (H1299-EI-p53G245S and H1299-EI-p53R248Q) were also imaged after the same procedures (with or without Pon-A induction). For nutlin protection in cells, H1299-EI-WTp53 cells were pre-incubated with Nutlin-3a (1 μM) for 2 hours, followed by incubation with TPECM-12.1Pep (2 μM) for 4 hours before imaging.

Flow cytometry study. H1299-EI-WTp53 cells were cultured overnight to the desired confluence followed by 24 hours of Pon-A (2 $\mu g \cdot mL^{-1}$) treatment to induce p53 expression. TPECM-12.1Pep (2 μM) was then added thereafter to both induced or uninduced (control) H1299-EI-WTp53 population of cells for 4 hours before all cells were rinsed with DMEM three

times, trypsinized, pelleted and resuspended in FBS free DMEM medium (2 mL) at a density of ~1.5 × 10^5 cells·mL^{-1}. Flow cytometry measurements were conducted using Cyan-LX (DakoCytomation). The mean fluorescence was determined by counting 10,000 events (λ_{ex} = 405 nm, 605/40 nm band pass filter).

Cytotoxicity of TPECM-12.1Pep. The metabolic activity of H1299-EI cells was evaluated using the MTT assay. H1299-EI cells were seeded onto 96-well plates (Costar, IL, USA) at 4 × 10^4 cells·mL^{-1}. After a day, culture media was replaced with that containing TPECM-12.1Pep at different concentrations, and further grown for 24 and 48 h, respectively. After the desired duration, the wells were washed twice with DMEM and 100 μL of freshly prepared MTT (0.5 mg·mL^{-1}) solution in culture medium was added into each well. The MTT medium solution was carefully removed after 3 hour incubation at 37°C. DMSO (100 μL) was then added into each well and the plate was gently shaken for 10 minutes at room temperature to dissolve all the precipitates formed. The absorbance of MTT at 570 nm was monitored by the microplate reader (Genios Tecan). Cell viability was expressed by the ratio of absorbance of the cells incubated with probe suspensions to that of the cells incubated with culture medium only.

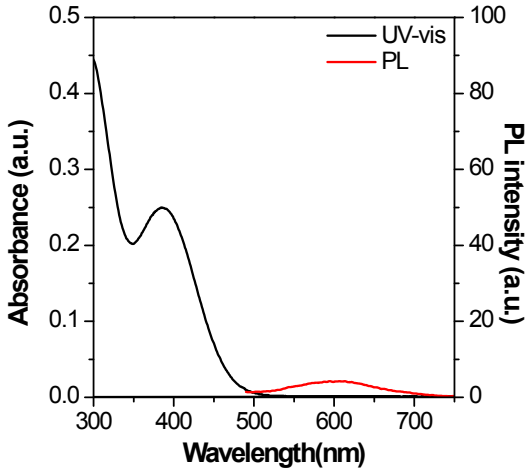
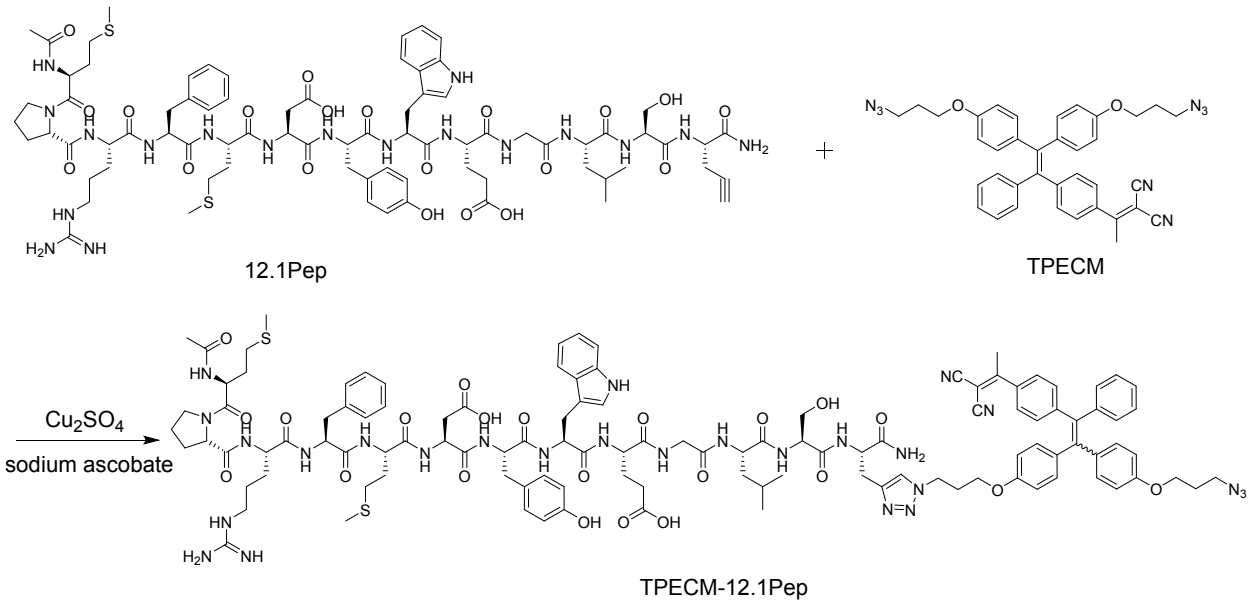


Figure S1. UV-vis (black) and PL (red) spectra of TPECM at 2 μM in THF.



Scheme S1. Click synthesis of TPECM-12.1Pep.

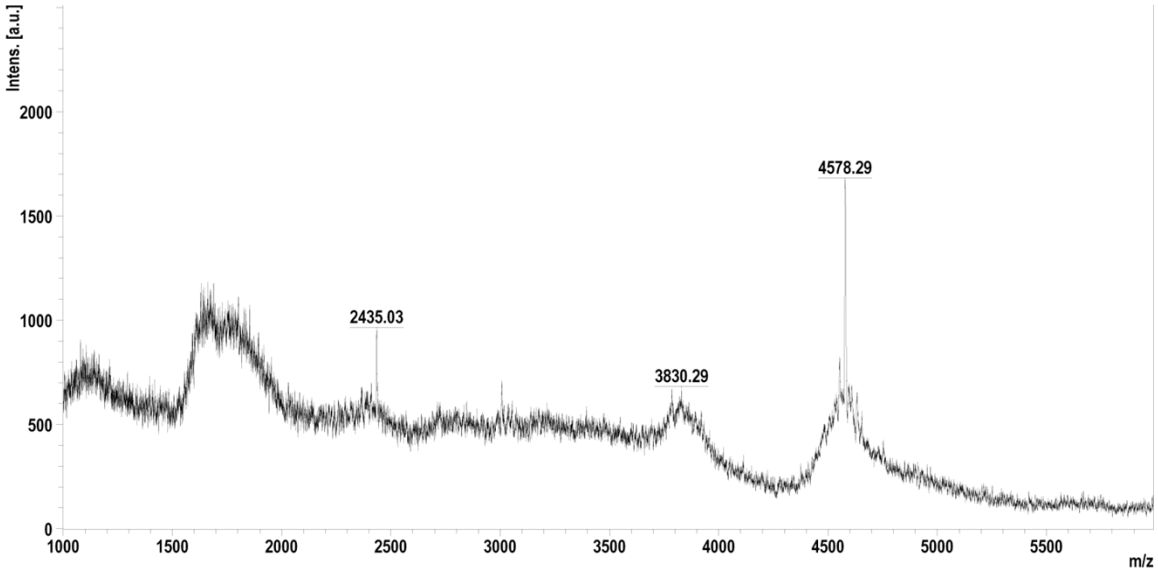


Figure S2. Mass spectrum (MALDI-TOF) of TPECM-12.1Pep.

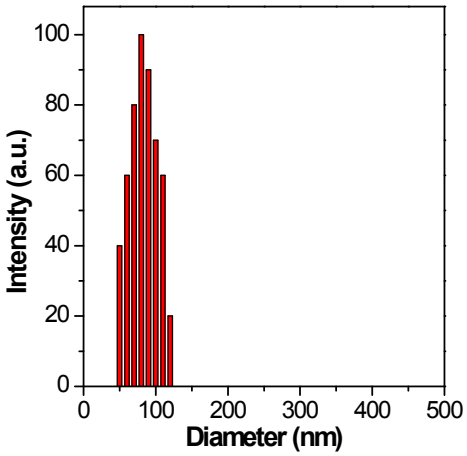


Figure S3. Hydrodynamic diameter of TPECM in DMSO/water (V/V = 1/199) obtained from laser light scattering.

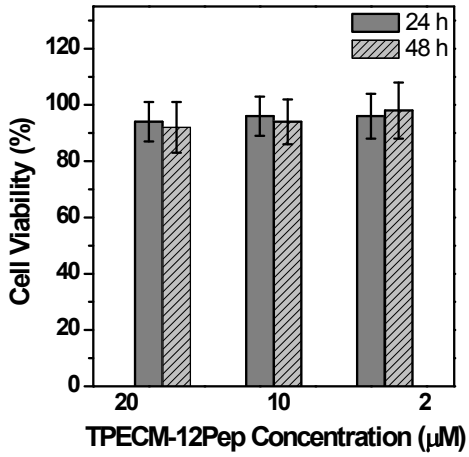


Figure S4. Metabolic viability of H1299-EI cells after incubation with TPECM-12.1Pep at various concentrations for 24 h (gray) and 48 h (shadow).

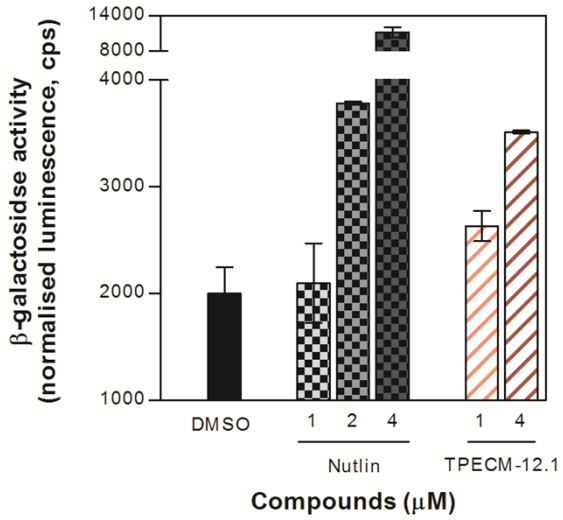


Figure S5. T22 reporter cells carrying a stably integrated β-galactosidase reporter gene downstream of a p53-sensitive promoter are treated with equi-molar concentrations of either nutlin (racemic) or TPECM-12.1Pep probe for 20 h before measuring β-galactosidase activity. Error shows S.D. of 3 individual reporter experiments.

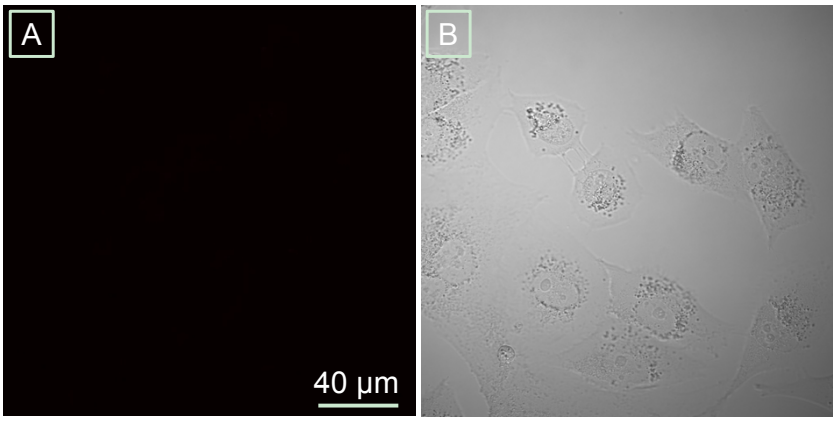


Figure S6. CLSM fluorescence image of H1299-EI-WTp53 cells treated with PonA (2 μg/mL) for 24 h without incubation with TPECM-12.1Pep.

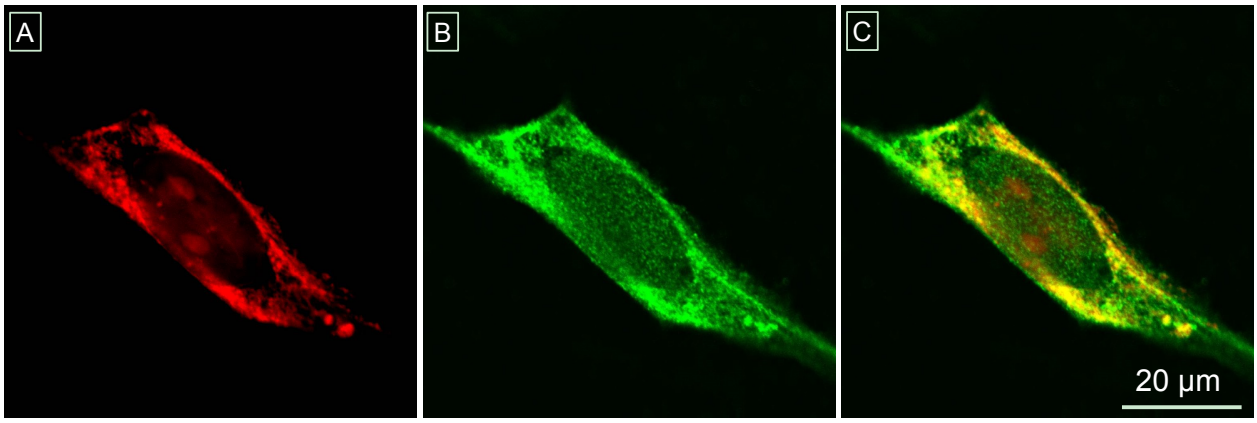


Figure S7. CLSM images of fixed H1299-EI-WTp53 cells treated with both TPECM-12.1Pep probe and Mdm2 antibody (2A9). (A) was taken under excitations at 405 nm using optical filters with long pass of >560 nm, and (B) was taken under excitations at 488 nm with a band pass of 505-520 nm. (C) is the overlay of TPECM-12.1Pep channel and antibody channel.

References

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