Supplemental Materials and Methods

PRMT5:MEP50 cloning and protein production

Full length human PRMT5 was cloned into pFastBac1 (ThermoFisher), and human MEP50 (WDR77) was cloned into a modified pFastBac1 vector with an N-terminal 6xHis and TEV cleavage site (MASHHHHHHDYDGATTENLYFQGS). Human pIClN (CLSN1A) and SMD3(SNRNPD3) were cloned into pET vectors (Novagen). The human pIClN expression vector is untagged and SmD3 expression constructs were modified with an N-terminal 6xHis and TEV cleavage site (MASHHHHHHDYDGATTENLYFQGS). PRMT5 and MEP50 proteins were co-expressed in the Bac-to-Bac baculovirus (ThermoFisher) system using manufacturer's protocol in Sf21 cells for 72 hrs in ESF-21 protein free media. SmD3 and pICLN were co-transformed into the BL21-AI strain of *E. Coli* in TB media at 37°C. Once the culture reached density, ITPG (0.4 mM) and arabinose (0.02%) was added and grown at 16°C overnight.

Cell pellets of expressed PRMT5/MEP50 complex or SmD3/pICLN complex were lysed in 50 mM Tris pH7.4, 150 mM NaCl, 10% glycerol and 0.25 mM TCEP with protease inhibitor, then stirred at 4°C for 1 hr. Lysate was centrifuged at 10,000g for 1 hr at 4°C. Supernatant was incubated with Ni-probond resin for 2 hr, resin, washed with lysis buffer containing 20 mM imidazole, and eluted with 200 mM imidazole buffer. After TEV digest overnight to remove His-tags on MEP50 or pICLN, the sample was passed through Ni-probond column (Invitrogen #R80101) to remove TEV. The flow through was concentrated and further purified on a Superdex 200 26/60 column.

PRMT5 Biochemical assay

Full length PRMT5/MEP50 protein complex was combined with H4(1-21) peptide (SGRGKGGKGLGKGGAKRHRKV) or SmD3 (99-119) peptide (KAAILKAQVAARGRGRGMGRG) in PRMT5 assay buffer (50 mM Tris pH 8.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM TCEP or DTT) and 44 µl was added to the microtiter plate containing compound. S-Adenosyl-L-methionine (SAM) was prepared by combining ^3H labeled SAM with unlabeled SAM in PRMT5 assay buffer such that the total SAM concentration was 5 times the desired SAM concentration for the assay, the concentration of ^3H labeled SAM was 0.5 µM. The reaction was initiated by adding 10 µl of SAM stock to the microtiter plate. The final reaction conditions were 0.75 - 3 nM PRMT5/MEP50 complex, 200 nM H4 peptide or 250 nM SmD3 peptide and various SAM concentrations typically between 1 and 10 µM. Following a 25-60 minute incubation at room temperature, the reaction was stopped with the addition of 100 µL of 20% TCA. The ^3H-peptide product was captured using a 96-well filter plate (MSIPN4B, Millipore) and washed 5 times with PBS buffer. Scintillation fluid (100 µl) was added to the dried filter plate and ^3H-methyl peptide product was quantified using a liquid scintillation counter.

For SAM K_m^{app} and k_{cat}^{app} determinations, enzyme activity was monitored as a function of SAM concentration and a fixed (100 nM) concentration of SmD3 (99-119) peptide substrate. Peptide K_m^{app} is estimated to be 10 nM. Enzyme concentration was 0.75 nM for WT complex, 1.6 nM for F327L complex and 1.1 nM for M420T complex. Cold SAM was mixed with ^3H SAM, such that the final SAM concentration was 5x the highest SAM concentration desired for the titration, followed by 2x serial dilution in assay buffer or into assay buffer with 0.5-1 uM ^3H SAM. For WT and F327L enzyme complexes, the highest SAM concentration used was 2.5 µM (2.375 µM

cold SAM, 0.125 μM ^{3}H SAM). For the M420T mutant complex, the top SAM concentration used was 20 μM (19 μM cold, 1 μM hot). Data were fit to the Michaelis-Menton equation using GraphPad Prism software. SAM specific activity (cpm/pmol of SAM) was calculated by spotting a small amount of the 5x SAM solution onto a wet filter after filtration was completed and processing alongside the reaction wells. Active enzyme concentration was estimated by active site titration using multiple tight binding inhibitors [32], including PF-06855800 and PF-06939999.

For inhibitor K_i determinations, compounds were solubilized in DMSO and serially diluted 3-fold into 100% DMSO at a concentration 50-fold greater than the desired final assay concentration. Following dilution, 1 μl was added to an empty 96-well microtiter plate prior to addition of enzyme or substrates. Compounds with IC_{50} values below 10 nM were tested at 10 μM SAM concentration, all others were tested at a final SAM concentration of 1 μM.

K_i values were determined by fitting the data to the tight binding competitive inhibition equation using Pfizer proprietary software. For EPZ015666, inhibition was measured at saturating concentrations (33x K_m) of SAM and K_i values were estimated by fitting the data to the equation for competitive inhibition with respect to the peptide substrate. The lower limit of quantitation for the assay with WT PRMT5/MEP 50 was conservatively estimated to be 5 pM based on 0.75 nM active enzyme concentration and [S]/K_m for SAM = 33.

For mechanism of inhibition studies with PF-06855800, K_i^{app} values were determined as multiple different SAM concentrations using the same assay as for K_i determination except that the data

were fit to the Morrison equation for tight binding inhibitors without the competitive inhibition component, generating K_i^{app} [32]. K_i^{app} (=IC_{50}) was plotted as a function of [SAM]/K_m. For PF-06939999, the K_i cannot be accurately determined, particularly at low [SAM]/K_m ratios. The off-rate for the enzyme inhibitor complex is about 10 hours so equilibrium K_i^{app} values cannot be determined in a 2 hour assay. In order to investigate whether PF-06939999 is competitive against SAM, an experiment was set up so that the on-rate of PF-06939999 would be decreased by the presence of competing substrate. PRMT5/MEP50 (0.75 nM) was incubated with 0.3-30 µM SAM for 15 minutes at RT. The enzyme:SAM mix was added to serially diluted PF-06939999 and the reaction was immediately initiated with SMD3 peptide (250 nM). K_i apparent values were determined by fitting the data to the Morrison equation for tight binding inhibitors [32]. K_i apparent was plotted as a function of [S]/K_m for SAM.

SPR

Biacore T200 instrument was desorbed and loaded with a Series S Sensor Chip SA. Biotinylated PRMT5·MEP50 complex was diluted to 50 µg/mL with assay buffer (25 mM HEPES, 150 mM NaCl, 1 mM TCEP, 0.02 % Tween-20, 1% DMSO, pH 7.4) and injected into ligand channel at a flow rate of 5 µL/min and a contact time of 5 min at 25 ºC. Approximately 3000 RU of PRMT5·MEP50 complex was captured on the streptavidin-coated biosensor chip. The functionalized surface was then equilibrated with assay buffer for approximately 2 hours. An un-functionalized channel was used as a reference surface for binding kinetic analysis. A five-fold, 5-point serial dilution of test compounds was set-up in a deep 96-well microplate (Greiner; Cat # 780201). Binding kinetics was measured at 25 ºC or 37 ºC in a single-cycle kinetics format by

injecting serial dilution of compounds onto reference and ligand channel at a flow rate of 100 µL/min and association time of 200 seconds. Compound dissociation was monitored for 3000-5000 seconds. Two buffer blanks were also run in a single-cycle kinetics format before the compound run for double referencing. No additional regeneration was used. DMSO calibration curve was obtained before and after compound analysis by injecting 0-2% of DMSO in running buffer. Data analysis was performed using Biacore T200 analysis software. The double-referenced and solvent-corrected data was fit to 1:1 Langmuir model to obtain binding constant (K_D) and binding kinetics (k_{on} and k_{off}) information. The adequateness of the fit was judged by χ^2 values (lower than 5% of the R_{max}) and the randomness of residue distribution. The raw kinetic data as well as the fit were then imported into Prism software (GraphPad Software, Inc.) for visualization.

Pharmacokinetic assay in rats

PF-06939999 was administered to 8 week old male Wistar-Han rats (n = 2/group) at the doses of 2 mg/kg intravenously and 10 mg/kg orally. Blood samples were collected at 0.033 (intravenous only), 0.083, 0.25, 0.5, 1, 2, 4, 7 and 24 hours post-dose to quantify plasma concentrations of PF-06939999 by liquid-chromatography tandem mass spectrometry. Pharmacokinetic parameters including maximal plasma concentration (Cmax), time to Cmax (tmax), area under the plasma concentration-time curves (AUC) and half-lives (t1/2), were determined by non-compartment pharmacokinetic analysis.

Elastic Net Analysis

Dose response curves were fit as previously described. [46]

The elastic net analysis was performed as previously described [46] using cell line AUC and EC_{50} data as input response vectors in which to identify predictive features. Feature datasets were extracted from the CCLE [47] using log2 transformed TPM data from RNA sequencing and SNP copy number profiles. Non-synonymous mutation annotations (De_novo_Start_OutOfFrame, Missense_Mutation, Nonsense_Mutation, Splice_Site, Start_Codon_SNP) were extracted from maf files using the 1,346 cell lines that were profiled with whole exome sequencing. EC50 vectors were log transformed prior to input into the elastic net. Supplementary data S3 provided list elastic net calculated weights and bootstrapped frequencies. No feature reached a minimum bootstrapped frequency of .5 to be considered.

RBM10 stable clone generation

NCI-H1975 cells were seeded in 6-well plate at 10^6 cells/well (RPM11640 + 10% FBS + 1% Antibiotic-Antimycotic (ThermoFisher, Cat# 5240096)) and incubated at 37˚C, 5% CO_2 overnight. Prior to transfection, medium was replaced with 1.5 ml/well fresh RPMI medium without antibiotics. Transfection was performed using Lipofectamine 2000 (ThermoFisher 1901464) following manufacturer's recommended protocol with 3 ug RBM10 cDNA plasmid (Origene, Cat# RC200150, NM_005676). Transfection was stopped by feeding cells with fresh medium in the presence of 800 ug/ml G418 (ThermoFisher, Cat No 10131-027) and continued culture for 8 days in the 6-well plate before further expansion and selection. The transfected cell pool was maintained in complete RPMI1640 medium plus 800 ug/ml G418 with medium change every 4 days. For stable clone generation, cells from each transfected pool were seeded in three 150 mm dishes at 2000 cells/dish and cultured with complete RPMI1640 medium plus 800 ug/ml

G418 until the appearance of colonies. Individual clones were picked up and expanded in complete RPMI1640 medium with G418. Clones which displayed RBM10 protein expression, similar to endogenous RBM10 in NSCLC cell lines were selected for further studies.

Cellular Fractionation –

Cells were washed with cold PBS, resuspended in 4x volume of swelling buffer (10 mM Tris pH 8.0, 1.5 mM $MgCl_2$, 10mM KCl, protease inhibitor cocktail). Following a 15 minute incubation on ice, 1x swelling buffer + 1% Triton X-100 was added and mixed. Lysate was centrifuged at 1000g for 10 minutes at 4°C. Supernatant containing cytoplasmic fraction was put in a new tube and NaCl added to a final concentration of 200 mM. The nuclear pellet was washed 5x with swelling buffer + 0.2% Triton X-100 by vortexing and centrifuging pelled at 1000g for 3 minutes at 4°C, then suspended in nuclear extraction buffer (10 mM Tris pH 8.0, 1.5 mM $MgCl_2$, 10 mM KCl, 400 mM NaCl, 0.4% Triton X-100, protease inhibitor cocktail) and vortexing for 30 minutes at 4°C. Nuclear lysate was centrifuged at 16,000g for 15 minutes at 4°C and supernatant removed.

Immunofluorescence

Cells were grown on chamber slides, fixed in ice-cold methanol for 10 minutes at -20°C, then washed twice with ice-cold PBS. Slides were incubated with PBS + 0.25% Triton X-100 for 10 minutes at room temperature, then washed three times in PBS. Slides were blocked using 1% BSA in PBST (PBS + 0.1% Triton X-100) for 60 minutes at room temperature. Blocking buffer

was removed, replaced with PRMT5 antibody (Millipore #07-405) diluted 1:100 in 1% BSA in PBST and incubated at 4°C overnight. PRMT5 antibody was removed and slides washed three times in PBS for 5 minutes each. Fluorochrome-conjugated antibody was diluted in 1% BSA in PBST and slides incubated at room temperature for 1 hour in the dark. Secondary antibody was removed and samples washed three times in PBS for 5 minutes each in the dark. Chamber was removed and slides were coverslipped with Prolong Diamond Antifade Mountant with DAPI (Thermofisher #P36971) prior to imaging.

Western blotting

Cells were lysed in RIPA buffer + protease/phosphatase inhibitor, sonicated and cleared by centrifugation. Histones were purified using the Histone Purification Kit (Active Motif). Protein lysates were loaded at 20 μg and histones at 500 ng. Proteins were separated on 4-12% bis-tris polyacrylamide gels in MOPS running buffer and transferred to nitrocellulose membranes. Membranes were blocked in Odyssey blocking buffer, then incubated with primary antibody overnight at 4°C. Primary antibody dilutions were as follows: anti-SDMA (CST #13222), PRMT5 (Millipore #07-405), Lamin A/C (CST #2032), H3R8me2s (Abcam #ab130740), H2AR3me2s (Abcam #ab22397), H4R3me2s (Abcam #ab5823), H3K27me3 (Cell Signaling #9733), PARP (CST #9452), cleaved PARP (CST #5625), p53 (Millipore #05-224), p21 (CST #2947), cleaved caspase-3 (CST #9664), and RBM10 (Abcam #ab26046) at 1:1000, anti-tubulin (Sigma #T6199), total histone H3 (Abcam #ab10799) and beta-actin (CST #3700) (1:5000), total histone H4 (Abcam #ab31830) (1:10,000). Membranes were washed 5X with PBS + Tween 20 (0.1%) (PBST), and incubated with secondary antibody (anti-rabbit or anti-mouse at 1:20,000) (LI-COR #926-68070, 926-32211) for 1 hour at room temperature. Membranes were washed 5X

in PBST and imaged using a LI-COR Odyssey CLx imager. Bands were quantified using the LI-COR Image Studio software.

Senescence assays

A549 cells were seeded in 6-well plates (10,000 cells/well). Media and compound were refreshed every 3-4 days, and cells were passaged upon reaching ~90% confluence. After 10 days of treatment, cells were fixed in 4% paraformaldehyde (w/v) for 15 minutes at room temperature and stained for SA-β-Galactosidase positivity with the Senescence β-Galactosidase Staining Kit (CST #9860).

Lowest Cytotoxic Concentration Assay

For lowest cytotoxic concentration determination, A427 or NCI-H441 cells were seeded at 100,000 cells per well in a 12-well plate and incubated overnight at 37°C, 5% CO_2. The following day, DMSO or PF-06939999 was added and incubated at 37°C, 5% CO_2 for 3-4 days. At each timepoint, cells were removed with 100 µl trypsin and neutralized by adding 1.9 ml culture media. 1 ml of the cell dilution was re-plated for continued incubation at 37°C, 5% CO_2 and 1 ml counted using a Vi-Cell cell counter. Cells were counted every 3-4 days up to 20 days to determine the lowest dose that demonstrated cytotoxicity.

Pharmacokinetic Analysis

Blood samples were collected at 0.5, 1, 2, 4, 7 and 24 hours post-dose to quantify plasma concentrations of PF-06939999 by liquid-chromatography tandem mass spectrometry.

Pharmacokinetic parameters of PF06939999 including maximal plasma concentration (C_{max}), time to C_{max} (t_{max}), area under the plasma concentration-time curves (AUC) and half-lives ($t_{1/2}$), were determined by non-compartment pharmacokinetic analysis (Watson LIMS, Thermo Electron, Philadelphia, PA).

Symmetric dimethyl arginine ELISA protocol

Flash frozen tumors are lysed in RIPA buffer with protease/phosphatase inhibitors in Lysing Matrix A tubes (MP Bio) by homogenizing using a FastPrep-24 instrument, incubating on ice for 30 minutes, and sonicated. Protein lysate (150 ng) was added to a 96 well plate and incubated at 4°C overnight. Wells were washed with PBS–Tween 20 (0.1%) (PBST), then blocked with 5% BSA in PBS at room temperature for 2 hr. A second round of washes with PBST was followed by incubation with anti-SDMA (CST #13222) at 1:250 or SmD3 (Abgent AP12451a) at 1:500 at 4 °C overnight. Plates were washed with PBST, anti-rabbit IgG peroxidase conjugate at 1:10,000 (CST #7074S) was added, and incubated 60 min. Plates were washed with PBST, chemiluminescent substrate (SuperSignal ELISA Pico -Thermofisher #37070) was added, and incubated shaking for 1 minute. Total luminescence was measured using an EnVision reader (PerkinElmer). SDMA/SMD3 ratios were calculated for each sample and then normalized to the average of the vehicle controls. Values were plotted in GraphPad Prism 7.

Supplemental Data:

Table S1. Crystallographic Data Collection and Refinement Statistics

	WT PRMT5:MEP50 adensoine	WT PRMT5:MEP50 PF-06939999	M420T PRMT5:MEP50 PF-06939999	WT PRMT5:MEP50 PF-06855800	M420T PRMT5:MEP50 PF-06855800
Data collection statistics					
Wavelength (Å)	1.00	1.00	1.0	1.00	1.00
Resolution range (Å)	109.03 – 2.41	89.13 – 2.49	109-2.54	82.12– 2.7	109 – 2.39
Space group	I222	I222	I222	I222	P2(1)2(1)2
Cell edge a, b, c (Å) angle (°)	99.9 137.9 177.94 90.0 90.0 90.0	101.6 137.5 178.3 90.0 90.0 90.0	100.7 137.8 178.9 90.0 90.0 90.0	102.1 138.2 178.6 90.0 90.0 90.0	98.4 138.4 178.4 90.0 90.0 90.0
Complexes per asymmetric unit	1	1	1	1	2
Total reflections (outer shell)	286564 (38612)	295441 (44173)	272915 (41049)	229328 (32634)	623563 (95183)
Unique reflections (outer shell)	47892 (6892)	44025 (6374)	41147 (5958)	34500 (4976)	96383 (13880)
Multiplicity (outer shell)	6.0 (5.6)	6.7 (6.9)	6.6 (6.9)	6.6 (6.6)	6.5 (6.9)
Completeness (%) (outer shell)	99.7 (99.7)	99.9 (99.9)	99.9 (99.9)	99.9 (99.4)	97.8 (99.8)
Mean I/sigma(I) (outer shell)	11.9 (2.4)	19.3 (2.2)	13.3 (2.6)	12.5 (2.3)	12.5 (2.4)
R-merge (outer shell) †	0.090 (0.477)	0.059 (0.625)	0.068 (0.437)	0.058 (0.561)	0.082 (0.622)
Rpim‡	0.057 (0.327)	0.027 (0.278)	0.043 (0.268)	0.036 (0.357)	0.053 (0.384)
$CC_{1/2}$ (outer shell)	0.995 (0.941)	0.999 (0.954)	0.997 (0.993)	0.999 (0.956)	0.997 (0.950)
Refinement statistics					
Resolution range (Å)	88.97 – 2.41	89.13 – 2.49	109 - 2.54	54.66 – 2.71	109 – 2.4
R-work / R-free ±	0.234/0.294	0.222/0.279	0.236/0.276	0.221 / 0.303	0.213 / 0.275
Number of atoms (protein/ligand/water)	7286/19/214	7341/32/249	7304/32/237	7341/27/162	14652/54/811
Rmsd bond length (Å)	0.010	0.09	0.08	0.010	0.010
Rmsd bond angle (°)	1.18	1.07	1.1	1.19	1.15

†Rmerge = $\Sigma_{hkl}\ \Sigma_i\ |I_i - \langle I \rangle\ | / - \Sigma_{hkl}\ \Sigma I_i$, where I_i is the intensity of the i^{th} observation, $\langle I \rangle$ is the mean intensity of the reflection and the summations extend over all unique reflections (hkl) and all equivalents (i), respectively.

‡Rpim is a measure of the quality of the data after averaging the multiple measurements and Rpim = $\Sigma_{hkl}\ [n/(n-1)]^{1/2}\ \Sigma_i\ |I_i(hkl) - \langle I(hkl) \rangle| / \Sigma_{hkl}\ \Sigma_i\ I_i(hkl)$, where n is the multiplicity, other variables as defined for $R_{merge.}$

±R-work = $\Sigma|F_o - F_c| / \Sigma F_o$, where F_o and F_c are observed and calculated structure factors, respectively, R-free was calculated from a randomly chosen 5% of reflections excluded from the refinement, and R-work was calculated from the remaining 95% of reflections.

Rmsd is the root mean square deviation from ideal geometry.

Table S2. Selectivity of PF-06855800 and PF-06939999 across a panel of protein methyltransferases.

	Adenosine		PF-06855800		PF-06939999	
Histone Methyltransferase	**% Inhibition**	**SEM**	**% Inhibition**	**SEM**	**% Inhibition**	**SEM**
ASH1L	ND		ND		-8	9
DOT1L	4	5	26.7	0.5	-10	2
EHMT1	23.1	0.8	-1.3	3	2.4	0.9
EZH1/EED/SUZ12	-10	4	-40	7	-16	5
EZH2/EED/SUZ12	-9	15	7	2	-13	10
G9a	10	5	-0.8	5	0.5	0.5
MLL Complex	-4.7	0.5	ND		-13	9
PRDM9	ND		-7	10	1.1	3
PRMT1	-0.1	0.3	20	3	4	4
PRMT3	-15	3	-14	3	8	5
PRMT4	13	14	17	2	0.14	4
PRMT5 Complex	79	1.5	99.3	0.4	98	1.1
PRMT6	-53	4	4.7	0.2	-0.2	2
PRMT7	ND		ND		-0.6	2
PRMT8	ND		10	6	-15	16
SET1B	ND		ND		18	1.3
SETD2	ND		-2	1.0	6	6
SETD7	-5	4	13	1.5	-4	6
SETD8	-16	2	-11	4	-11	1
SETDB1	-2	3	-2	7	-6	3
SMYD2	ND		8.5	0.7	4.3	0.2
SUV39H2	-1	5	-5	11	-9	5
SUV420H2	-8	5	1.9	0.9	-30	9

Percent inhibition was determined at a dose of 10 μM across a panel of protein methyltransferases. Data are the average of two measurements with the associated standard error of the mean (SEM). All data were generated at Eurofins-CEREP. ND = not determined

Table S3. Selectivity of PF-06855800 and PF-06939999 across a panel of 40 protein kinases.

	PF-06855800		PF-06939999	
Kinase	**% Inhibition**	**SEM**	**% Inhibition**	**SEM**
ABL	4	2	0.8	0.8
AKT	8.44	0.04	-2	3
BTK	-0.06	0.5	-8.3	0.4
CAMK2A	7.0	0.3	4	1
CDK2/Cyclin A	7	2	2.0	0.8
CHEK1 (CHK1)	10	2	6.1	0.2
CHEK2 (CHK2)	8.4	0.5	3.2	0.9
CSNK1A1 (CK1 alpha1)	5.9	0.5	5	3
CSNK2A2 (CK2 alpha 2)	5	1.1	3	1.2
EGFR (ErbB1)	4.5	0.5	-1.7	2
EPHA2	1.1	3	-0.2	1.7
FGFR1	8	1.5	0.7	1.9
GSK3B (GSK3 beta)	9.6	0.3	3.1	0.5
INSR	-0.4	4	4.1	0.9
IRAK4	10	4	-5.2	0.3
JAK3	-6	2	-1.3	4
KDR	8.1	0.5	5	2
LCK	29	5	13.0	0.2
MAP4K4	76.4	0.4	5.3	0.5
MAPK1 (ERK2)	7.0	0.17	2.9	0.2
MAPKAPK2	2.8	0.17	12.8	0.4
MARK1	20	1.3	6.3	0.9
MET	-11	1.4	-0.16	1.4
MST4	4	9	9	3
MYLK2	-11	6	-2	5
NTRK1 (TRK-A)	22.5	0.7	5.2	0.2
P38	-1.8	0.8	4.4	0.4
PAK4	12.4	0.9	-4	3
PDK1	-3	5	11.7	0.6
PIM2	-7	6	1.4	0.15
PRKACA (PKACa)	46	7	-4	9
PRKCB2	6	1.1	11	1.0
ROCK1	38	1.3	3	3
SGK	3.0	0.4	-0.5	0.6
SRC	9.6	0.19	4.9	0.5
STK3 (MST2)	21	7	-19	6
STK6 (Aurora-A)	1.1	4	1.2	3
TAOK2	4	1.9	-2	1.8
TEK (Tie2)	12.96	0.08	7	1
TGFBR2	1.0	5	ND	

Percent inhibition was determined at a dose of 10 μM across a panel of protein kinases. Data are the average of two measurements with the associated standard error of the mean (SEM). All data were generated using the SelectScreen Kinase Profiling service at ThermoFisher Scientific at the K_m concentration of ATP for each enzyme for PF-06855800 and at 1 mM ATP for PF-06939999. ND = not determined.

Table S4: Dose-response results for protein kinases with moderate inhibition by PF-06855800

Kinase	**IC_{50}**	**95% CI**
MAP4K4	3.3	2.6-4.1
PRKACA	9	2-43
ROCK1	13	0.9-200

Dose-response curves were generated for PF-06855800 against the three enzymes with moderate activity (>35% inhibition) as shown in Table S2. All data were generated using the SelectScreen Kinase Profiling service at ThermoFisher Scientific at the K_m concentration of ATP for each enzyme.