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Supplemental Data

A Small Molecule Primes Embryonic Stem Cells for Differentiation

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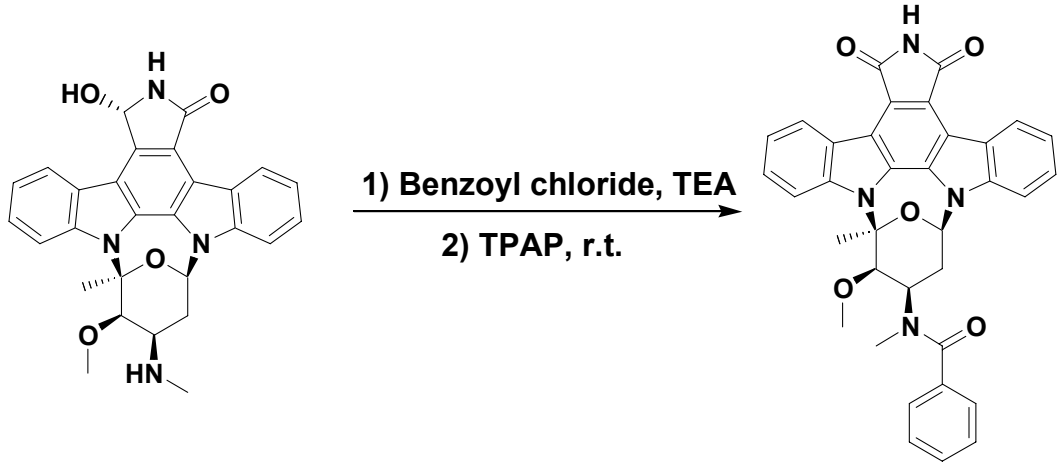
Supplemental Experimental Procedures

Schemes for Staurpimide and Biostauprimide Synthesis and Characterization.

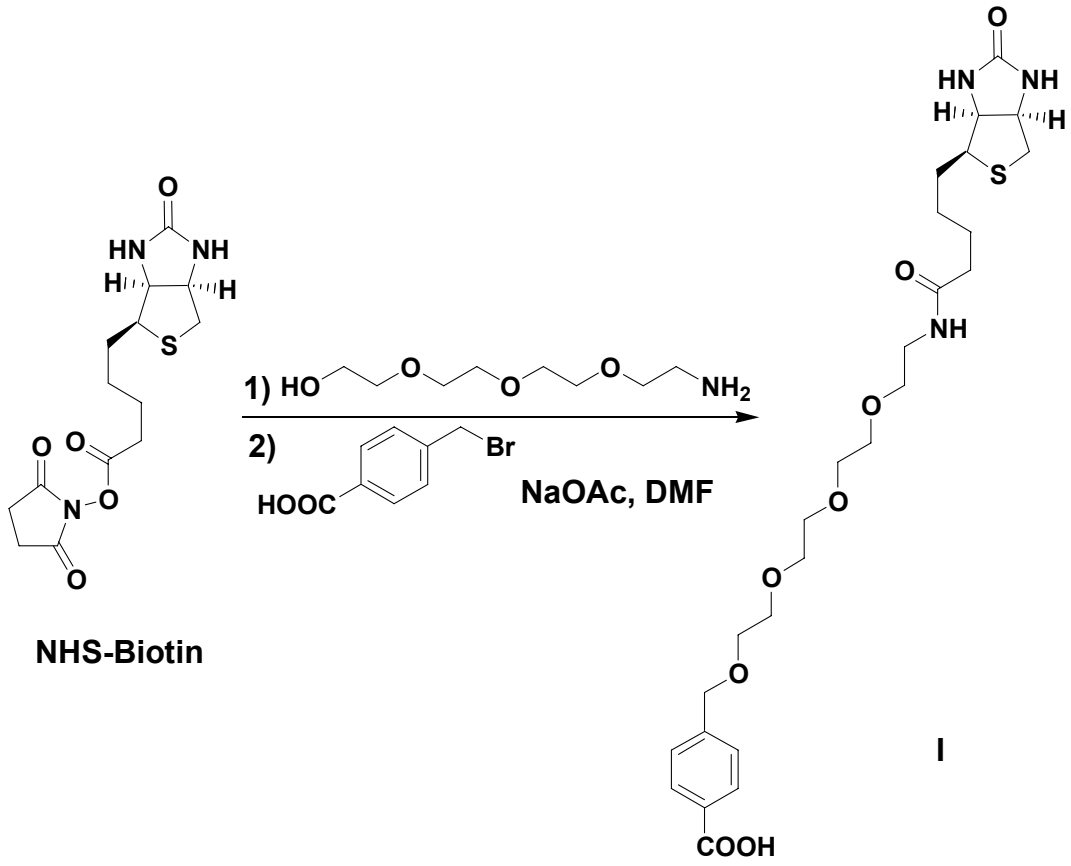


UCN-01 **Stauprimide**

Synthesis of Stauprimide

Benzoyl chloride (1.45 μl, 0.012 mmol) was added to a stirred solution of UCN-01 (5 mg, 0.011 mmol) in CH_2Cl_2 (1 ml) at room temperature. Triethyl amine (1.88 μl, 0.014 mmol) was then added slowly, and the reaction was stirred at room temperature for 1 h. Following the addition of tetrapropylammonium perruthenate (TPAP) (6.0 mg, 0.016 mmol), the reaction mixture was stirred for another 2 h and then concentrated under

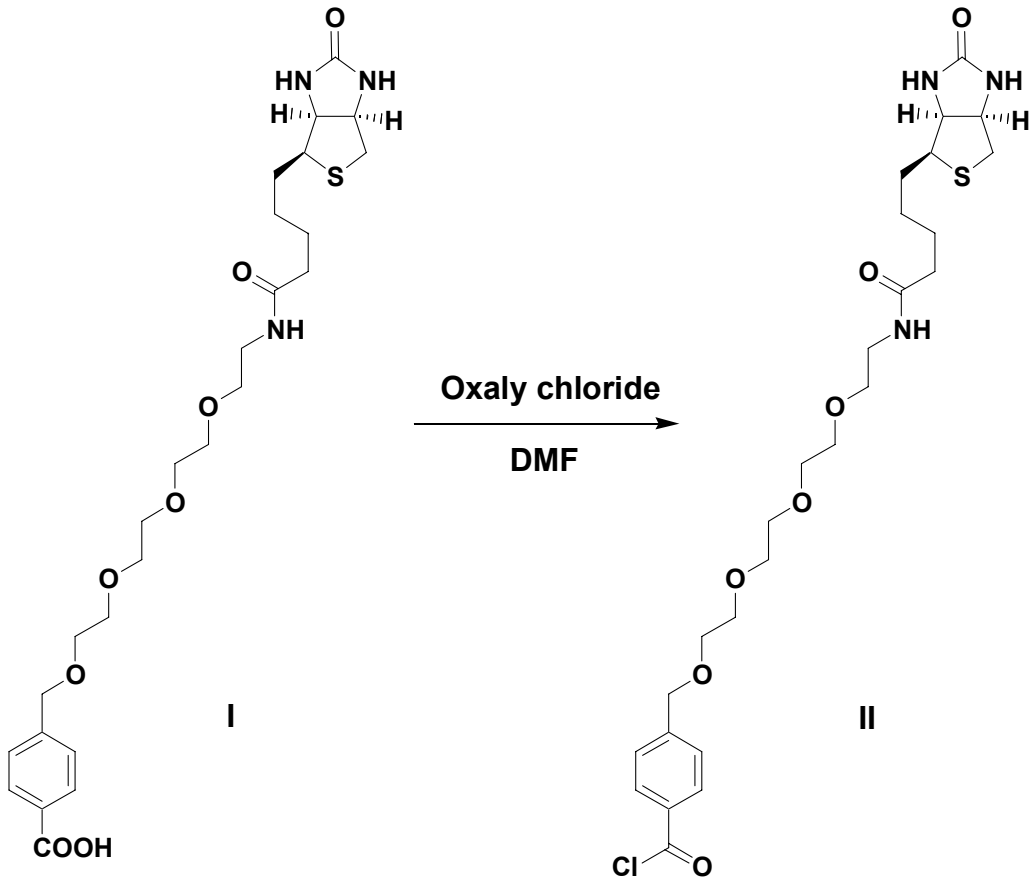
vacuum. The residue was purified by flash column chromatography over silica gel (EtOAc/hexane = 1:10 to 3:1) to yield stauprimide as a yellow solid (3.9 mg, 67% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.1 (br., 1H), 8.20-8.15 (m, 2H), 8.09-7.97 (m, 2H), 7.61-7.20 (m, 9H), 5.47 (t, J = 7.5 Hz, 1H), 4.05-4.00 (m, 1H), 3.49 (d, J = 10.5 Hz, 1H), 3.41 (s, 3H), 3.24 (s, 3H), 2.54-2.20 (m, 2H), 1.78 (s, 3H); HRMS (EI) calcd for $C_{35}H_{29}N_4O_5^+$ (M + H^+) 585.2132, found 585.2137.



Synthesis of I

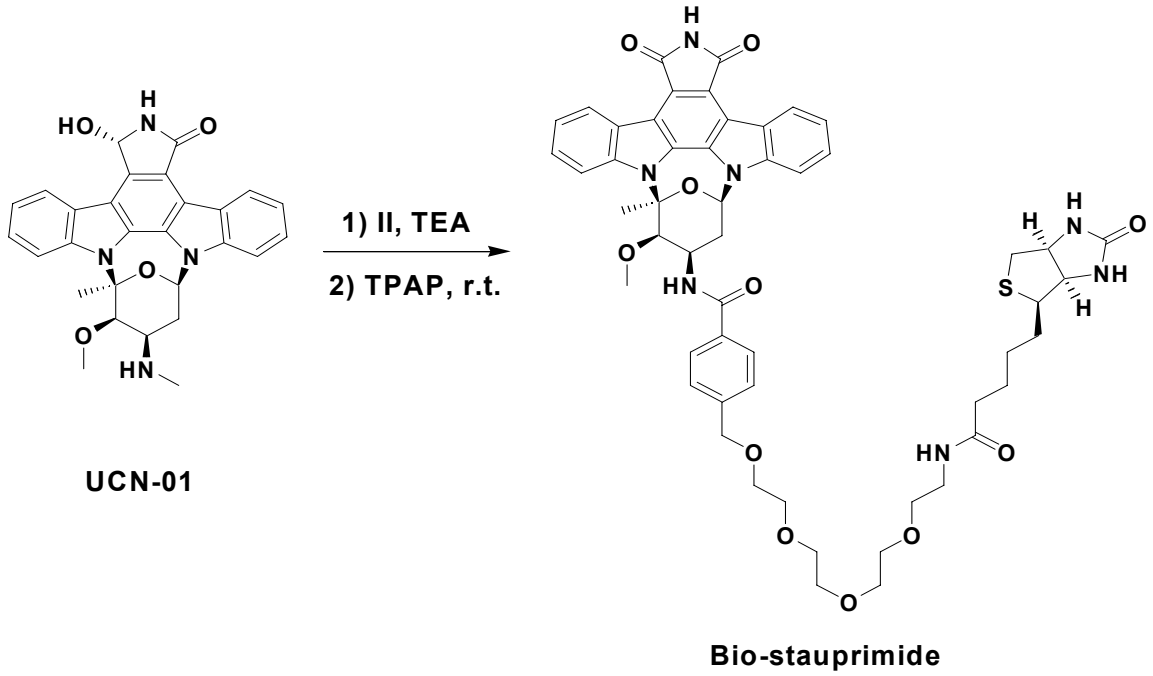
2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethanol (212 mg, 1.1 mmol) and triethyl amine (215 μl, 1.5 mmol) were added to a stirred solution of N-hydroxysuccinimidobiotin (NHS-Biotin) (340 mg, 1.0 mmol) in DMF (1.0 ml) at room temperature. After stirring at

room temperature for 24 h, 4-(bromomethyl)benzoic acid (215 mg, 1.0 mmol) and NaOAc (164 mg, 2.0 mmol) were added sequentially at room temperature. After stirring at 70 ^{o}C for 72 h, the reaction mixture was concentrated under vacuum. The residue was purified by HPLC (CH_3CN/H_2O = 10:90 to 90:10) to yield **I** as a slight yellow solid (116 mg, 21% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.17 (d, *J* = 10.5 Hz, 2H), 7.84 (br., 1H), 7.68 (d, *J* = 10.0 Hz, 2H), 6.32 (br., 2H), 5.04 (s, 2H), 4.87-4.75 (m, 2H), 3.91-3.83 (m 2H), 3.81 (s, 10H), 3.66-3.21 (m, 7H), 2.03-1.94 (m, 2H), 1.85-1.57 (m, 6H); ESI-MS *m/*z 554.2 ($M + H^+$).



Synthesis of II

Oxalyl chloride in CH_2Cl_2 (2 M, 2 ml) was added to a vial containing compound **I** (116 mg, 0.21 mmol). Then 2 drops of DMF was added additionally and the mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated under vacuum and then used for the next step without further purification.



UCN-01

Bio-stauprimide

Synthesis of Biostauprimide

Compound II (6.9 mg, 0.012 mmol) was added to a stirred solution of UCN-01 (5 mg, 0.011 mmol) in a mixture of 1, 4-dioxane (0.2 ml) and CH_2Cl_2 (0.2 ml) at room temperature. Triethyl amine (1.88 μl, 0.014 mmol) was then added slowly and the mixture stirred at room temperature overnight. Following the addition of tetrapropylammonium perruthenate (8.5 mg, 0.022 mmol), the reaction mixture was stirred for another 4 h, and then concentrated under vacuum. The residue was purified by

flash column chromatography over silica gel (EtOAc/hexane = 1:10 to 3:1 and followed with MeOH/CH_2Cl_2 = 1:7) to yield Biostauprimide as a slight yellow solid (3.1 mg, 28% overall yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.94 (br., 1H), 8.29-8.18 (m, 2H), 8.17-8.01 (m, 3H), 7.54-7.15 (m, 8H), 6.04 (br., 2H), 5.84-5.78 (m, 1H), 4.37-4.32 (m, 1H), 4.12 (s, 3H), 4.03-3.97 (m, 1H), 3.75-3.67 (m, 2H), 3.65 (s, 10H), 3.62-3.54 (m, 3H), 3.44 (s, 3H), 3.38-3.31 (m, 3H), 3.26 (s, 3H), 3.10-3.02 (m, 2H), 2.87-2.80 (m, 2H), 2.50-2.27 (m, 4H), 1.86 (s, 3H) , 1.62-1.32 (m, 5H); HRMS (EI) calcd for $C_{54}H_{62}N_7O_{11}S^+$ (M + H^+) 1016.4223, found 1016.4225.

Figure S1. Dose-Dependent Curve of Stauprimide on ESC Endoderm Differentiation

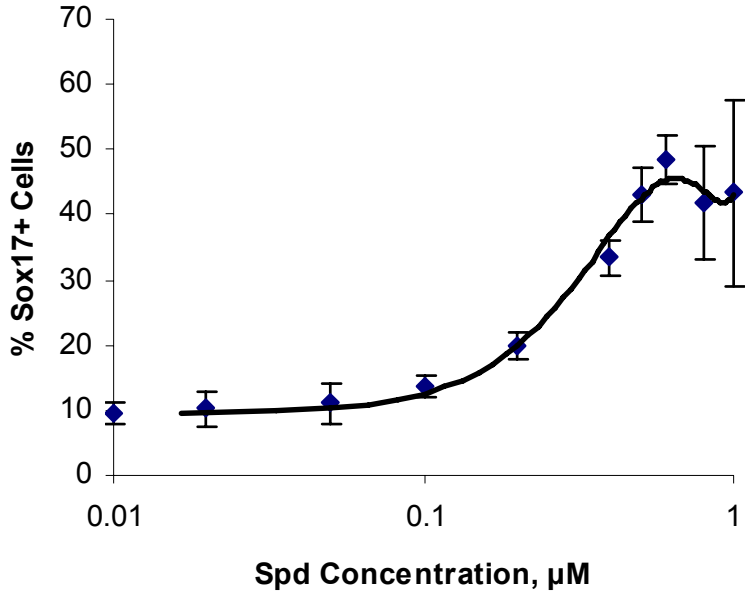
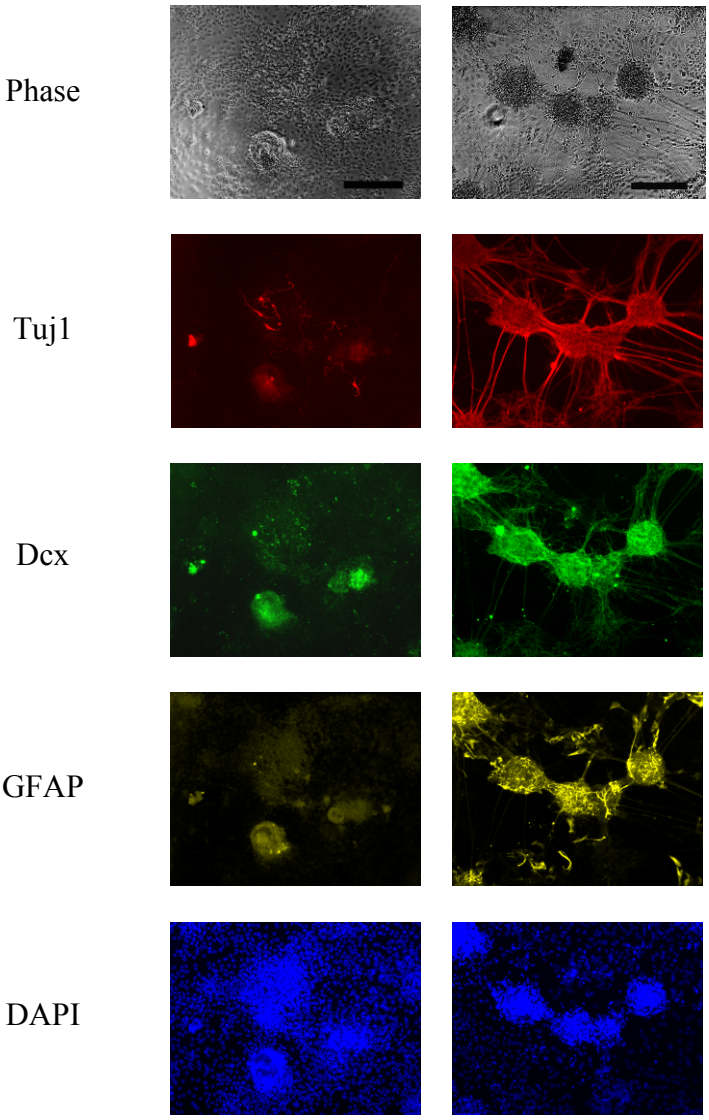


Figure S2. Immunostaining of Neural Cell Lineages

(A) Low resolution immunostaining for neurons and astrocytes. Scale bar: 100 µm.
(B) High resolution immunostaining for neurons and astrocytes differentiated from stauprimide-primed cells. Scale bar: 25 µm.

A



B

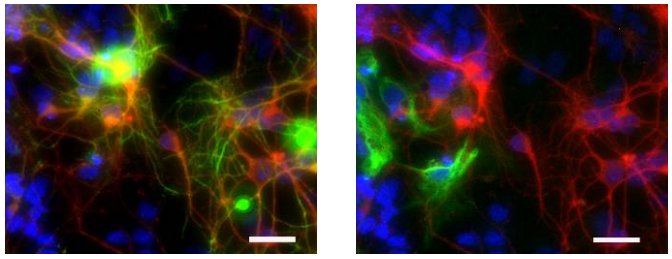


Figure S3. Kinase Inhibitor Effects on ESC Differentiation

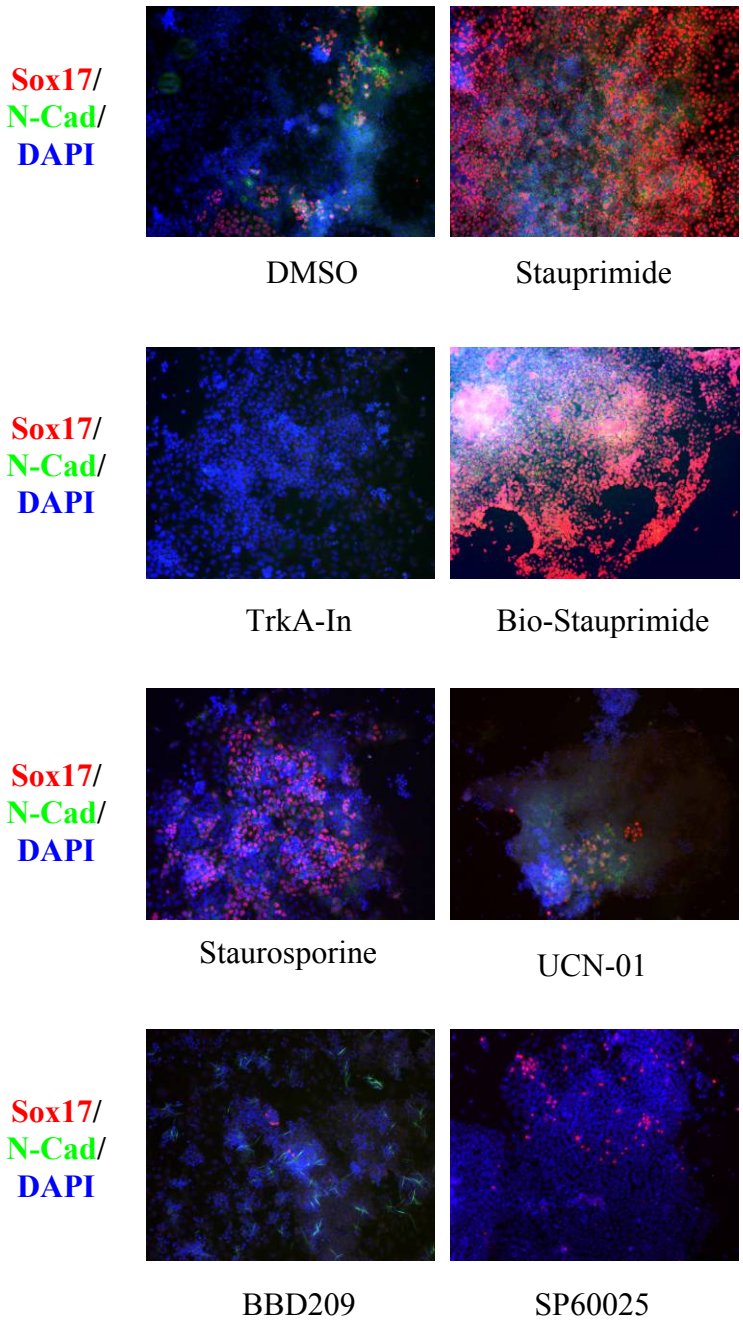


Table S1. Primers Used for Semiquantitative RT-PCR

Gene	Forward Primer	Reverse Primer
Sox17	5’-tttgtgtataagcccgagatgg-3’	5’-aagattgagaaaacacgcatgac-3’
Sox7	5’-tcatgtcaggaggagcatgg-3’	5’-caggactgagatgaggctggt-3’
Brachyury	5’-aactttcctccatgtgctgagac-3’	5’-tgacttcccaacacaaaaagct-3’
E-Cadherin	5’-aaacttggggacagcaacatcag-3’	5’-tcttttggtttgcagagacaggg-3’
GSC	5’-atgctgccctacatgaacgt-3’	5’-cagtcctgggcctgtacatt-3’
Claudin6	5’-acaaagctgaccgagcact-3’	5’-agcagcaaaaggcctgag-3’
FoxA2	5’-tggctgcagacacttcctact-3’	5’-caacatcagtacaaccctctggt-3’
Mixl1	5’-actttccagctctttcaagagcc-3’	5’-attgtgtactccccaactttccc-3’
Flk1	5’-taggtgcctccccataccctgg-3’	5’-tggccggctctttcgcttactg-3’
N-Cadherin	5’-cccaagtccaacatttccatcc-3’	5’-aaagcctccagcaagcacg-3’
Oct4	5’-agaaggagctagaacagtttgc-3’	5’-cggttacagaaccatactcg-3’
Snail	5’-cagctggccaggctctcggt-3’	5’-gcgagggcctccggagca-3’
Sox1	5’-cacaactcggagatcagcaa-3’	5’-tgtaatccgggtgttccttc-3’
Sox2	5’-cacaactcggagatcagcaa-3’	5’-ctccgggaagcgtgtactta-3’
c-Myc	5’-atgcccctcaacgtgaacttc-3’	5’-cctcttctccacagacaccac-3’
Tuj1	5’-tcagcgatgagcacggcata-3’	5’-cactctttccgcacgacatc-3’
Doublecortin	5’-agagggtcacggatgaatg-3’	5’-aaacggtcagaagaaacagc-3’
Albumin	5’-ccactgttgaagaaagccca-3’	5’-cagatagtcttccacacaaggca-3’
AFP	5’-tgcagaaacacatcgaggagag-3’	5’-gcttcaccaggttaatgagaagct-3’
Pdx1	5’-ccggacatctccccatacgaa-3’	5’-gaggtcaccgcacaatcttgc-3’
Cyp7A1	5’-tacgcatgtttctcaacgatac-3’	5’-tcttggacagcaaatagtcttc-3’
β-Actin	5’-cctaaggcaaccgtgaaaag-3’	5’-tcttcatggtgctaggagcca-3’
GFAP	5’-catcaccattcctgtacagactttc-3’	5’-ccacgatgttcctcttgaggtg-3’
Ngn3	5’-gcgcaacaggcccaagagcg-3’	5’-tcacaagaagtctgagaaca-3’
NeuroD	5’-gaaagccccctaactgactgc-3’	5’-gcactttgcagcaatcttagcaaaa-3’
α-Actin	5’-tgttacgtcgccctggattttgag-3’	5’-agagagagacatctcagaagc-3’
MLC2v	5’-gccaagaagcggatagaag-3’	5’-ctgtggttcagggctcagtc-3’
KCNH2	5’-atggcgattccagccgggaa-3’	5’-atgtccacgatgaggtc-3’

Table S2. Kinase Profiling for Stauprimide

Kinase	% Inhibition		
	stauprimide - 5 μM	stauprimide - 2 μM	stauprimide – 0.5 μM
ABL	20	ND	ND
ADRBK1	ND	8	8
AKT1	59	ND	ND
ALK	ND	24	14
AMPK	98	52	32
AURB	94	30	16
AXL	75	ND	ND
BRSK1	ND	38	13
BTK	87	23	20
CAMKIIa	100	48	27
CDC42BPA	ND	2	-1
CDK2	79	ND	ND
CHK2	46	ND	ND
CK1a	5	ND	ND
CK2a1	ND	13	16
CLK1	ND	9	7
cKIT	78	ND	ND
cRAF	1	ND	ND
CSK	17	ND	ND
DAPK3	ND	5	1
DYRK1a	75	ND	ND
EGFR	43	ND	ND
EPHA3	6	ND	ND
FAK2	80	5	5
FGFR3	84	8	4
FLT3	106	84	64
FMS	99	ND	ND

FYN	92	18	10
GSK3B	98	60	30
IGF1R	10	ND	ND
IKKb	95	6	4
INSR	27	ND	ND
IRAK4	39	ND	ND
JAK2	98	17	3
JNK2	6	ND	ND
KDR	97	52	30
LCK	89	37	27
LTK	ND	9	6
LYN	85	31	22
MAPK1	0	ND	ND
MAPK13	14	ND	ND
MAPK14	-8	ND	ND
MAPKAPK2	6	ND	ND
MARK1	ND	37	16
MATK	ND	9	8
MEK1	ND	13	10
MERTK	ND	13	9
MET	71	ND	ND
MLK1	ND	82	55
NEK2	62	ND	ND
P70S6K	48	ND	ND
PAK2	73	ND	ND
PASK	ND	1	-3
PDGFRa	93	42	20
PDK1	104	32	18
PHKG1	ND	38	18
PIM2	85	8	-1

PKA	92	11	0
PKCa	90	58	31
PKCm	ND	18	14
PKD3	ND	24	22
PKN1	ND	74	37
PLK1	48	ND	ND
PRKG1	ND	41	20
RET	86	30	16
ROCK2	42	ND	ND
RSK1	95	62	42
SGK1	72	ND	ND
SRC	91	18	13
SRPK1	ND	8	5
STK3	ND	32	8
TAOK2	ND	9	5
TBK1	ND	65	36
TEK	ND	23	18
TRKA	100	56	44
TYRO3	ND	18	13
ZAP70	92	-2	-4

* ND: Not Determined.