SUPPLEMENTARY INFORMATION

Targeted Protein Oxidation Using a Chromophore-Modified Rapamycin Analog

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1. Synthesis of BORap

All chemicals obtained from commercial sources Acros, Alfa Aesar and Fisher Scientific were used without further purification. All reactions were run in flame-dried glassware under an argon athmosphere. ¹H and ¹³C NMR spectra were acquired on a Bruker Avance III 400 MHz, 500 MHz, or 600 MHz NMR spectrometer with chemical shifts reported relative to either residual CDCl₃ (7.26 ppm) or CD₃OD (3.30 ppm). High resolution mass spectrometry (HRMS) was performed on a Q-Exactive (Thermo Scientific) mass spectrometer.



2-(2-(2-Hydroxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (5). Potassium iodide (43 mg, 0.3 mmol, 0.1 eq), silver oxide (713 mg, 3.1 mmol, 1.2 eq), and *p*-toluenesulfonyl chloride (489 mg, 2.6 mmol, 1 eq) were added to a solution of tri-ethylene glycol (350 µL, 2.6 mmol, 1 eq) in dry dichloromethane (5 mL). The reaction mixture was stirred at room temperature overnight then filtered over celite (4 g). The solvent was evaporated, and the residue was purified via column chromatography on silica gel (15% acetone in dichloromethane) to yield the product as a pale, yellow oil (687 mg, 88% yield), matching the previously reported literature spectra.¹ ¹H NMR (500 MHz, CDCl₃) δ 7.81 (d, 2 H, J = 8.0 Hz), 7.36 (d, 2 H, J = 7.9 Hz), 4.18 (t, 2 H, J = 4.5 Hz), 3.72 (t, 4 H, J = 4.2 Hz), 3.61 (m, 4 H), 3.58 (t, 2 H, J = 4.2 Hz), 2.45 (s, 3 H). ¹³C NMR (125 MHz, CDCl₃) δ 144.9, 129.9, 127.9, 72.5, 70.8, 70.2, 69.2, 68.7, 61.7, 30.9, 21.6. HRMS (M+H)⁺ calcd for C₁₃H₂₁O₆S (M+H)⁺ 305.1053, found 305.1059.

2-(2-(2-Azidoethoxy)ethoxy)ethan-1-ol (1). Sodium iodide (34 mg, 0.3 mmol, 0.1 eq) and sodium azide (220 mg, 3.4 mmol, 1.5 eq) were added to a stirring solution of the tosylate **5** (687 mg, 2.3 mmol, 1 eq) in water/acetone (1:1, 3 mL). The reaction mixture was stirred at 60 °C overnight. The solution was concentrated in vacuo and the remaining aqueous phase was extracted (3 x 5 mL) with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to yield the product as a colorless oil (385 mg, 97% yield), matching the previously reported literature spectra.¹ ¹H NMR (500 MHz, CDCl₃) δ 3.72 (t, 2 H, J = 4.5 Hz), 3.67 (t, 6 H, J = 5.0 Hz), 3.60 (t, 2 H, J = 4.5 Hz), 3.39 (t, 2 H, J = 5.0

Hz). ¹³C NMR (125 MHz, CDCl₃) δ 72.6, 70.7, 70.5, 70.1, 61.8, 50.7. HRMS (M+H)⁺ calcd for C₆H₁₄O₃N₃ (M+H)⁺ 176.10297, found 176.10364.



4-(Prop-2-yn-1-yloxy)benzaldehyde (7). Propargyl bromide (620 μL, 8.2 mmol, 2 eq) was added to a stirring solution of 4-hydroxybenzaldehyde (**6**, 500 mg, 4.1 mmol, 1 eq) and potassium carbonate (793 mg, 5.7 mmol, 1.4 eq) in acetonitrile (8 mL) at reflux and the mixture was heated at reflux for 2 hours. The reaction mixture was allowed to cool to room temperature and was concentrated in vacuo. The residue was redissolved in water (10 mL) and extracted with dichloromethane (3 x 10 mL). The combined organic fractions were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified via column chromatography on silica gel (20% ethyl acetate in hexanes) to yield the product as a white solid (634 mg, 97% yield), matching the previously reported literature spectra.² ¹H NMR (400 MHz, CDCl₃) δ 9.89 (s, 1 H), 7.91 (d, 2 H, J = 12 Hz), 7.19 (d, 2 H, J = 8 Hz), 4.95 (d, 2 H, J = 4 Hz), 3.66 (s, 1 H). ¹³C NMR (125 MHz, CDCl₃) δ 190.8, 162.4, 131.9, 130.6, 115.2, 77.5, 76.3, 55.9. HRMS (M+H)⁺ calcd for C₁₀H₉O₂ (M+H)⁺ 161.0597, found 161.0601.

4,4-Difluoro-1,3,5,7-tetramethyl-8-(4-(propargyloxyphenyl)-4-bora-3a,4a-diaza-s-indacene

(8). A solution of the benzaldehyde 7 (180 mg, 1.1 mmol, 1 eq) in dry tetrahydrofuran (6 mL) was prepared. Trifluoroacetic acid (3 drops) and 2,4-dimethylpyrrole (500 µL, 4.5 mmol, 4 eq) were added and the reaction mixture was stirred overnight at room temperature. A solution of DDQ (510 mg, 2.2 mmol, 2 eq) in dry tetrahydrofuran (8 mL) was added and the reaction mixture was stirred for 4 hours in the dark. Triethylamine (6.5 mL, 46.0 mmol, 40 eq) was added and the reaction mixture was stirred overnight. The mixture was filtered through celite (4 g), the celite was washed with dichloromethane (3 x 5 mL), and the solvents were removed in vacuo. The residue was purified via flash chromatography on silica gel (dichloromethane) to yield the product as a dark red solid (302 mg, 71% yield), matching the previously reported literature spectra.^{3 1}H NMR (500 MHz, CDCl₃) δ 7.19 (d, 2 H, J = 8.5 Hz), 7.08 (d, 2 H, J = 8.5 Hz), 5.97 (s, 2 H), 4.75 (s, 2 H), 2.56 (s, 6 H), 2.54 (s, 1 H), 1.42 (s, 6 H). ¹³C NMR (125 MHz, CDCl₃) δ 158.2, 155.4, 143.1, 141.5, 131.8, 129.3, 128.1, 121.2, 115.7, 56.1, 14.5. HRMS (M+H)⁺ calcd for C₂₂H₂₀ON₂BF₂ (M+H)⁺ 379.1788, found 379.1797.

4,4-Difluoro-2,6-diiodo-1,3,5,7-tetramethyl-8-(4-propargyloxyphenyl)-4-bora-3a,4a-diazas-indacene (4). A solution of the BODIPY alkyne **8** (200 mg, 0.5 mmol, 1 eq) and *N*iodosuccinimide (637 mg, 1.1 mmol, 2 eq) in dry dichloromethane (5 mL) was prepared. The reaction mixture was stirred for 10 hours in the dark, concentrated in vacuo, and the residue was purified via flash chromatography on silica gel (50% dichloromethane in hexanes) to yield the product as a purple solid (296 mg, 89% yield), matching the previously reported literature spectra.³ ¹H NMR (500 MHz, CDCl₃) δ 7.16 (d, 2 H, J = 8.5 Hz), 7.11 (d, 2 H, J = 8.5 Hz), 4.77 (s, 2 H), 2.64 (s, 6 H), 2.57 (s, 1 H), 1.44 (s, 6 H). ¹³C NMR (125 MHz, CDCl₃) δ 158.5, 156.7, 145.3, 141.2, 131.7, 129.2, 127.7, 116.0, 56.1, 17.1, 16.0. HRMS (M+H)⁺ calcd for C₂₂H₂₀ON₂BF₂I₂ (M+H)⁺ 630.9721, found 630.9724.



Triethylene glycol azido rapamycin (3). A solution of triethylene glycol azide **1** (50 mg, 0.3 mmol, 1 eq) and bis(pentafluorophenyl) carbonate (134 mg, 0.4 mmol, 1.3 eq) in dichloromethane (3 mL) was prepared. N,N-Diisopropylethylamine (160 µL, 0.9 mmol, 3 eq) was added and the reaction mixture was stirred for 1 hour. The reaction mixture was concentrated in vacuo and the residue was purified via flash chromatography on silica gel (gradient 10-20% ethyl acetate in hexanes) to yield the carbonate 2 as a colorless oil (106 mg, 91% yield). Identity was confirmed [¹H NMR (500 MHz, CDCl₃) δ 4.47 (p, 2 H, J = 4.5 Hz), 3.80 (p, 2 H, J = 4.8 Hz), 3.67 (m, 6 H), 3.37 (t, 2 H, J = 5.1 Hz)] and the compound was directly used in the next step due to limited stability. A solution of 2 (96 mg, 0.3 mmol, 2 eq), rapamycin (128 mg, 0.1 mmol, 1 eq), and 4dimethylaminopyridine (75 mg, 0.6 mmol, 4 eq) in dry dichloromethane (2 mL) was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo and purified via flash chromatography on silica gel (gradient 30 - 50% ethyl acetate in dichloromethane) to yield the product as a white solid (47 mg, 30% yield). ¹H NMR (500 MHz, CD₃OD) δ 6.36 (dd, 1 H, J = 14.5, 10 Hz), 6.19 (dd, 1 H, J = 14.5, 9 Hz), 6.10 (d, 1 H, J = 10 Hz), 6.02 (d, 1 H, J = 9 Hz), 5.39 (dd, 1 H, J = 16.5, 11 Hz), 5.17 (d, 1 H, J = 8 Hz), 5.00 (m, 2 H), 4.39 (m, 1 H), 4.16 (t, 4 H, J = 6.5 Hz), 4.05 (m, 1 H), 3.90 (d, 1 H, J = 7 Hz), 3.69 (m, 1 H), 3.65 (p, 4 H, J = 6.5 Hz), 3.49 (p, 4 H, J = 6.5 Hz), 3.40 (m, 1 H), 3.39 (s, 2 H), 3.25 (s, 3 H), 3.19 (m, 2 H), 3.05 (m, 3 H), 2.9 (m, 1 H), 2.72 (m, 2 H), 2.55 (m, 1 H), 2.39 (dd, 1 H, J = 16.5, 11 Hz), 2.22 (t, 1 H, J = 6 Hz), 2.16 (m, 1 H), 2.06 (m, 2 H), 2.01 (m, 2 H), 1.81 (d, 2 H, J = 6.5 Hz), 1.75 (s, 3 H), 1.63 (m, 5 H), 1.52 (d, 2 H, J = 7 Hz), 1.32 (q, 2 H, J = 6.5 Hz), 1.21 (m, 2 H), 1.10 (m, 2 H), 0.98 (d, 2 H, J = 6.5 Hz), 0.95 (d, 2 H, J = 7 Hz), 0.90 (d, 2 H, J = 6.5 Hz), 0.81 (d, 2 H, J = 6.5 Hz), 0.77 (d, 2 H, J = 7 Hz). ¹³C NMR (125 MHz, CD₃OD) δ 219.4, 208.2, 169.2, 166.8, 154.8, 140.2 136.1, 135.6, 133.7, 130.2, 129.6, 126.6, 126.4, 98.8, 98.5, 84.8, 84.4, 80.8, 80.3, 77.4, 77.2, 77.0, 76.7, 75.5, 72.5, 69.1, 67.2, 66.8, 61.8, 59.4, 57.5, 55.9, 51.3, 50.7, 46.6, 44.2, 41.5, 40.7, 40.2, 38.8, 38.2, 35.7, 35.1, 33.8, 33.2, 32.88, 31.3, 29.7, 27.3, 27.0, 25.3, 21.5, 20.7, 16.2, 16.0, 15.9, 13.8, 13.1, 10.3, 10.2; HRMS $(M+H)^+$ calcd for $C_{58}H_{91}O_{17}N_4$ $(M+H)^+$ 1115.6374, found 1115.6362.

BORap. A solution of the BODIPY alkyne 4 (10 mg, 0.02 mmol, 2 eq), copper(I) iodide (6 mg, 0.3 mmol, 4 eq), and azido rapamycin 3 (7 mg, 0.07 mmol, 1 eq) in dry dichloromethane (1.75 mL) was prepared. DIPEA (500 µL, 0.3 mmol, 4 eq) was added and the solution was stirred in the dark at room temperature for 2 hours. The reaction mixture was concentrated in vacuo and the residue was purified via flash chromatography on silica gel (gradient 30 - 50% ethyl acetate in dichloromethane) to yield the product as a purple solid (10 mg, 91% yield). ¹H NMR (500 MHz, CD₃OD) δ 8.23 (s, 1 H), 7.31 (s, 4 H), 6.36 (dd, 1 H, J = 14.5, 10 Hz), 6.19 (dd, 1 H, J = 14.5 9 Hz), 6.10 (d, 1 H, J = 10 Hz), 6.02 (d, 1 H, J = 9 Hz), 5.51 (s, 2 H), 5.39 (m, 1 H), 5.17 (d, 1 H, J = 6.5 Hz), 4.98 (m, 2 H), 4.38 (m, 1 H), 4.16 (t, 4 H, J = 6.5 Hz), 4.05 (m, 1 H), 3.88 (d, 1 H, J = 3 Hz), 3.70 (m, 1 H), 3.65 (p, 4 H, J = 6.5 Hz), 3.48 (p, 4 H, J = 6.5 Hz), 3.40 (m, 1 H), 3.39 (s, 2 H), 3.22 (s, 3 H), 3.16 (m, 2 H), 3.05 (m, 3 H), 2.90 (m, 1 H), 2.72 (m, 2 H), 2.62 (s, 6 H), 2.55 (m, 1 H), 2.38 (dd, 1 H, J = 10, 8 Hz), 2.22 (t, 1 H, J = 6.5 Hz), 2.15 (d, 1 H, J = 7 Hz), 2.06 (m, 2 H), 2.00 (m, 2 H), 1.81 (d, 2 H, J = 5 Hz), 1.74 (s, 3 H), 1.63 (m, 5 H), 1.55 (d, 2 H, J = 6.5 Hz), 1.44 (s, 6 H), 1.30 (q, 2 H J = 5 Hz), 1.21 (m, 2 H), 1.11 (m, 2 H), 0.97 (d, 2 H, J = 6.5 Hz), 0.95 (d, 2 H, J = 7 Hz), 0.89 (d, 2 H, J = 5 Hz), 0.81 (d, 2 H, J = 6.5 Hz), 0.77 (d, 2 H, J = 7 Hz). ¹³C NMR (125 MHz, CD₃OD) δ 219.4, 208.2, 169.2, 166.8, 159.4, 156.6, 154.8, 145.3, 141.4, 136.1, 135.6, 133.7, 130.2, 129.8, 127.2, 126.4, 124.3, 115.8, 98.5, 85.6, 84.4, 80.8, 80.3, 77.4, 75.5, 70.5, 69.5, 69.0, 66.7, 62.1, 59.4, 57.5, 55.9, 51.3, 50.4, 46.6, 44.2, 41.5, 40.5, 38.8, 38.2, 35.7, 35.1, 33.8, 33.2, 32.88, 31.3, 29.7, 27.3, 25.3, 21.5, 20.7, 17.2, 16.0, 15.9, 13.8, 13.2, 10.2. HRMS $(M+H)^+$ calcd for $C_{80}H_{110}O_{18}N_6BF_2I_2$ $(M+H)^+$ 1745.6022, found 1745.6071.

2. Photochemical and stability characterization of BORap

HPLC analysis of compound stability. A solution of BORap in phosphate buffered saline (PBS) at pH 7.4 was made at 20 μ M with 10% DMSO and maintained in a 37 °C incubator. Every 60 minutes, 100 μ L was removed for HPLC analysis. HPLC analysis was performed on an Agilent 1200 Series instrument with Agilent InfinityLab Poroshell 120 EC-C18 column (4.6 x 150 mm, 4 μ m particles, P/N 693970-902) with Phenomenex SecurityGuard C18 kit (KJ0-4282). Each sample (50 μ L) was analyzed at a gradient of 35 – 95% acetonitrile in water ran over 40 minutes. The UV detector was set to 280 nm. Each HPLC trace was plotted, along with the integral of each compound peak relative to the integral of the initial peak without incubation.

A solution of **BORap** in 10% fetal bovine serum (FBS) in PBS (pH 7.4) was made at 40 μ M with 10% DMSO and maintained in a 37 °C incubator. Every 60 minutes, 100 μ L was removed and 100 μ L of ice-cold acetonitrile was added to precipitate proteins. Precipitates were pelleted at 15,000 g for 5 minutes at 4 °C. A portion of the supernatant (100 μ L) was removed and transferred to an HPLC vial. HPLC analysis was performed and analyzed as described above.

DPBF singlet oxygen assay. DPBF (Acros) was prepared at 100 mM in DMSO and aliquots were frozen at –20 °C. A working stock solution (10 mM) was prepared by diluting 10 μ L of the 100 mM solution into 90 μ L of DMSO. The "DPBF only" sample was prepared by combining 15 μ L of 10 mM DPBF, 60 μ L of DMSO, and 1425 μ L of 1X PBS (pH 7.4). The "DPBF + **BORap**" sample was prepared by combining 15 μ L of 10 mM DPBF, 5 μ L of 2.86 mM of **BORap**, 55 μ L of DMSO, and 1425 μ L of 1X PBS (pH 7.4). This preparation yielded 100 μ M of **BORap** in a 5% DMSO solution. To a black 96-well flat-bottomed plate, 100 μ L of each prepared solution was transferred to 12 separate wells (24 wells total). Wells were irradiated for 0, 10, 30, or 60 seconds in triplicate using a 530 nm LED (Mouser Electronics, LUMILEDS LXML-PM01-0100) with an output of 130 mW. DPBF fluorescence was monitored using ex. 417/5 and em. 487/5 nm on a Tecan M1000 plate reader. The three independent wells were averaged, and values were normalized such that the 0 second sample was equal to 100. Error bars represent standard deviation of the three replicates.

3. Biological Studies with BORap

Stock solutions of rapamycin and **BORap** were generated at 2.86 mM (5 mg/mL) in sterile-filtered DMSO and stored at –20 °C. HEK293T cells were obtained from ATCC and monitored every three months for mycoplasma contamination. BL21 (DE3) competent cells were prepared in-house.

Cloning of DNA constructs. Top10 cells were used for cloning. FRB-CFP-6xHis and FKBP-YFP-6xHis were generated using Gibson assembly. See **Supplementary Table 1** for a list of all primers used. The FRB fragment (template: mCherry-NLSx3-FRB, primers: P1/P2), the CFP fragment (template: CFP-FRBx5-His, primers P3/P4), the FKBP fragment (template: GFP-FKBP, primers P5/P6), the YFP fragment (template: YFP-FKBP_{x5}-His, primers P7/P8) were PCR amplified using DreamTaq (Thermo EP0713). PCR products were electrophoresed on a 0.8% agarose gel and gel extracted following the manufacturer's protocol (E.Z.N.A. Gel Extraction Kit – Omega BioTek). CFP-FRB_{x5}-His was linearized with Ncol and Notl (from NEB) to generate the backbone fragment. Equimolar amounts of backbone, FRB, and CFP were used to generate FRB-CFP-6xHis, while equimolar amounts of backbone, FKBP, and YFP were used to generate FKBP-YFP-6xHis by Gibson assembly.⁴ Both constructs were confirmed by Sanger sequencing performed by Genewiz using their "T7" forward and "T7 term" reverse sequencing primers.

Bacterial expression of FKBP-YFP-His and FRB-CFP-His. BL21 (DE3) were transformed with either FKBP-YFP-His or FRB-CFP-His and grown on LB agar supplemented with kanamycin (50 µg/mL). Starter expression cultures in LB/kanamycin were prepared and incubated with shaking (250 rpm) overnight at 37 °C. Expression cultures (100 mL for each) were prepared by diluting 1 mL of the saturated starter culture in 100 mL of LB/kanamycin and cells were incubated with shaking (250 rpm) at 37 °C until OD₆₀₀ reached 0.5. Protein expression was induced by the addition of 10 µL of 1 M IPTG (for final 0.1 mM IPTG) and flasks were shaken at 250 rpm overnight at room temperature. After 18 hours, cells were collected into 50 mL conical tubes (2 tubes per expression) and pelleted at 4000 g for 10 minutes at 4 °C. Cell pellets were resuspended in 10 mL of PBS (pH 7.4) supplemented with 1X protease inhibitor (Sigma P8849) via vortexing and kept on ice, then the two identical samples were pooled (20 mL per sample). Cell lysis was performed using an EmulsiFlex C3 Homogenizer (Avestin) in a cold room. Cells were passed through without pressure for 5 minutes, then cycled through with pressure (~20,000 psi) for 15 minutes. Cellular debris was pelleted at 21,000 g for 15 minutes at 4 °C, and the soluble protein fraction was transferred to a fresh tube and kept on ice. Ni-NTA resin (400 µL; G-Biosciences) was added to each sample and incubated with rocking for 90 minutes in a cold room. The immobilized protein on the resin was pelleted at 800 g for 5 minutes, then the supernatant was discarded. The resin was washed thrice with wash buffer (PBS containing 75 mM imidazole). The proteins were eluted in four fractions with PBS plus 250 mM imidazole. Protein purity was assessed using a 10% SDS-PAGE gel, followed by Coomassie staining. After confirming purity, the elution fractions were combined and buffer exchanged in storage and assay buffer (20 mM Tris-HCl pH 7.4, 100 mM NaCl, 10% glycerol) using centrifuge filters (Amicon Ultra, 10 kDa cutoff). Protein concentrations were determined using a BSA standard curve from densitometry analysis of a Coomassie stained protein gel (Supplementary Figure 4).

Gel-shift dimerization assay. FKBP-YFP-His and FRB-CFP-His were diluted to 10 μ M in assay buffer (20 mM Tris-HCl pH 7.4, 100 mM NaCl, 10% glycerol). A master mix of 1:1 FKBP-YFP-His and FRB-CFP-His was prepared by mixing 300 μ L of each 10 μ M protein sample (600 μ L total of

5 μ M FKBP/FRB) and 18 μ L were transferred to 0.2 mL tubes. Compound solutions (rapamycin, **3** or **BORap**) were prepared at 10X (2.5 mM to 0.01 mM) in water (except 1 and 2.5 mM were in DMSO) of the desired final concentrations (250 μ M to 1 μ M) and 2 μ L was added to the protein mixture. Samples were incubated for 2 hours at room temperature. After, 8 μ L of 4X Laemmli buffer without reducing agent (200 mM Tris-HCl pH 6.8, 40% glycerol, 0.08% bromophenol blue) was added to each sample. The entire 28 μ L of sample was loaded and resolved on 10% native-PAGE gels with a 4% stacking gel on ice for 20 minutes at 60 V followed by 80 minutes at 150 V. The gel was Coomassie stained and imaged using a BioRad ChemiDoc system. Images were processed using the BioRad Image Lab software.

Split-luciferase reporter assays. HEK293T cells were plated at 200,000 cells per well in a 6well clear bottom plate and grown at 37 °C, 5% CO₂ in 2 mL per well of Dulbecco's modified Eagle's media (DMEM) supplemented with 10% FBS and 1% streptomycin/penicillin. At 80% confluence, the media on the cells was replaced with 2 mL of DMEM (+ 10% FBS) in preparation for transfection. CLuc-FKBP (Addgene plasmid 31184) and FRB-NLuc (Addgene plasmid 31181) in a 2:1 ratio (2667 ng FKBP:1333 ng FRB) at 4000 total ng (2000 ng/well) were diluted into 500 µL of OptiMEM. Lipofectamine 2000 (10 µL) was diluted into 500 µL of OptiMEM in a separate tube. These two solutions were incubated at room temperature for 5 minutes, the Lipo/OptiMEM solution was mixed into the DNA/OptiMEM solution and mixed by inverting. Following a 20-minute room temperature incubation, 500 µL of transfection mix was added to each well (two wells were transfected identically). Approximately 18 hours after transfection, the media was carefully removed, and the cells were lifted with 500 µL of TrypLe and combined in a 15 mL conical tube. The addition of 9 mL of DMEM inactivated TrypLe. Cells were pelleted at 1,000 g for 10 minutes at room temperature. DMEM was removed and cells were resuspended in 1 mL of Live Cell Imaging Solution (LCIS). Cells were counted on a hemocytometer using Trypan Blue (to avoid counting dead cells). Cells were re-plated at 10,000 cells per well in a black, clear bottom 96-well plate in 90 µL of LCIS.

Split-luciferase reporter – **validation of dimerization.** To determine the optimal compound concentration for dimerization, three 10X stock solutions in LCIS were prepared for each compound, rapamycin and **BORap** (1 μ M, 10 μ M and 100 μ M). A DMSO negative control solution in LCIS was also prepared. The appropriate stock solution (10 μ L) was added to each of three wells in triplicate to obtain a total volume of 100 μ L in each well and final concentrations of 100 nM, 1 μ M, or 10 μ M. Compound treatment was followed by a 2-hour incubation period. After, 90 μ L of BrightGlo reagent was added to each well and incubated for 2 minutes, then luminescence was measured on a Tecan M1000 plate reader. Raw luminescence values were normalized such that the average value of the DMSO treated wells was equal to 1. Average fold-change with error bars representing standard deviation of triplicates was calculated.

Split-luciferase reporter – light induced deactivation. HEK293T cells were plated and transfected in 3 wells of a 6-well clear bottom plate as described above. Compound solutions in 500 μ L of DMEM (– phenol red, + 10% FBS) each were prepared for DMSO, rapamycin (100 nM) and **BORap** (10 μ M). Media was removed and the appropriate compound solution (500 μ L) was added to each of 3 wells. Compound treatment was followed by a 2-hour incubation period, and two 1-hour washes with fresh LCIS to remove any additional compound from the well. Cells were counted and replated in a black, clear bottom 96-well plate as described previously for 15 wells

from each treatment condition (triplicate for five irradiation conditions). Wells were irradiated using a 530 nm LED (130 mW) for 0, 10, 30, 60, or 90 seconds in triplicate. After a 2-hour incubation period in the absence of light, 90 μ L of BrightGlo reagent was added to each well and incubated for 2 minutes, then luminescence was measured on a Tecan M1000 plate reader. Raw luminescence values were normalized such that the average value of the DMSO treated wells was equal to 1. Average fold-change with error bars representing standard deviation of triplicates was calculated.

Split-TEV reporter – light-induced deactivation. HEK293T cells were plated at 10,000 cells per well in a PDK-treated, black, clear bottom 96-well plate and grown at 37 °C, 5% CO₂ in 100 µL per well of DMEM (+ 10% FBS, + 1% streptomycin/penicillin). At 80% confluence, media was replaced with 100 µL of DMEM (+ 10% FBS) in preparation for transfection. A batch transfection mix was prepared for 12 wells using pFKBP-TEVpCT, pFRB-TEVpNT, and PCS2-GFP-TEV in a 2:1:1 ratio (1200 ng FKBP:600 ng FRB: 600 ng GFP-TEV) at 2400 total ng (200 ng/well) diluted into 240 µL of OptiMEM. LPEI (24 µL of a 0.5 mg/mL stock solution) at 12,000 total ng (1000/well) was added and the transfection solution was mixed by inverting, followed by a 20-minute room temperature incubation. Transfection mix (20 µL) was added to each well (12 wells were transfected identically). Compound solutions (500 µL each) in DMEM (- phenol red, + 10% FBS) were made for DMSO, rapamycin (20 µM), and **BORap** (20 µM). Approximately 18 hours after transfection, media was removed and replaced with the appropriate compound solution, followed by a 6-hour incubation period. Media was carefully removed and replaced with 100 µL of LCIS for two 1-hour washes to remove any residual compound. The plate was imaged with a 40X objective and GFP (filter set 38 HE; ex. BP 470/40; em. BP 525/50 nm) filter cube. Cells were irradiated using a dsRed (filter set 43 HE; ex. BP 550/25; em. BP 605/70) filter cube on the microscope for 0 or 90 seconds. Another set of images was acquired the next day (18 hours post-irradiation). Image processing and analysis was performed in FIJI. Images were processed by using the subtract background, despeckle, and smooth functions in FIJI. Images were pseudocolored green to represent GFP fluorescence. Integrated density (mean fluorescence x area of region) was guantified for 20 cells from each condition, averaged, and normalized to the DMSO control. Error bars represent standard deviation.

Membrane translocation reporter – **validation of dimerization.** Hela cells were plated at 10,000 cells per well in a clear bottom 96-well plate and grown at 37 °C, 5% CO₂ in 100 μ L per well of DMEM (+ 10% FBS, + 1% streptomycin/penicillin). At 80% confluency, cells were transfected using linear polyethyleneimine (LPEI). The media on the cells was replaced with 100 μ L of DMEM (+ 10% FBS) in preparation of transfection. A batch transfection mix was prepared for 12 wells as to avoid pipetting small volumes of reagents. Lyn₁₁-FKBP_{x2}-CFP (Addgene plasmid 20149) and YFP-FRB (Addgene plasmid 20148) in a 3:1 ratio (1800 ng FKBP:600 ng FRB) at 2400 total ng (200 ng/well) were diluted into 240 μ L of OptiMEM. LPEI (24 μ L of a 0.5 mg/mL stock solution) at 12,000 total ng (1000/well) was added and the transfection mix (20 μ L) was added to each well (12 wells were transfected identically). Approximately 18 hours after transfection, a 10X stock solution of each rapamycin and **BORap** were made at both 50 μ M and 200 μ M in LCIS. The media was carefully removed and replaced with 90 μ L of LCIS. The plate was imaged on a Zeiss Axio Observer Z1 microscope (20X objective, NA 0.8 Plan-Apochromat)

with a GFP (filter set 38 HE; ex. BP 470/40; em. BP 525/50 nm) filter cube, with an HBO 100 lamp as a light source. Images were acquired every 15 minutes (from 30 minutes pre-treatment to 120 minutes post-addition). Cells were treated at 5 μ M and 20 μ M concentrations of each compound by carefully adding 10 μ L of the appropriate 10X stock solution to each well. Image processing and analysis was performed in FIJI. Images were processed by using the subtract background, despeckle, and smooth functions in FIJI. Images were pseudocolored yellow to represent YFP fluorescence.

Membrane translocation reporter – **light-induced deactivation.** Hela cells were plated and transfected as described above. Approximately 18 hours after transfection, 20 μ M solutions of rapamycin and **BORap** were made in DMEM (– phenol red, + 10% FBS) to allow for 100 μ L of compound solution for each well. The media was carefully removed and replaced with 100 μ L of the appropriate compound solution, followed by a 2-hour incubation period. Media was carefully removed and replaced with 100 μ L of LCIS for two 1-hour washes to remove any residual compound. The plate was imaged on same microscope described above with GFP (filter set 38 HE; ex. BP 470/40; em. BP 525/50 nm. Images were acquired every 15 minutes (from 30 minutes pre-irradiation to 120 minutes post-irradiation). Cells were irradiated using a dsRed (filter set 43 HE; ex. BP 550/25; em. BP 605/70) filter cube on the microscope for 90 seconds. Image processing and analysis was performed in FIJI. Images were processed by using the subtract background, despeckle, and smooth functions in FIJI. Images were pseudocolored yellow to represent YFP fluorescence. Integrated density (mean fluorescence x area of region) was quantified for 20 cells from each condition, averaged, and normalized to the DMSO, non-irradiated control. Error bars represent standard deviation.

Western blot analysis of light-induced deactivation. HEK293T cells were plated in triplicate at 100,000 cells per well in 8 wells of a clear bottom 12-well plate and grown at 37 °C, 5% CO₂ in 1 mL per well of DMEM (+ 10% FBS, + 1% streptomycin/penicillin) until 80% confluent. Media was replaced with 1 mL of DMEM (+ 10% FBS) in preparation for transfection. A batch transfection mix was prepared for each treatment condition (4 wells per condition) as to avoid any variation in transfection efficiency. Lyn₁₁-FKBP_{x2}-CFP and YFP-FRB in a 3:1 ratio (3000 ng FKBP:1000 ng FRB) at 4000 total ng (1000 ng/well) were diluted into 400 µL of OptiMEM. LPEI (40 µL of a 0.5 mg/mL stock solution) at 20,000 total ng (5000/well) was added and the transfection solution was mixed by inverting, followed by a 20-minute room temperature incubation. Transfection mix (100 µL) was added to each well. Approximately 18 hours after transfection, 20 µM solutions of rapamycin and **BORap** were made in DMEM (- phenol red, + 10% FBS) to allow for 500 µL of compound solution for each well. The media was carefully removed and replaced with 500 µL of the appropriate compound solution, followed by a 2-hour incubation period. Media was carefully removed and replaced with 1 mL of LCIS for two 1-hour washes to remove any residual unbound compound. Cells were irradiated for 0, 10, 30, or 60 seconds using a 530 nm LED (using a foil mask to ensure illumination of only the specified well), followed by a 2-hour incubation period in the absence of light.

After this time, the 12-well plate was placed on ice and the cells were lifted and transferred to Eppendorf tubes using the 1 mL of media, and cells were pelleted at 10,000 g for 2 min at 4 °C and the supernatant discarded. Cells were resuspended in 100 μ L of ice-cold RIPA lysis buffer (150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris pH 8.0) with 1X

Halt protease inhibitor cocktail and placed on an orbital shaker (still on ice) for 15 minutes to completely lyse cells. Cellular debris was pelleted at 15,000 g for 7.5 min at 4 °C and 72 µL of each supernatant was transferred to a 0.2 mL tube. Samples were mixed with 30 µL of 4X Laemmli sample buffer (recipe above + 4% β -mercaptoethanol). At this point, 15 μ L of each sample was removed for in-gel fluorescence analysis. The remaining samples were then heated at 95 °C for 10 minutes to denature proteins. Each sample (15 µL) was resolved on a 10% SDS-PAGE gel with a 4% stacking gel on ice at 60 V for 20 minutes followed by 150 V for 80 minutes, then transferred to a 0.45 µM PVDF membrane at 80 V for 105 minutes in Towbin buffer (25 mM Tris-HCl, 192 mM glycine, 20% methanol). Membranes were blocked for 2 hours with 5% milk in TBST (0.1% Tween-20 in 1X tris-buffered saline). After this time, one membrane was cut in half horizontally at the 70 kDa marker. The top half of the membrane was probed with anti-nucleolin and the bottom half was probed with anti-GAPDH, while another entire membrane was probed with anti-GFP. Anti-GFP was diluted 1:5000 and anti-GAPDH was diluted 1:5000 in 5% milk in TBST, while anti-nucleolin was diluted 1:2000 in TBST. The membranes were incubated with the appropriate primary antibodies overnight with rocking in a cold room. The following day, membranes were washed with TBST, then a secondary antibody solution (goat anti-rabbit HRP) was prepared using 1:10000 dilution in TBST. The secondary antibody solution was applied, and membranes were incubated at room temperature for 1 hour with rocking. Membranes were again washed with TBST. Membranes were then developed using SuperSignal West Pico PLUS Chemiluminescent Substrate for 5 minutes and imaged on a BioRad ChemiDoc system using the Chemi Hi Sensitivity setting. Images were processed using the BioRad Image Lab software.

Statistical analysis of triplicate western blot experiments. Statistical analysis of triplicate western blot experiments. Densitometry was performed using the Analyze > Gel feature of ImageJ (FIJI). As the purpose of this experiment is to assess degradation of both the proteins of interest and of a housekeeping protein, individual data sets were normalized to the band intensity of the rapamycin (0 sec) sample, rather than the traditional method of normalizing each band to its corresponding housekeeping intensity. The normalized intensities of the corresponding band for each of the three blots was averaged, and statistical analysis was performed in GraphPad Prism. A two-way ANOVA test was utilized to compare the average band intensities of FKBP, FRB, or nucleolin of irradiated vs non-irradiated samples within each treatment condition (10, 30, or 60 sec vs 0 sec).

In-gel fluorescence analysis of deactivation. The set aside samples from above (15 μ L) were resolved on a 10% SDS-PAGE gel with a 4% stacking gel on ice at 60 V for 20 minutes followed by 150 V for 80 minutes. The gel was imaged on a BioRad ChemiDoc system using the GFP setting. Images were processed using the BioRad Image Lab software.

Western blot analysis of cells treated with proteasome inhibitors. HEK293T cells were plated and transfected in 8 wells of a 12-well clear bottom plate as described previously. Approximately 18 hours after transfection, 6 mL of 20 μ M of **BORap** was prepared in DMEM (– phenol red, + 10% FBS) and 500 μ L of compound solution was used per well, followed by a 2-hour incubation period. The media was carefully removed and replaced with 1 mL of LCIS (supplemented with 10 μ M of epoxomicin or MG132 or no inhibitor) for two 1-hour washes. After the two washes, the media was replaced with a fresh 1 mL of LCIS plus the respective proteasome inhibitor such that

it would be maintained through irradiation and post-irradiation incubations. Cells were irradiated, incubated, lysed and analyzed by western blot as previously described.

Western blot analysis of cells treated with scavengers. HEK293T cells were plated and transfected in 12 wells of a 12-well clear bottom plate as described previously. Approximately 18 hours after transfection, 8 mL of a 20 μ M solution of **BORap** was prepared in DMEM (– phenol red, + 10% FBS) and 500 μ L was used per well, followed by a 2-hour incubation period. ROS scavengers (sodium azide or sodium pyruvate) were prepared at 100 mM in water. Working solutions (10 mM) were prepared in LCIS (1.2 mL of 100 mM scavenger into 10.8 mL of LCIS). The media was carefully removed and replaced with 1 mL of LCIS (supplemented with scavenger) for two 1-hour washes. After the two washes, the media was replaced with a fresh 1 mL of LCIS plus the respective scavenger such that it would be maintained through irradiation and post-irradiation incubations. Cells were irradiated, incubated, lysed and analyzed by western blot as previously described.

Cell viability assay. HEK293T cells were plated at 10,000 cells per well in a PDK-treated, black, clear bottom 96-well plate and grown at 37 °C, 5% CO₂ in 100 µL per well of DMEM (+ 10% FBS, + 1% streptomycin/penicillin). At 80% confluence, half of the plate of cells was transfected using LPEI as detailed previously. The other half of the plate had the media changed and was maintained as a non-transfected sample. The next day, cells were treated with 20 µM of rapamycin or **BORap** for two hours, followed by two 1-hour washes. The media was replaced with 100 µL of DMEM (– phenol red, + 10% FBS), then a subset of wells was irradiated for 0, 10, 30, or 60 seconds (in both the membrane reporter transfected wells and the non-transfected samples). The plate was maintained in the 37 °C, 5% CO₂ incubator for an additional 72 hours. An XTT cell viability assay was performed by adding 40 µL of the activated XTT reagent (8 µL of 1.7 mg/mL menadione diluted into 1 mL of 1 mg/mL XTT reagent solution) to each well. Absorbance was measured at 450 nm and 630 nm (background) on a Tecan M1000 plate reader immediately following reagent addition. Cells were placed back in the incubator for four hours, then final absorbance measurements were taken. The background absorbance was subtracted from each well, then absorbance was normalized such that the non-irradiated sample equaled 100% cell viability. Averages are reported with standard deviation.

4. Supplementary Figures and Tables

Primer	Sequence $(5' \rightarrow 3')$
P1	GTTTAACTTTAAGAAGGAGATATACCATGGGCATCCTCTGGCATGAGATGTGG
P2	TTGCTCACCATACCACCAGCACTAGTCTTTGAGATTCGTCGGAAC
P3	TCTCAAAGACTAGTGCTGGTGGTATGGTGAGCAAGGGCGAGGA
P4	GTGGTGGTGGTGGTGCTCGAGTGCGGCCGCCTTGTACAGCTCGTCCATGCCG
P5	TTGTTTAACTTTAAGAAGGAGATATACCATGGGAGTGCAGGTGGAAACCATCTCC
P6	CACTGCCATACCACCAGCACTTTCCAGTTTTAGAAGCTCCACATCG
P7	AACTGGAAAGTGCTGGTGGTATGGCAGTGAGCAAGGGCGA
P8	GTGGTGGTGCTCGAGTGCGGCCGCCTTGTACAGCTCGTCCATGCCGAG

Supplementary Table 1. List of primers used to generate DNA constructs.



Supplementary Figure 1. BODIPY-rapamycin **BORap** was diluted in phosphate buffered saline at 20 μ M in the absence (A) and presence (B) of 10% fetal bovine serum (FBS) and maintained at 37 °C for 8 hours. Each hour, an aliquot of this solution was analyzed by HPLC. The integration of each peak was normalized to the initial reading and plotted over time (subset). This compound is stable in the presence or absence of FBS for at least 8 hours.



Supplementary Figure 2. Rapamycin and **BORap** were diluted in water at 100 μ M in a 96-well plate and an absorption spectrum of each was obtained using a Tecan M1000 plate reader. The absorbance maximum (λ_{max}) for **BORap** was measured at 542 nm, and the extinction coefficient (ϵ) was determined to be 46,208 M⁻¹cm⁻¹.



Supplementary Figure 3. Rapamycin and **BORap** were diluted in water at 100 µM. **BORap** samples were irradiated with 530 nm light in a 96-well format for 0, 10, 30, 60, or 90 seconds. Samples were incubated at room temperature for 2 hours, followed by analysis by HPLC. **BORap** is stable to the irradiation/incubation conditions used in subsequent experiments.



Supplementary Figure 4. In order to determine protein concentrations, recombinant FKBP-YFP-His (A) and FRB-CFP-His (B) were loaded on an SDS-PAGE gel using three different dilution factors (1:2, 1:5, and 1:10). BSA standards were loaded on each gel (1, 0.5, 0.25, 0.125, and 0.0625 mg/mL) in order to generate a standard curve. After Coomassie staining each gel, protein concentrations were determined to be 58.2 (FKBP-YFP-His) and 73.1 μ M (FRB-CFP-His) using the BSA standard curve from densitometry analysis.



Supplementary Figure 5. A) The fluorescent sensor, DPBF, reacts with singlet oxygen to generate an unstable peroxide intermediate that rapidly decomposes into a non-fluorescent product. B) DPBF was utilized to measure the singlet oxygen generation capabilities of **BORap**. DPBF was kept in 10-fold excess of **BORap** such that 100 μ M of DPBF and 10 μ M of **BORap** was used. Irradiation with 530 nm light was tested in a dose-response manner, showing a significant decrease in fluorescence in response to increasing irradiation times, thus demonstrating that **BORap** generates singlet oxygen when irradiated with 530 nm light. C) To validate 530 nm is an ideal irradiation wavelength for generation of singlet oxygen, seven other commonly utilized irradiation wavelengths were also tested, specifically 365 nm (66 mW), 390 nm (60 mW),

405 nm (70 mW), 415 nm (70 mW), 448 nm (70 mW), 490 nm (60 mW), and 655 nm (85 mW), and compared to 530 nm (75 mW). Light intensity measurements were performed using a ThorLabs power and energy meter console (PM200) with sensor (S170C). For all wavelengths, a 60 second irradiation was utilized, as to produce the maximum amount of singlet oxygen. Irradiation of the DPBF reporter alone showed minimal change in fluorescence, while irradiation of the sample containing **BORap** showed largest decreases in fluorescence when irradiated with 365 nm, 405 nm, 490 nm, or 530 nm.



Supplementary Figure 6. Recombinant FKBP-YFP-His and FRB-CFP-His (5 µM each) were used for dimerization studies. Solutions of FKBP/FRB were incubated with A) rapamycin, B) BODIPY-rapamycin **BORap**, and C) azido-rapamycin **3** at increasing concentrations for two hours, then analyzed via native PAGE to assess protein dimerization through ternary complex formation. While **BORap** is an efficient dimerizer, it requires 50-fold higher concentration than rapamycin.



Supplementary Figure 7. Hela cells expressing both Lyn₁₁-FKBP_{x2}-CFP and YFP-FRB in a 3:1 ratio were treated with either rapamycin or **BORap** at both 5 and 20 μ M concentrations. Images were acquired over the course of two hours to monitor for membrane translocation and the YFP images are provided here pseudocolored in yellow. Treatment with 20 μ M of **BORap** resulted in the most rapid membrane accumulation, thus this concentration was utilized in subsequent experiments. Scale bar equals 10 μ M.



Supplementary Figure 8. Hela cells expressing both Lyn₁₁-FKBP_{x2}-CFP and YFP-FRB in a 3:1 ratio were treated with either rapamycin or **BORap** at 20 μ M concentrations. Cells were irradiated through a DsRed filter for 0 or 90 seconds. Images were acquired over the course of two hours. The YFP images are provided here pseudocolored in yellow. Treatment with 20 μ M of **BORap** resulted in a decrease in YFP signal. Scale bar equals 10 μ M.

rapamycin				BORap				rapamycin				BORap					rapar	n	BORap					
0	10	30	60	0	10	30	60	0	10	30	60	0	10	30	60	0	10	30	60	0	10	30	60	irradiation time (sec)
-	-	-	-	-	-	-	-	-	-	-	-	-	-	***	-	-	-	-	-	-		-		Lyn ₁₁ -FKBP _{x2} -CFP
-	-	-	-	-	-	-		-	-			-	$\hat{\mathbf{e}}_{i'}$	$\{ r^{\ast} \}_{i}$	14	-	-	-	-	-	-			YFP-FRB
_	-	-	-	_	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nucleolin

Supplementary Figure 9. Biological replicates of western blot analyses shown in Figure 4.



Supplementary Figure 10. Prior to denaturing samples for western blot analysis, an aliquot of native proteins was collected and used to analyze in-gel fluorescence. As in the western blot experiment, a decrease in signal is observed with **BORap**, but not rapamycin treatment.



Supplementary Figure 11. Non-transfected (A) or membrane reporter transfected (B) HEK293T cells were incubated with 20 µM of rapamycin or **BORap** for two hours, then washed twice for one hour each time, and irradiated for the indicated time. Cells were maintained in the incubator for an additional 72 hours prior to cell viability testing with an XTT assay. No apparent toxicity was observed for the irradiated **BORap**-treated samples. Average signal intensity is plotted with error bars representing standard deviation.

5. NMR Spectra of New Compounds







6. References

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