Supporting Information

A fluorescent ligand for human progesterone receptor imaging in live cells

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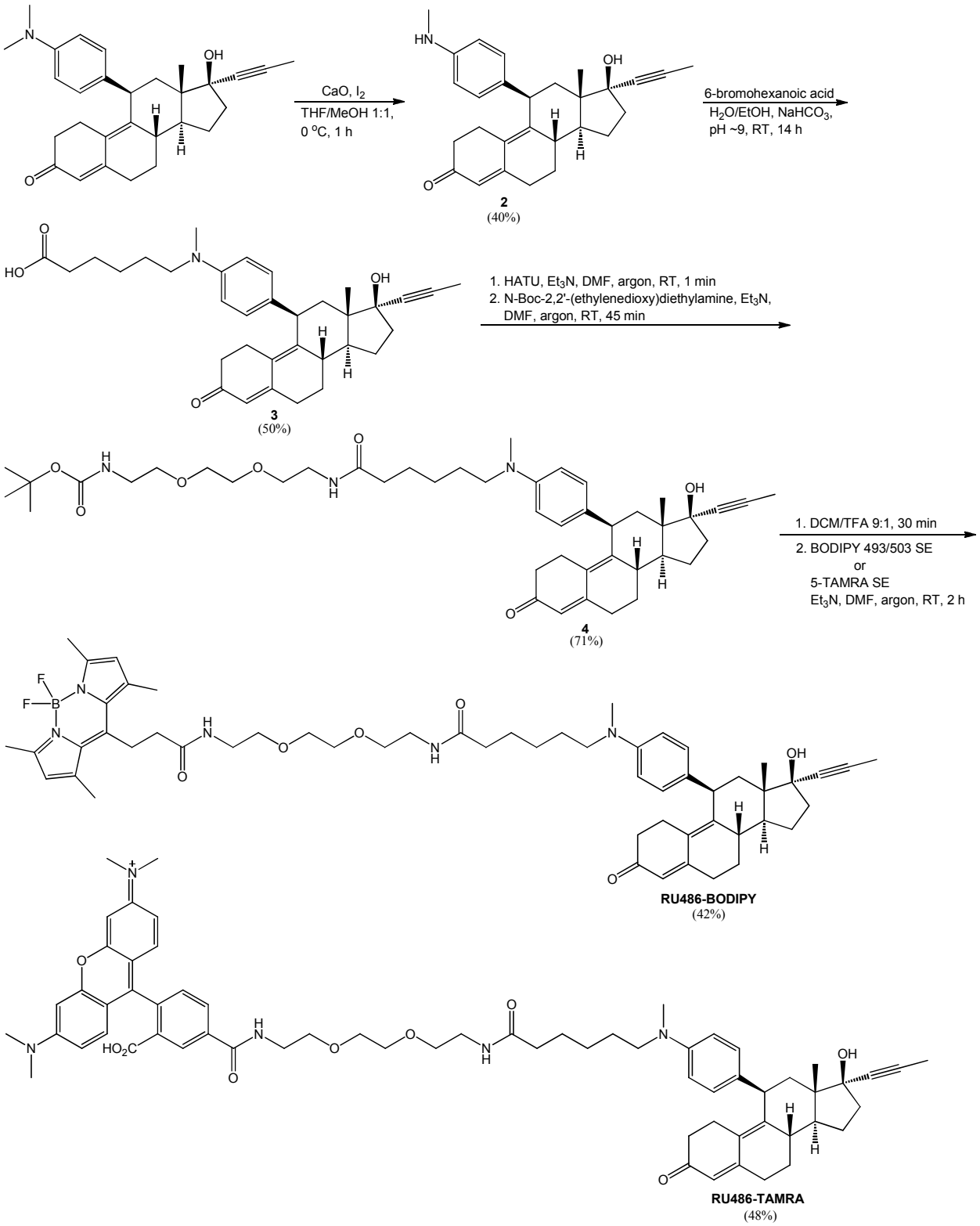
General synthetic and analytical methods

RU486 (Mifepristone) was purchased from OChem. BODIPY® 493/503, SE (4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene-8-propionic acid, succinimidyl ester) and 5-TAMRA, SE (5-carboxytetramethylrhodamine, succinimidyl ester) were purchased from Invitrogen. 17-AAG (17-(Allylamino)-17-demethoxygeldanamycin), vorinostat, panobinostat and FK506 were purchased from LC laboratories. VER-155008 was purchased from Santa Cruz Biotechnology. Femto ELISA-AP substrate was purchased from G Biosciences. All other chemicals were purchased from Sigma-Aldrich and used as received unless otherwise noted. Anhydrous solvents and reagents (DMF) were obtained as SureSeal bottles from Sigma-Aldrich. Thin-layer chromatography and flash chromatography were performed using EMD pre-coated silica gel 60 F-254 plates and silica gel 60 (230-400 mesh). UV absorbance and fluorescence spectra were recorded on a Cary 3E (Varian) and Fluorolog 2 (Spex) fluorimeter, respectively. Analytical and preparative HPLCs were performed on Agilent HPLCs, with Luna C18(2) columns (Phenomenex) using water (solvent A) and acetonitrile (solvent B) with 0.05% TFA as an additive. Low resolution ESI mass spectrometry was performed on an Agilent LC/MSD Trap XCT coupled to an Agilent HPLC. ^{1}H- and ^{13}C-NMR spectra were collected in d_6-DMSO (Cambridge Isotope Laboratories, Cambridge, MA) at 25 ºC using a Varian Unity Inova spectrometer at 500 MHz at the Department of Chemistry and Biochemistry NMR Facility at the University of California, San Diego. All chemical shifts are reported in the standard δ notation of parts per million using the peak of residual proton signals of d_6-DMSO as an internal reference.

Abbreviations: DMF: dimethylformamide, THF: tetrahydrofuran, Et_3N: triethylamine, HATU: 2-(7-aza-1H-benzotriazole -1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, DCM: dichloromethane, DMSO: dimethyl sulfoxide, TFA: trifluoroacetic acid, RT: room temperature, HBSS: Hank's balanced salt solution.

Synthesis and characterization



Scheme S1. Synthetic scheme for the synthesis of **RU486-BODIPY** and **RU486-TAMRA**.

Compound 1: Compound **1** was synthesized according to published procedure.[1]

Compound 2: Compound **2** was synthesized from compound **1** according to published procedure.[2]

Compound 3: Compound **2** (20 mg, 38 μmol, 1 eq) was dissolved in 1 mL dry DMF under argon atmosphere and Et_3N (5.5 μL, 40 μmol, 1.05 eq) and HATU (15 mg, 40 μmol, 1.05 eq) were added. The solution was stirred for 1 minute, then N-Boc-2,2'-(ethylenedioxy)diethylamine (10 μL, 41 μmol, 1.1 eq) and Et_3N (5.8 μL, 41 μmol, 1.1 eq) were added, and the reaction mixture was stirred for 45 minutes. The reaction mixture was diluted with EtOAc (25 mL) and washed with sat. NH_4Cl, brine and water. The organic layer was separated, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified on silica gel column (DCM:MeOH 98:2 to 93:7). After removal of solvents under reduced pressure, 21 mg (27 μmol, yield 71%) of **3** as a yellowish solid were obtained.

^{1}H-NMR ($CDCl_3$, 500 MHz): δ = 0.55 (3H, s), 1.18-1.36 (3H, m), 1.39 (9H, s), 1.47-1.74 (7H, m), 1.85 (3H, s), 1.88-2.09 (2H, m), 2.10-2.36 (6H, m), 2.37-2.48 (2H, m), 2.49-2.62 (2H, m), 2.63-2.78 (1H, m), 2.83 (3H, s), 3.16-3.36 (4H, m), 3.37-3.46 (2H, m), 3.48-3.65 (8H, m), 3.98-4.09 (1H, m), 4.30 (1H, d, J = 6.8 Hz), 5.00 (1H, s), 5.71 (1H, s), 6.05 (1H, s), 6.55 (2H, d, J = 8.3 Hz), 6.94 (2H, d, J = 8.3 Hz). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 3.8, 13.8, 23.3, 25.8, 26.4, 26.8, 27.3, 28.4, 31.1, 36.8, 38.2, 38.8, 39.0, 39.1, 39.5, 40.3, 46.8, 49.8, 52.7, 69.9, 70.1, 70.2, 80.1, 82.3, 82.4, 112.3, 122.6, 127.5, 129.0, 146.9, 147.2, 157.1, 199.8. ESI-MS (positive mode) calculated ($C_{45}H_{65}N_3O_7$) 759.5, found m/z $[M+H]^+$ 760.7.

RU486-BODIPY: Compound **3** (10 mg, 13 μmol, 1 eq) was dissolved in 0.5 mL DCM:TFA (9:1) and stirred for 30 minutes at room temperature (NOTICE: prolonged reaction time leads to dehydration at 17α). The solvents were removed under reduced pressure, the residue was triturated with Et_2O and dried again under reduced pressure. The crude product was used without further purification. Thus, the residue was dissolved in 750 μL dry DMF under argon atmosphere and Et_3N (7.3 μL, 52 μmol, 4 eq) and BODIPY® 493/503 SE (6 mg, 14.3 μmol, 1.1 eq) were added. The reaction was stirred at room temperature for 2 hours then solvent was removed under reduced pressure. The crude residue was dissolved in 2 mL DMSO:ACN:water 2:1:1 and purified on preparative HPLC (gradient 20 to 80% ACN in 20 minutes, for more details see

Preparative HPLC conditions below). Retention time: 15.68 minutes. After removal of solvents, 5.3 mg (5.5 μmol, yield 42%) of **RU486-BODIPY** as an orange solid were obtained.

^{1}H-NMR ($CDCl_3$, 500 MHz): δ = 0.53 (3H, s), 1.16-1.34 (3H, m), 1.47-1.74 (7H, m), 1.84 (3H, s), 1.88-2.09 (2H, m), 2.10-2.36 (10H, m), 2.37-2.48 (2H, m), 2.41 (6H, s, partially obscured by 2.37-2.48 signal), 2.45 (6H, s, partially obscured by 2.37-2.48 signal), 2.50-2.62 (2H, m), 2.63-2.78 (1H, m), 2.83 (3H, s), 3.16-3.36 (4H, m), 3.37-3.46 (2H, m), 3.48-3.65 (8H, m), 3.98-4.09 (1H, m), 4.40 (1H, d, J = 6.8 Hz), 5.76 (1H, s), 6.01 (2H, s), 6.95 (1H, s), 7.02 (1H, s), 7.27 (2H, d, J = 8.8 Hz), 7.37 (2H, d, J = 8.8 Hz). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 3.8, 13.9, 14.5, 16.4, 23.3, 23.8, 23.9, 24.3, 25.0, 25.8, 27.3, 29.6, 31.0, 35.1, 36.6, 37.0, 38.9, 39.1, 39.2, 39.3, 39.4, 40.1, 45.2, 46.8, 49.5, 58.6, 69.7, 69.8, 70.1, 70.2, 79.9, 81.9, 82.9, 121.3, 121.9, 123.4, 129.1, 130.1, 131.2, 138.4, 140.9, 144.1, 145.0, 147.2, 154.3, 156.3, 171.0, 199.3. ESI-MS (positive mode) calculated ($C_{56}H_{74}BF_2N_5O_6$) 961.6, found m/z $[M+H]^+$ 962.8.

RU486-TAMRA: Prepared similarly to **RU486-BODIPY** from 5-TAMRA SE (7.5 mg, 14.3 μmol, 1.1 eq). The crude product was purified on preparative HPLC (gradient 10 to 100% ACN in 20 minutes, for more details see Preparative HPLC conditions below). Retention time: 14.93 minutes. After removal of solvents, 6.7 mg (6.2 μmol, yield 48%) of **RU486-TAMRA** as a purple solid were obtained.

^{1}H-NMR ($CDCl_3$, 500 MHz): δ = 0.38 (3H, s), 1.02-1.18 (2H, m), 1.20-1.61 (8H, m), 1.61-1.80 (2H, m), 1.88 (3H, s), 1.97-2.09 (2H, m), 2.10-2.29 (4H, m), 2.30-2.49 (4H, m), 2.50-2.62 (2H, m), 2.67-2.79 (1H, m), 2.94 (3H, s), 3.18-3.32 (14H, m), 3.38-3.46 (2H, m), 3.56-3.64 (2H, m), 3.65-3.70 (4H, m), 3.71-3.82 (4H, broad singlet), 4.40 (1H, d, J = 7.3 Hz), 5.77 (1H, s), 6.77 (2H, t, J = 2.5 Hz), 6.86 (2H, dd, J_1 = 9.2 Hz, J_1 = 2.0 Hz), 7.10 (2H, dd, J_1 = 9.2 Hz, J_1 = 6.9 Hz), 7.26 (expected 2H partially obscured by $CDCl_3$ signal), 7.31 (1H, d, J = 8.3 Hz), 7.46 (2H, d, J = 8.3 Hz), 7.60 (1H, broad triplet), 8.22 (1H, s), 8.33 (1H, d, J = 7.3 Hz), 8.77 (1H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ = 3.8, 13.8, 15.2, 23.2, 23.9, 24.5, 25.4, 25.8, 27.3, 29.2, 29.4, 31.0, 35.6, 36.2, 36.7, 38.7, 39.0, 39.05, 39.1, 40.0, 40.9, 44.8, 46.8, 49.6, 53.7, 58.3, 66.0, 69.4, 69.6, 70.2, 70.4, 79.9, 82.1, 82.5, 96.4, 113.7, 114.2, 115.3, 117.6, 121.3, 123.2, 128.9, 129.4, 129.8, 129.9, 131.2, 131.5, 131.9, 132.5, 135.8, 136.8, 139.0, 144.6, 156.5, 157.2, 157.5, 166.0, 174.1, 199.4. ESI-MS (positive mode) calculated ($C_{65}H_{77}N_5O_9$) 1071.6, found m/z $[M]^+$ 1072.7.

Preparative HPLC conditions. Preparative separations were performed on Agilent HPLC system (1200 series) with Luna 10 μm PREP C18(2) column (250.0 X 21.2 mm, 100 Å), using a water-acetonitrile solvent system (solvent A = water, solvent B = acetonitrile, both with 0.05% TFA as an additive) at flow rate of 15 mL/min.

Molecular modeling

The 3D structure of human PR co-crystalized with RU486 [PDB: 2W8Y] was used as a template. RU486-BODIPY was inserted into the protein and its steroidal skeleton was superimposed on RU486 using Biopolymer mode in Insight II. RU486 was then extracted from the complex. The RU486-BODIPY-PR complex was refined using Discover 3 in Insight II. For this energy minimization step, Discover 3 was run for 10,000 iterations, using CVFF force field and a distance dependent dielectric constant of 2 to approximate for water in the protein. Images were rendered using UCSF Chimera 1.5.3.

LogP measurement and calculation

LogP was measured using the shake flask method. Approximately 0.5 mg of the tested compound was dissolved in 1 mL of a 1:1 mixture of water/1-octanol in a glass vial. The solution was vortexed for ~1 minute until completely dissolved and then placed on a rotator for gentle mixing over 6 h. The vial was then removed and allowed to sit for 16 h. A sample was removed from each layer and the concentration of the tested compound was determined by comparing the sample's fluorescence intensity against a calibration curve (calibration curve was prepared for water and 1-octanol separately). The logP was calculated using the following equation: $\log P = \log_{10}(C_{octanol}/C_{water})$, where $C_{octanol}$ is the concentration in the 1-octanol layer, and C_{water} is the concentration in the water layer.

Cell cultures methods

T47-D and MDA-MB-231 cells were purchased from ATCC (HTB-133 and HTB-26, respectively). MDA-MB-231 cells were maintained in DMEM medium (Cellgro) supplemented with 10% fetal bovine serum (FBS, Atlanta Biologicals) and T47-D cells were maintained in RPMI 1640 medium (Cellgro) supplemented with 10% FBS and human insulin (0.2 unit/mL, Sigma). Both cell lines were cultured in a humidified atmosphere containing 5% CO_2 at 37 °C.

Three days before experiments, cells were seeded in 8-well borosilicate cover-glass chambers (Nunc* Lab-Tek* Chambered Coverglass, Thermo Scientific) or in 48-well plates (Corning* Costar* cell culture plates) with their respective maintenance medium (above). 24-36 hours prior to experiments, maintenance medium was removed and cells were washed twice with HBSS. Then, for MDA-MB-231, DMEM medium (without phenol red) supplemented with 5% dextran charcoal stripped (CDS) FBS was added and for T47D, RPMI 1640 medium (without phenol red) supplemented with 5% CDS FBS and human insulin (0.2 unit/mL) was added.

Stock solutions of all reported and used molecules (1 or 10 mM) were prepared in DMSO and stored at −20 °C. Before use, stocks were diluted with either DMEM or RPMI 1640 (both without phenol red, supplemented with 5% CDS FBS) to the final working concentration (final DMSO content was always > 0.1% v/v).

Alkaline phosphatase assay

Alkaline phosphatase was assayed using the multiwall format described by Markiewicz and Gurpide.[3] T47D cells were plated in 48-well dishes, and 24 hours before the start of the experiment, media was changed to the phenol red-free formulation with reduced and charcoal-treated serum as described in the cell culture section above, to avoid any baseline activation of the progesterone receptor. On the day of the experiment, Progesterone was added, with or without experimental test compounds in the range of doses shown in figure S2. 3 days later, hormone treatment was ended by washing the plates twice in PBS (immersion in a tub of .5 liter PBS), plate was inverted to dry, and cells were lysed by 2 rounds of freeze- thaw cycle in a -80^{o}C freezer. Alkaline phosphatase activity was determined by adding 200 microliters of a chromogenic substrate based on *p*-nitrophenyl phosphate (Femto ELISA-AP substrate). Yellow color developed over a period of minutes to hours, and was read at 405 nm on a Tecan Safire plate reader when max OD was between 0.5 and 1 absorbance units.

Fluorescence imaging

Unless otherwise stated, cells were incubated with either RU486-BODIPY or RU486-TAMRA for 10 minutes in their respective medium (see cell cultures) then Hoechst stain (10 μg/mL) was added and incubation was resumed for additional 5 minutes. The incubation medium was then removed, cells were washed twice with HBSS and fresh medium (without phenol red,

supplemented with 5% CDS FBS and insulin) was introduced. Cells were kept in a humidified atmosphere containing 5% CO_2 at 37 °C until imaged. Cells were imaged on a Zeiss Axiovert 200M microscope with a 40x (oil immersion 1.3 NA) objective and cooled EMCCD camera (Cascade II:1024, Photometrics) controlled by METAFLUOR 6.1 software (Universal Imaging). Imaging experiments of RU486-BODIPY were performed with excitation at 495 nm (10 nm bandwidth), emission at 535 nm (45 nm bandwidth), and dichroic mirror at 515 nm. Imaging experiments of RU486-TAMRA were performed with excitation at 540 nm (25 nm bandwidth), emission at 595 nm (50 nm bandwidth), and dichroic mirror at 560 nm. Images were processed with ImageJ (image processing and analysis software; National Institutes of Health). Contrast and brightness were adjusted manually (ImageJ) with no background subtraction. Images were similarly scaled unless otherwise noted.

Antibody staining

T47D or MDA-MB-231 cells were cultured and treated with RU486-BODIPY and Hoechst dye as described in the Fluorescence Imaging method above. After washing off the dyes, cells were incubated in a humidified atmosphere containing 5% CO_2 at 37 °C for 1 hour. Cells were then washed twice with HBSS and fixed with IHC Zinc Fixative (BD Pharmingen, #550523) for 30 minutes. The fixed cells were washed twice with PBS containing 1 % BSA and incubated in this medium for 15 minutes. Cells were then washed again with HBSS (twice) and incubated with polyclonal rabbit anti-human progesterone receptor primary antibody (Dako, #A0098), at 1:500 dilution in PBS containing 1 % BSA, for 18 hours at room temperature. Cells were then washed three times (x 5 minutes) with PBS 1 % BSA and incubated with AlexaFluor®594 goat anti-rabbit IgG (Life Technologies #A11037), at 1:500 dilution in PBS 1 % BSA, for 3 hours at room temperature. Cells were then washed three times (x 5 minutes) with PBS 1 % BSA and kept in this medium for imaging. Imaging was performed as described in the Fluorescence Imaging method above with the addition of excitation at 580 nm (20 nm bandwidth), emission at 653 nm (95 nm bandwidth) and dichroic mirror at 600 nm for AlexFluor®594 detection.

Inhibition of PR complex components

T47D cells were incubated with 10 μM Vorinostat, Panobinostat**,** VER155008 or methylene blue for 12 hours, 10 μM FK506 for 2 hours, 500 μM EHNA for 1 hour or 0.1 % v/v DMSO for 12

hours as control, before treatment with 5 nM RU486-BODIPY for 15 minutes. Cell were then washed twice with HBSS containing the respective inhibitor at similar concentration and incubated with fresh media containing the respective inhibitor at similar concentration for an additional 45 minutes. Cells were then imaged and the ratio of nuclear vs. cytoplasmic fluorescence was determined by drawing regions of interest for each cellular compartment of a specific cell and quantifying the mean fluorescence intensity (using ImageJ). This was repeated for 30 cells in each experimental setting.

***In vivo* tissue distribution**

FVB/N female mice were injected intravenously with either 1 or 10 nmol RU486-BODIPY and anesthetized 4 hours post-injection. Blood was collected by cardiac puncture and the mice perfused with 20 mL PBS. Tissues were flash frozen and stored at -80 °C prior to processing. Animal studies followed the NIH guidelines for the care and treatment of experimental laboratory rodents. Preparation for analysis: frozen tissues were weighed and diluted 9-fold with PBS containing 0.1 mg/mL DNAase I (Sigma #DN25) then probe-sonicated (Fisher Scientific Sonic Dismembrator 500) on ice until completely homogenized (1-2 cycles, 10 seconds each). To 100 μL homogenate, or blood, was added 300 μL ACN containing 2.5 % acetic acid. The mixture was vortexed, bath-sonicated for 5 minutes then centrifuged at 14,000 rpm for 5 minutes. The supernatant was used for further analysis. Analysis: samples were subjected to HPLC/MS/MS analysis and tissue concentration was quantified using an RU486-BODIPY standard calibration curve.

Statistical methods

Data were expressed as mean ± s.d. for all experiments. Statistical significance was determined using an unpaired two-tailed *t*-test compared to control except for *in vivo* tissue distribution analysis where a paired one-tailed *t*-test was applied (under the assumption that target organs are expressing higher levels of progesterone receptor.)

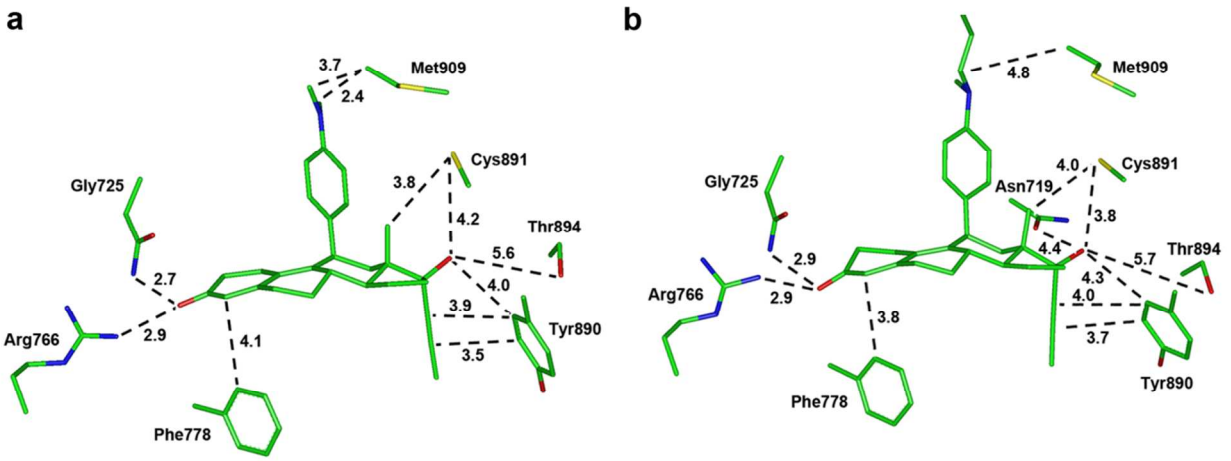


Figure S1. RU486-BODIPY maintains key interactions with PR ligand binding domain (LBD). **a**) Interactions of RU486 with key amino acids in PR LBD. Gly725 and Arg766 lock the ketone in position while Asn719 (not shown) and Cys891 interact with the 17β hydroxyl which points towards Thr894. Tyr890 is part of the hydrophobic pocket accommodating the 17α alkynyl group. **b**) RU486-BODIPY's orientation in PR LBD and interactions with key amino acids resembles that of RU486.

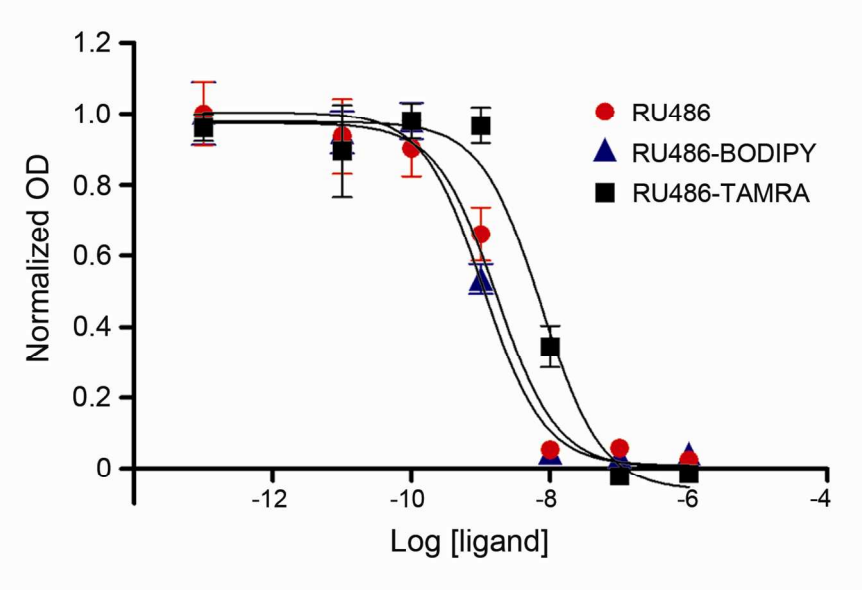


Figure S2. Antagonistic activity of RU486-BODIPY and RU486-TAMRA compared to RU486 as measured in the alkaline phosphatase assay. RU486-BODIPY IC_{50} is slightly lower than RU486 (IC_{50} = 1.0 ± 0.2 and 1.5 ± 0.2 nm, respectively) while that of RU486-TAMRA is higher, but still in the low nanomolar range (9.2 ± 1.1 nm). Each data point represents the mean of three experiments. Error bars represent ± SD.

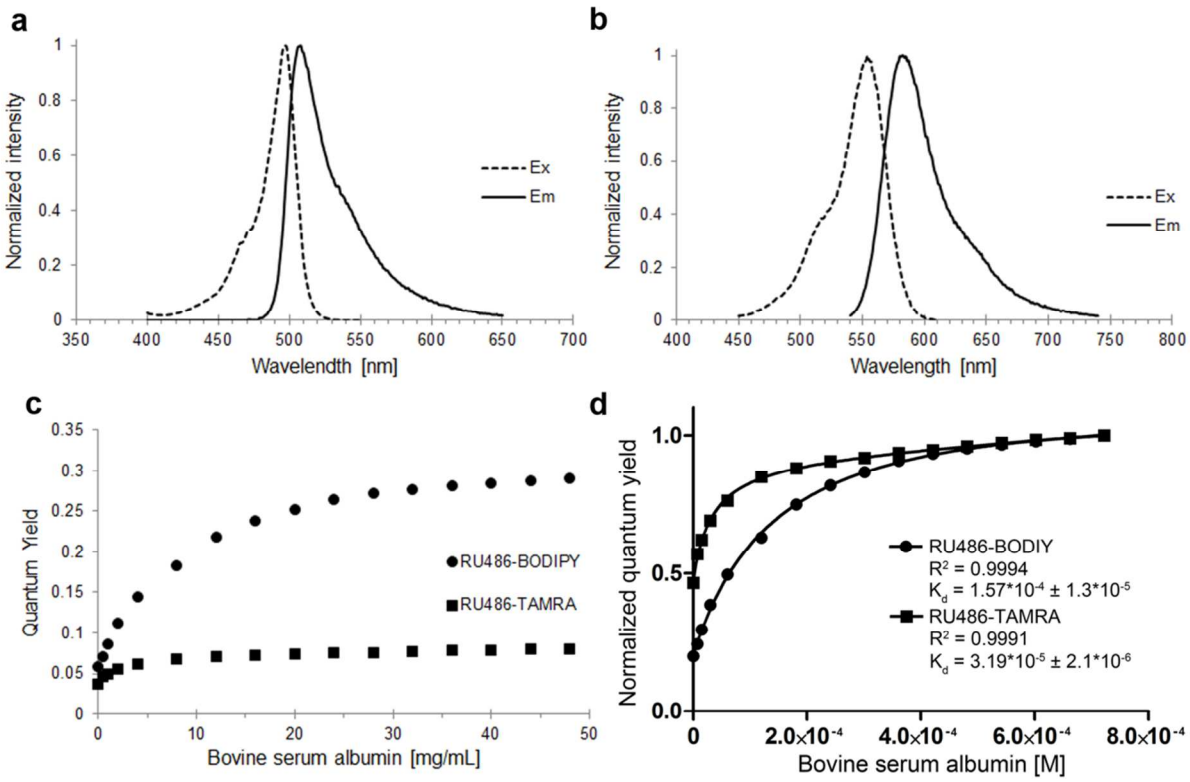


Figure S3. Spectral properties of RU486-BODIPY and RU486-TAMRA. Excitation (dashed line) and emission (solid line) spectra of 0.5 μM RU486-BODIPY (**a**) and RU486-TAMRA (**b**) in 10 mM MOPS buffer pH 7.2 at room temperature. **c**) Fluorescence quantum yields of RU486-BODIPY (squares) and RU486-TAMRA (circles) in 10 mM MOPS buffer pH 7.2 supplemented with BSA at indicated concentrations. **d**) RU486-BODIPY and RU486-TAMRA BSA binding. Data from quantum yield measurements were normalized and analyzed using GraphPad Prism5 software (binding saturation – total binding). The calculated K_d ± error are presented on the graph.

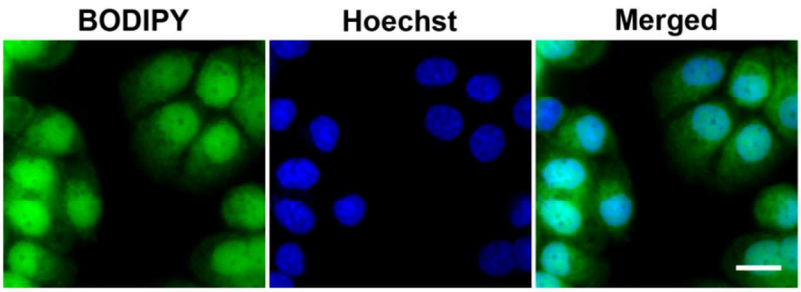


Figure S4. RU486-BODIPY distribution remains mostly nuclear after 24 hours in T47D cells. Cells were incubated with 5 nM RU486-BODIPY for 15 minutes, washed and incubated with fresh media for additional 24 hours before imaging. Scale bar represents 20 μm.

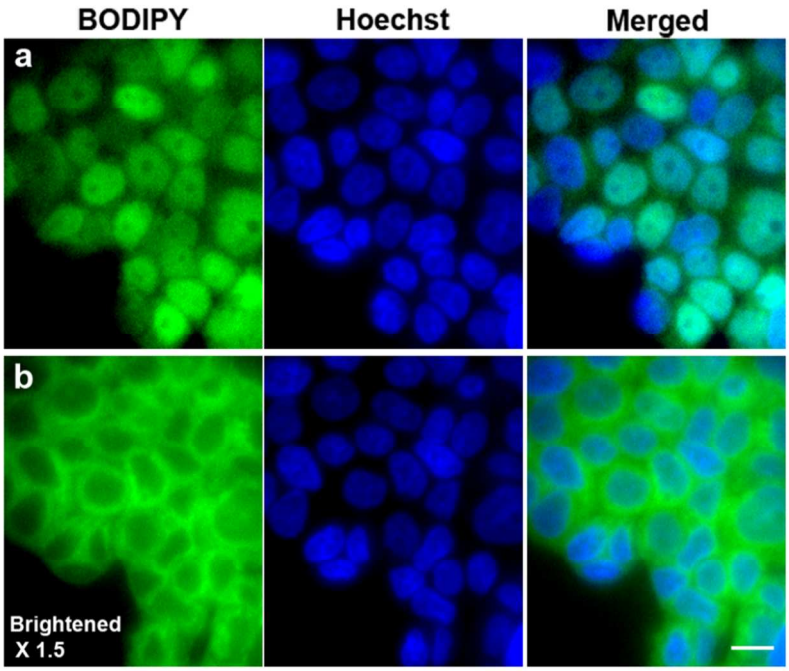


Figure S5. Nuclear RU486-BODIPY can be competed off by unlabeled RU486. **a**) T47D cells were treated with 5 nM RU486-BODIPY for 15 minutes then washed and re-incubated with fresh medium for 45 minutes. **b**) Same cells 30 minutes after addition of 100 nM unlabeled RU486.

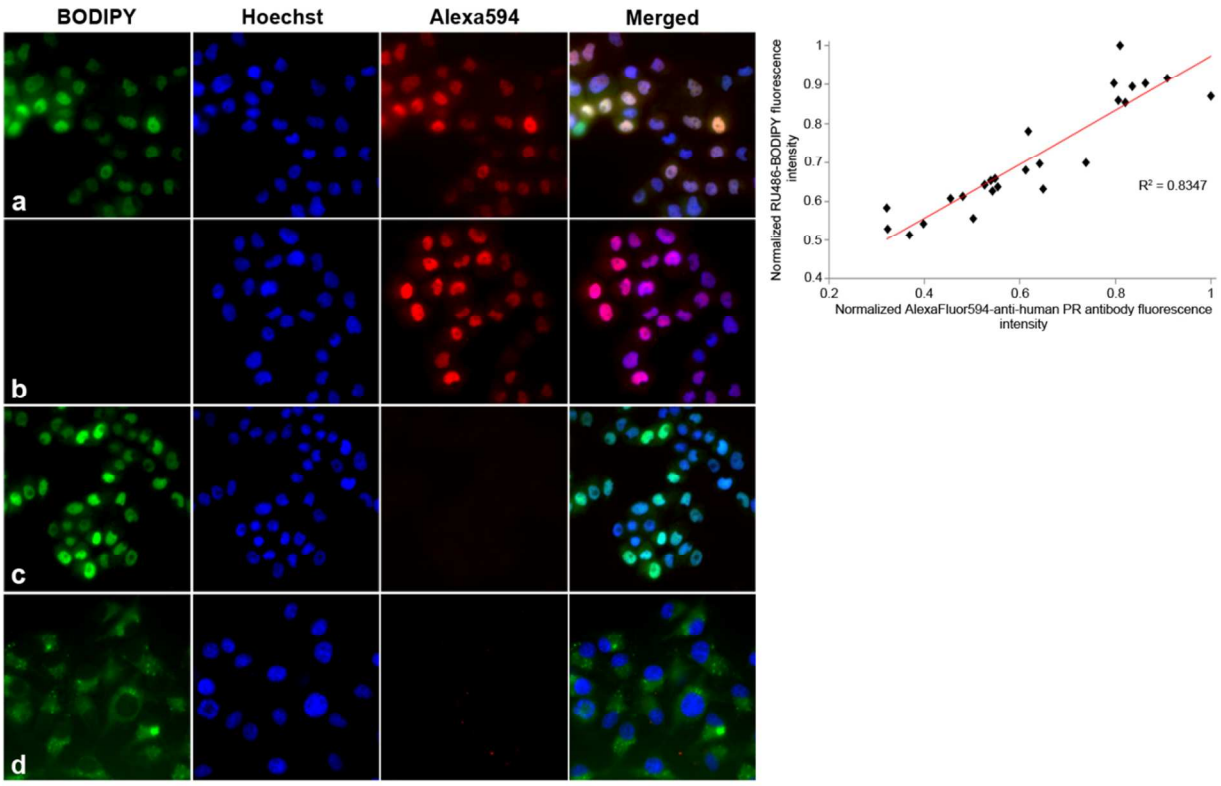


Figure S6. RU486-BODIPY nuclear distribution co-localizes with progesterone receptor. **a-c**) T47D cells treated with **a**) RU486-BODIPY, fixed then stained with rabbit anti-human PR antibody (primary) followed by AlexaFluor594 goat anti-rabbit IgG (secondary). **b**) same conditions as in **a** but without RU486-BODIPY. **c**) same conditions as in **a** but without primary antibody. **d**) MDA-MB-231 cells (PR negative) treated with the same conditions as in **a**. Complete experimental details appear on the Antibody staining Method above (page S8).

Right side graph: positive correlation between nuclear fluorescence intensity of RU486-BODIPY and AlexaFluor594 for T47D cells treated as in **a** (n = 25). For each cell, a ROI was drawn around the nucleus and the mean fluorescent intensity in both BODIPY and Alexa594 channels was quantified for the same region using ImageJ. For each set (BODIPY or Alexa594), fluorescence intensity was normalized against the brightest cell in the set.

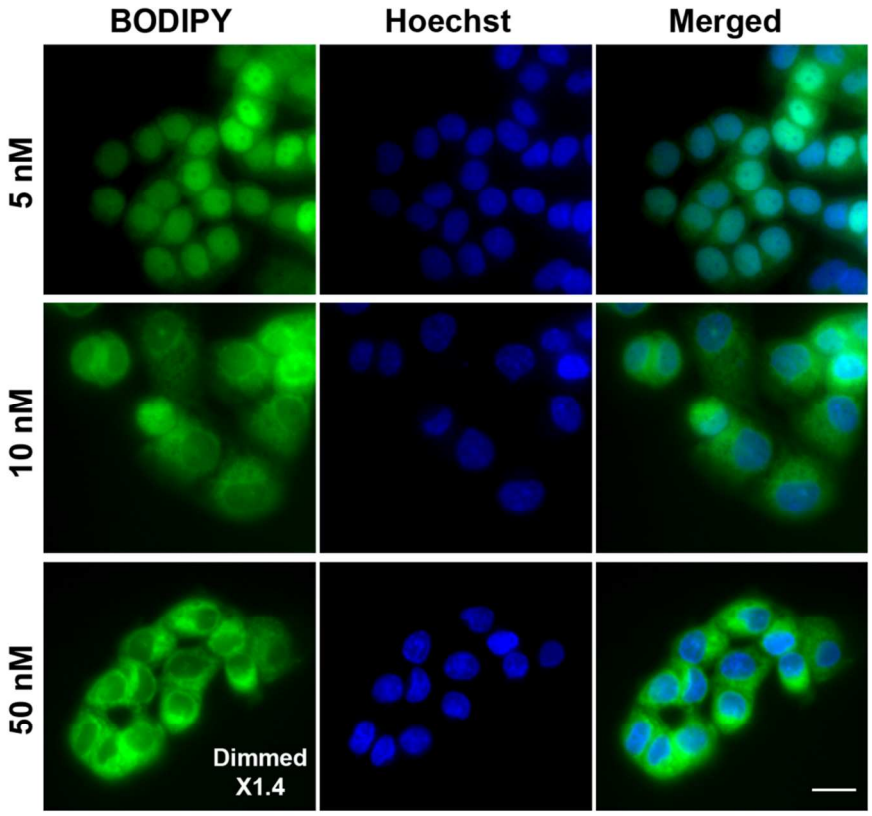


Figure S7. Cellular distribution of RU486-BODIPY at increasing concentrations in T47D cells. Cells were incubated with RU486-BODIPY at indicated concentrations for 15 minutes and imaged 45 minutes after. At 5 nM the distribution is mostly nuclear while at 10 nM it is almost uniform throught the cell. At 50 nM most of the fluorescent ligand is concentrated in membranes and cytosol. All images are with same field of view. Scale bar represents 20 μm.

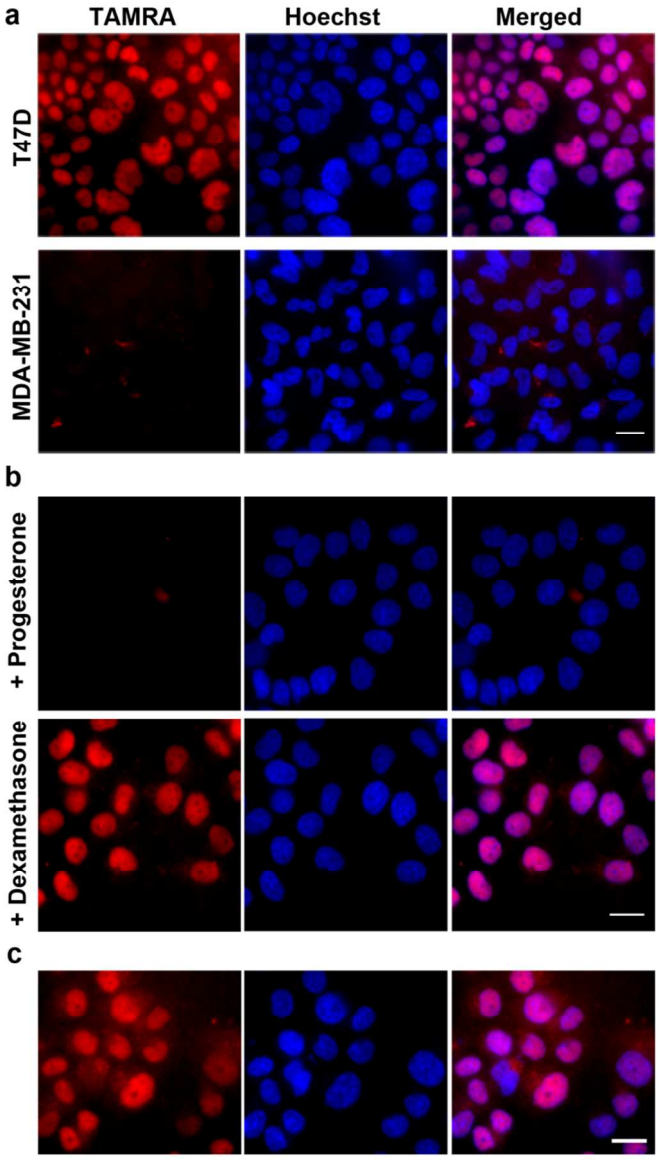


Figure S8. RU486-TAMRA nuclear accumulation is PR dependent. **a**) RU486-TAMRA accumulates in the nuclei of PR positive cells but not PR negative cells. T47D (PR positive) or MDA-MB-231 (PR negative) cells were incubated with 50 nM RU486-TAMRA for 15 minutes, washed and immediately imaged. **b**) Nuclear accumulation of RU486-TAMRA in T47D cells can be competed off with PR agonist but not with GR agonist. T47D cells were co-incubated with 50 nM RU486-TAMRA and 500 nM progesterone (PR agonist) or dexamethasone (GR agonist) for 15 minutes, washed and immediately imaged. **c**) RU486-TAMRA remains mostly nuclear after 24 hours in T47D cells. Cells were incubated with 5 nM RU486-TAMRA for 15 minutes, washed and incubated with fresh media for additional 24 hours before imaged. Scale bar represents 20 μm.

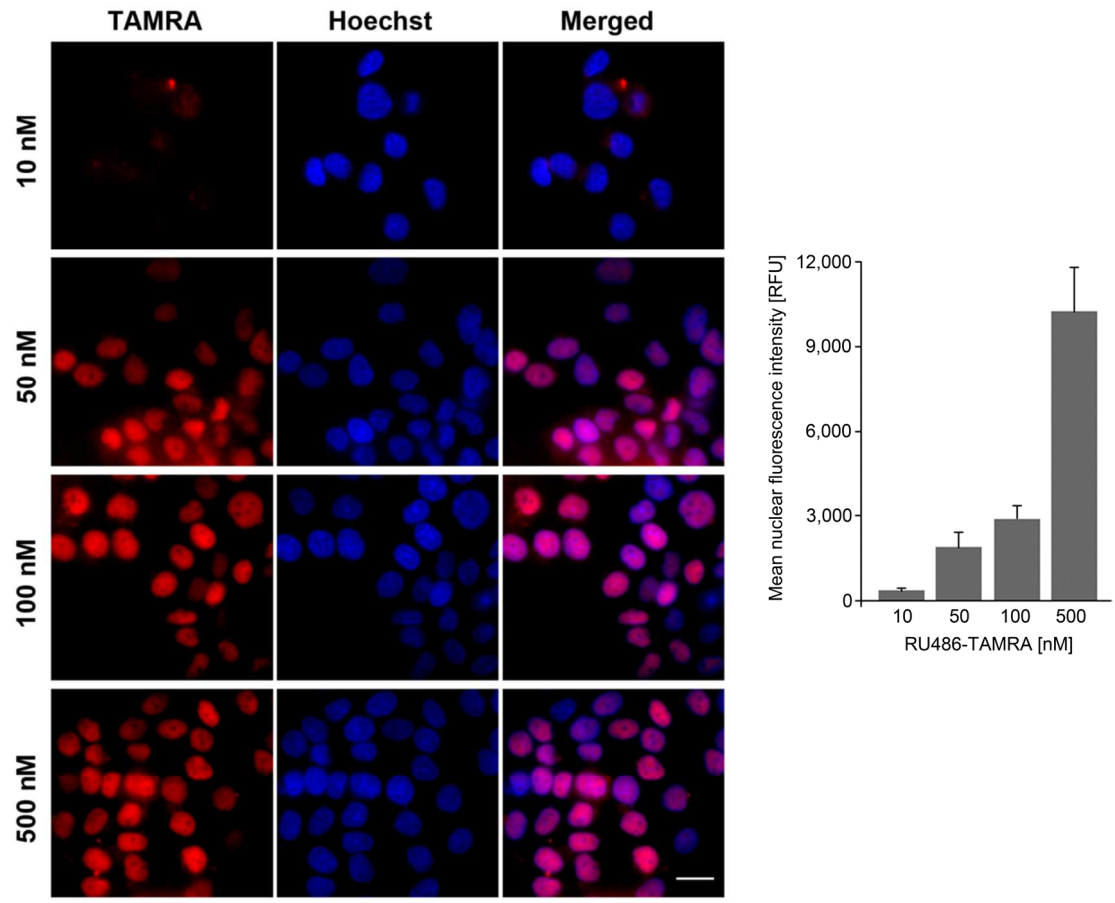


Figure S9. Cellular distribution of RU486-TAMRA at increasing concentrations in T47D cells. Cells were incubated with RU486-TAMRA at indicated concentrations for 15 minutes, washed and immediately imaged. The fluorescent ligand accumulates specifically in the nucleus at all tested concentrations. Almost no membranal or cytosolic accumulation can be observed even at the highest concentration tested (500 nM). All images were taken with same field of view and individually optimized. Scale bar represents 20 μm. Bar graph on right: quantitation of mean nuclear fluorescence intensity of images on left. Each bar represents mean of 10 cells. Error bars represent SD.

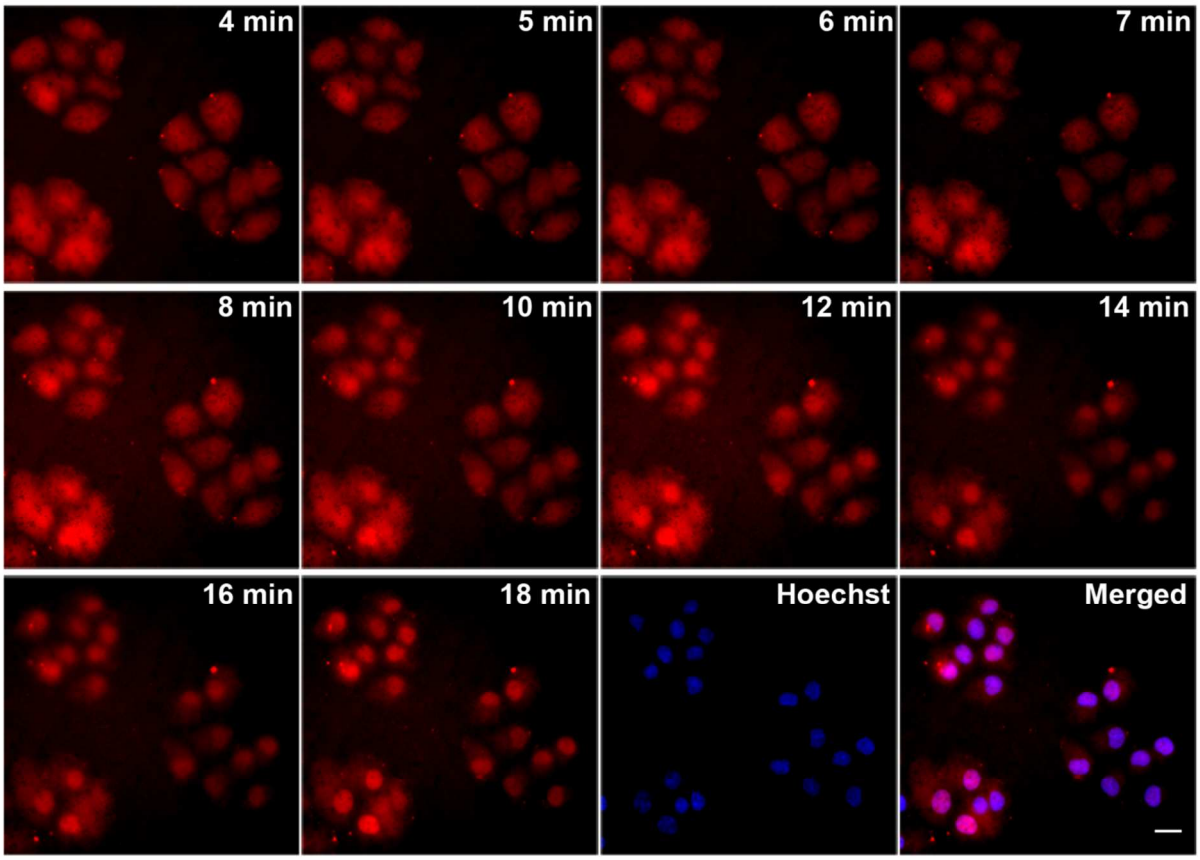


Figure S10. Real-time imaging of RU486-TAMRA mediated nuclear translocation of PR in T47D cells. T47D cells were incubated with 500 nM RU486-TAMRA and 10 μg/mL Hoechst dye for 90 seconds, then washed twice with HBSS, incubated in RPMI 1640 (with no phenol red, supplemented with 5% CDS-FBS) and imaged at indicated times (from start of incubation with the dyes). All images are of same field of view. Scale bar represents 20 μm.

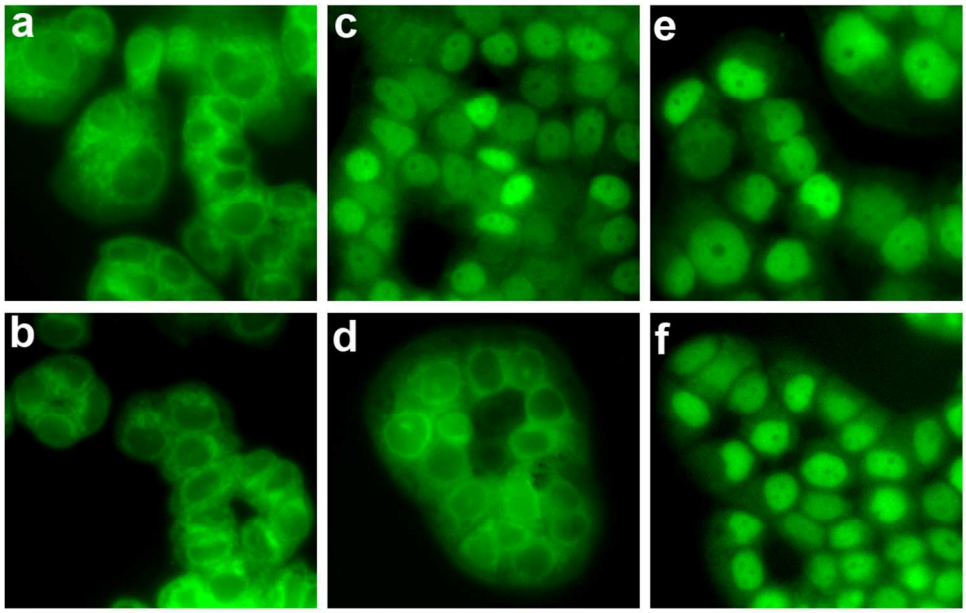


Figure S11. Modulation of PR nuclear translocation by inhibitors of the PR complex. T47D cells were incubated with 10 μM **a**) Vorinostat **b**) Panobinostat **c**) VER155008 or **d**) methylene blue for 12 hours **e**) 10 μM FK506 for 2 hours or **f**) 500 mM EHNA for 1 hour, before being treated with 5 nM RU486-BODIPY. All images are with same field of view.

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