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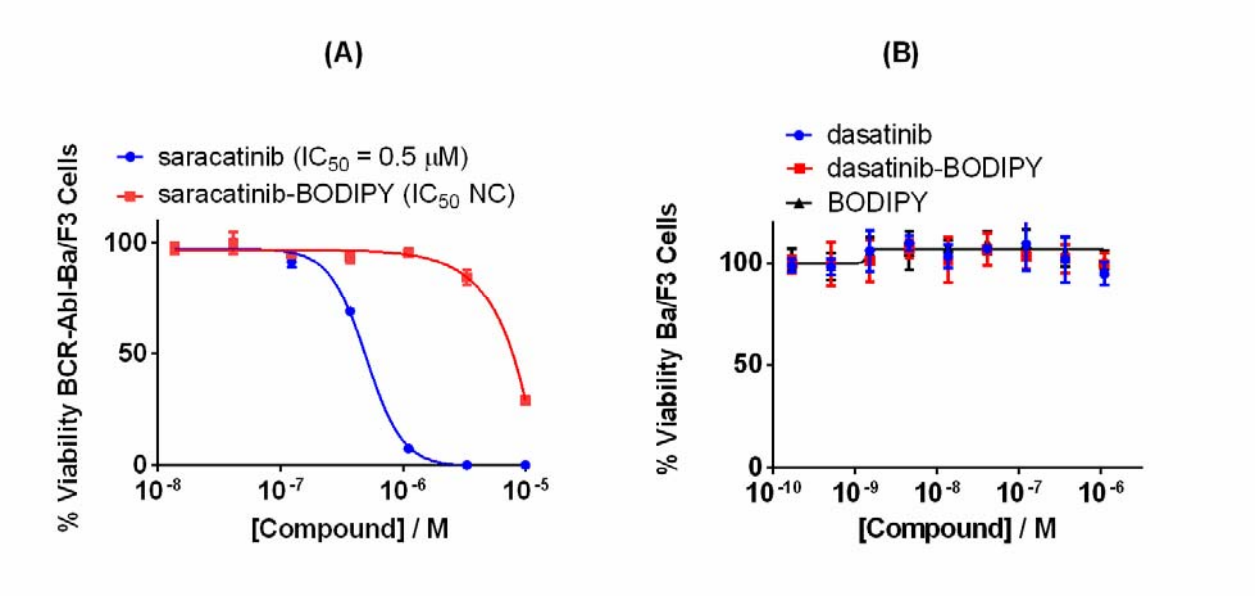
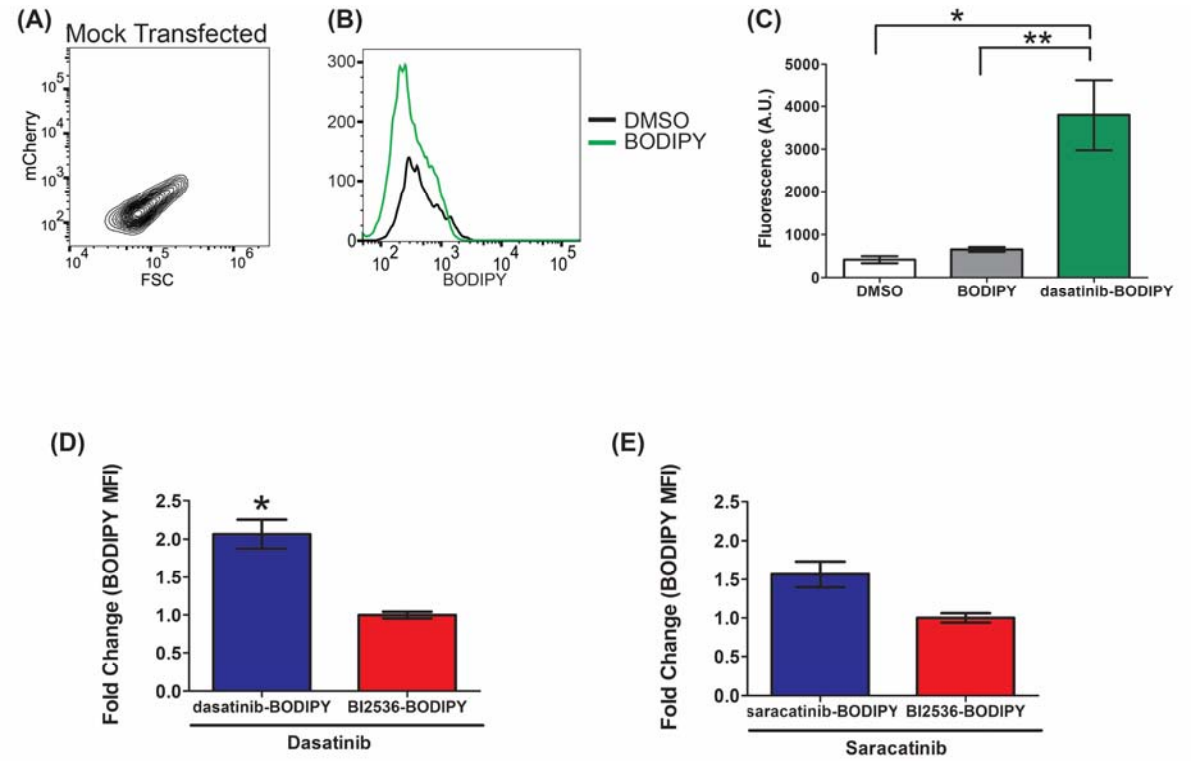
Supporting Data 1. Synthesis and characterization of dasatinib-BODIPY and saracatinib-BODIPY. Schematics are presented in Figure 1 of the main article.

Compound Characterization: ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.48 (s, 1H), 9.86 (s, 1H), 8.20 (s, 1H), 7.68 (s, 1H), 7.38 (d, *J* = 7.7 Hz, 1H), 7.26 (dd, *J* = 16.6, 7.5 Hz, 2H), 7.08 (d, *J* = 3.8 Hz, 1H), 6.42 (d, *J* = 3.9 Hz, 1H), 6.28 (s, 1H), 6.05 (s, 1H), 3.57 (s, 5H), 3.54 (s, 2H), 3.28 (s, 1H), 3.05 – 3.12 (m, 2H), 2.73 – 2.80 (m, 2H), 2.46 (s, 3H), 2.41 (s, 3H), 2.24 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.07, 165.64, 162.94, 162.66, 159.50, 158.37, 157.39, 149.09, 144.47, 139.25, 134.85, 133.95, 133.47, 132.86, 129.46, 129.35, 127.45, 125.77, 120.68, 117.60, 83.20, 44.49, 43.89, 43.66, 41.08, 31.74, 26.03, 24.22, 18.75, 14.96, 11.46; MS (ESI) 718.3 [M+H$^+$]; HRMS (ESI) [M+H$^+$] for $C_{34}H_{36}BClF_2N_9O_2S$ 718.2462 (Calc.), 718.2465 (found).

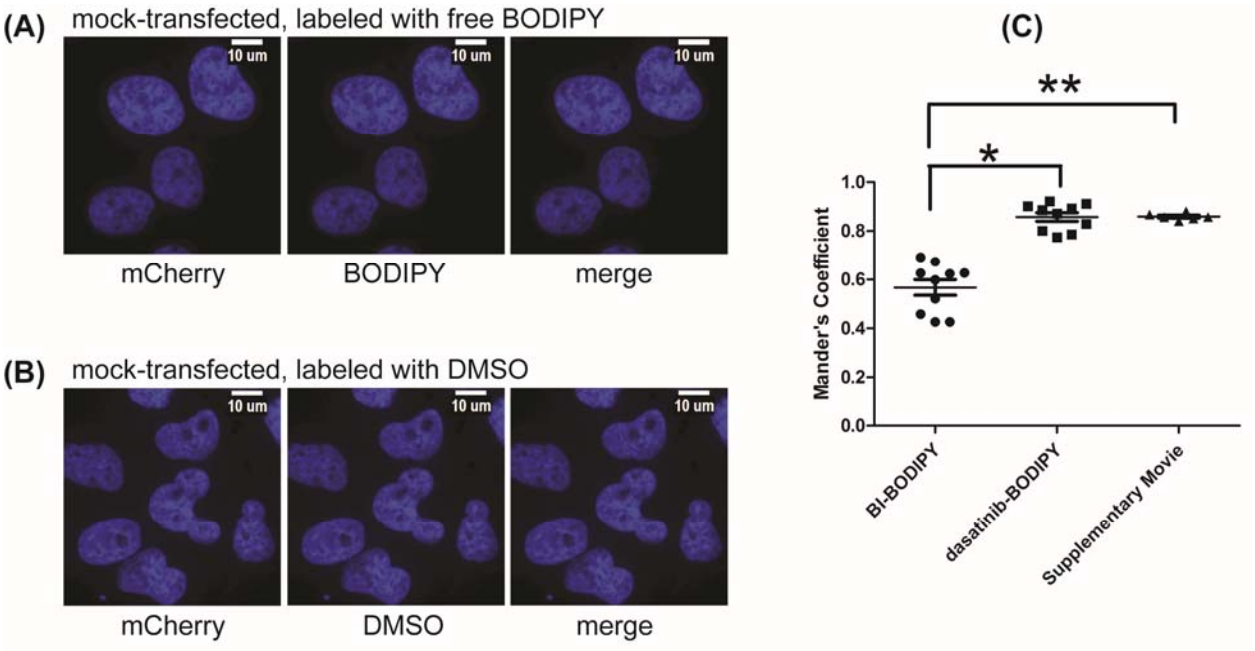
Compound Characterization: ^{1}H NMR (300 MHz, CD_3OD): δ 8.30 (s, 1H), 7.46 (s, 1H), 7.01-7.06 (m, 2H), 6.79-6.88 (m, 3H), 6.34 (d, *J* = 4.1 Hz, 1H), 6.22 (s, 1H), 6.06 (s, 2H), 4.28 (m,1H), 3.90-4.03 (m, 2H), 3.47-3.69 (m, 10H), 2.73-2.93 (m, 4H), 2.53-2.67 (m, 4H), 2.50 (s, 3H), 2.28 (s, 3H), 1.71 – 1.81 (m, 2H), 1.41-1.43 (m, 2H). MS (ESI) 802.2 [M+H$^+$]; HRMS (ESI) [M+H$^+$] for $C_{40}H_{44}BClF_2N_7O_6$ 802.3103 (Calc.), 802.3110 (found).



Supporting Figure 1. Cellular activities of saracatinib-BODIPY and dasatinib-BODIPY on the viability of BCR-Abl Ba/F3 and Ba/F3 cells. (A) Inhibition of the proliferation of BCR-Abl kinase-dependent Ba/F3 cells was used as a proxy to demonstrate cell penetrance as well as intracellular kinase recognition and inhibition. Cell viability was measured in the presence of increasing concentrations of saracatinib and saracatinib-BODIPY. Although and IC_{50} for saracatinib-BODIPY was not calculated due to lack at sufficiently high concentrations of compound, saracatinib-BODIPY appears to have lost approximately 10-fold of saracatinib's potency as an inhibitor of BCR-Abl Ba/F3 cell proliferation. **(B)** Increasing concentrations of dasatinib, dasatinib-BODIPY, and free BODIPY were added to parental Ba/F3 cells. No cell death was observed in the parental Ba/F3 cells supporting the conclusion that cell death seen in BCR-Abl-Ba/F3 cells is due to kinase inhibition. Note that data for dasatinib and dasatinib-BODIPY against BCR-Abl Ba/F3 cells are presented in Figure 2 of the main article.



Supporting Figure 2 Analysis of flow cytometric data. (A) Analysis of mCherry fluorescence versus forward scatter for mock transfected Huh7 cells demonstrates the absence of a mCherry^{hi} population (compare to Figure 3A in main article). **(B)** Src-mCherry-expressing Huh7 cells labeled with free BODIPY exhibit BODIPY fluorescence equal to that of the DMSO-labeled negative controls whereas **(C)** cells labeled with dasatinib-BODIPY exhibit an increase in BODIPY fluorescence intensity that is statistically significant (dasatinib-BODIPY versus DMSO, *p = 0.023; dasatinib-BODIPY versus free BODIPY, **Student's t-test p value = 0.028. **(D and E)** Statistical analysis of the competition experiments depicted in Figure 2B-D. Briefly, Src-mCherry-expressing Huh7 cells were stained with 100 nM dasatinib-BODIPY, saracatinib-BODIPY, or BI-BODIPY in the presence or absence of unlabeled 10 μM dasatinib or saracatinib as indicated below each plot. Cells were gated on the mCherry^{hi} population for data collection and analysis. The mean BODIPY fluorescence intensity (BODIPY MFI) of each sample was determined using FlowJo software, and the fold change in MFI caused by the addition of unlabeled dasatinib or saracatinib competitor was calculated. The graphs illustrate the average fold change in BODIPY MFI caused by the presence of the unlabeled competitor in four independent replicates of the experiment. **(D)** Excess, unlabeled dasatinib reduces the BODIPY MFI of Src-mCherry^{hi} cells labeled with dasatinib-BODIPY but has no effect on the BODIPY MFI of cells labeled with BI-BODIPY. The difference in the effect of the dasatinib competitor on the BODIPY MFI dasatinib-BODIPY and BI-BODIPY was statistically significant, with a Student's t-test p value of 0.001 (indicated by "*"). **(E)** The analogous experiment performed with excess saracatinib indicated that excess, unlabeled saracatinib has a comparable effect on the BODIPY MFI of mChery^{hi} cells labeled with saracatinib-BODIPY and BI-BODIPY (Student's t-test p value = 0.051), suggesting that saracatinib-BODIPY labeling of cells is not due to binding of the conjugate to known targets of saracatinib.



Supporting Figure 3. Analysis of confocal fluorescence microscopy data. To demonstrate that labeling of cells with dasatinib-BODIPY resulted in significant BODIPY signal over background, mock-transfected Huh7 cells were treated with **(A)** 100 nM free BODIPY or **(B)** DMSO for two hours and were then processed for imaging as described in Materials and Methods. For imaging, samples were sequentially excited with a laser at 405 nm, 488 nm and 561 nm, and signal was measured through emission filters of 452/45 nm (DAPI), 525/50 nm (BODIPY), and 607/36 nm (mCherry). The mCherry and BODIPY fluorescence of these cells was indistinguishable from background. **(C)** Colocalization analysis of the microscopy images depicted in Figure 4B-D was performed using the JACoP in ImageJ (*Journal of Microscopy* **2006**, *224*, 213-232). This coefficient relies on analysis of pixel intensities to determine the proportion of signal in the BODIPY channel that is coincident with signal in the mCherry channel. A coefficient of 0 represents no colocalization and a coefficient of 1 represents complete colocalization of dasatinib-BODIPY with Src-mCherry. Mander's coefficients were calculated for for ten representative images taken from the BI-BODIPY-labelled fixed cell samples (*e.g.,* Figure 4C), ten representative images for dasatinib-BODIPY-labelled fixed cell samples (*e.g.,* Figure 4B), and for each of the images collected for the live cell imaging movie (Supporting Movie 1). Analysis of the differences in Mander's coefficients of BI-BODIPY versus dasatinib-BODIPY (fixed cell) and BI-BODIPY versus the dasatinib-BODIPY (live cell) samples by the Student's *t*-test demonstrates that the colocalization of dasatinib-BODIPY with Src-mCherry is statistically significant (* $p = 6.2 \times 10^{-7}$, ** $p = 0.002$)

Supporting Movie 1. Live-cell imaging of Src-mCherry-expressing Huh7 cells labeled with dasatinib-BODIPY. Huh7 cells were transfected with the Src-mCherry expression plasmid, and then 48 hours post-transfection incubated with 100 nM dasatinib-BODIPY or BI-BODIPY for two hours. Samples were then placed in an AttoFluor Cell Chamber (Invitrogen) and perfused with DMEM medium lacking phenol red. The cell chamber was then placed in a heated stage plate over the objectives and maintained at 37°C. The air above the cells was humidified and maintained at 37°C, 5% CO_2 for the duration of the experiment. Samples were imaged every 30 seconds for 2.5 minutes. All images were acquired using a QuantEM (Photometrics) cooled charge-coupled device as part of a CSU-X1 spinning disk confocal system (Yokogawa Electric Corporation) using a 100X, 1.4NA objective (Zeiss). Samples are sequentially excited by 405nm (DAPI), 488 nm (BODIPY) and 561 nm (mCherry) Lasers (Coherent) and signal is collected through emission filters 452/45 nm, 525/50 nm and 607/36 nm (Semrock), respectively. Initial data capture was performed using Slidebook software (Intelligent Imaging Innovations). Further processing was performed using Metamorph (Molecular Devices). Arrowheads have been added to highlight colocalization of mCherry and BODIPY signals over time. These live-cell imaging results further demonstrate the utility of dasatinib-BODIPY in imaging applications as colocalization with the Src-mCherry target can be seen in live and fixed cell images.