Supporting Information

Materials and Methods

Chemicals: iPYrazine was obtained from an academic chemical screening collection.[1] VEGFRiII[2], RETi[3] and PP1[4] were purchased from Calbiochem and SB-431542[5] was purchased from Tocris. The following compounds were provided as generous gifts from collaborators: dasatinib[6] and sunitinib[7] (Prof. Vaughn Smider), ABLi/GNF-4[8] (Dr. Weijun Shen), Imatinib[9] (Dr. Anthony Boitano), LY-364947[10] (Dr. Jun Liu) and JK239[11] (Dr. Lubica Supekova).

Cell Culture and Differentiation Methods: All MEF, ES and iPS cells were cultured in knock-out DMEM containing 15% FBS, glutamax, beta-mercaptoethanol (0.1 mM) and nonessential amino acids (collectively hereafter referred to as growth media) in a humidified incubator with 5% CO_2. Feeder layer MEFs were generated from wild type MEFs (a generous gift from Prof. Enrique Saez) that had been inactivated with mitomycin C (4 hours at 37° using 10 µg/mL). Transgenic MEFs were isolated from NL[12], O4G[13] or O4NR[14] mice and selected from E13.5 embryos. ES and iPS cells were cultivated on mitotically-inactivated MEF feeder layers in growth media plus 20 ng/mL LIF (Chemicon). All media reagents were purchased from Life Science Technologies (formerly Invitrogen) unless otherwise specified.

Chemical Screening and Nanog Assay: The Nanog luciferase high throughput screening assay was performed as described[12] with one exception, the cells were assayed at day 7 instead of day 10. Primary hits were re-screened in triplicate in 1536-well format. Dose response curves were generated by screening OKM NL-MEFs in 8-pt dilution (1 mM stock, half log serial dilutions) in 384-well format. The HEK293T-based SV40-Fluc counter screen was also performed as described.[12]

Counter-screening, dose response experiments, structure and activity relationship analyses and cell number quantification assays were all run in 384-well plates (Greiner). Cells were plated at 1K cells/well in growth media and assayed 7 days later. All luciferase readings were acquired following addition of Bright-Glo reagent (Promega). All experiments were run in triplicate and repeated at least two times.

Virus Production for Colony Forming Assays: Maloney retrovirus was generated in GP2-293 packaging cells (Clontech) plated on poly-D-lysine–coated 15cm dishes. Cells were grown to ~90% confluence in 15 mL of media (DMEM + 10% FBS) and then transfected (FugeneHD, Roche) with pMXs-based moloney retroviral vectors (containing the cDNA of Oct4, Sox2, c-Myc and Klf4; Addgene, deposited by S. Yamanaka) and the VSV-G packaging plasmid in a 1-to-1 ratio (15 µg of parent vector and VSV-G each per 15cm dish). FugeneHD was used at a 1:4 ratio (µg of DNA : µL of FugeneHD) according to the manufacturers instruction. 24 hours post-transfection, 25mL of GP2-293 growth media was added to 15cm dishes. 72 post-transfection, media containing viral supernatant was collected, filtered (0.45 µm) and applied directly to cells. The latter is detailed below in the colony forming assay section. VSV-G pseudotyped lentiviruses were generated in 293T cells, as described.[15]

Colony Forming Assays: O4NR-MEFs (at passage 1-2; 10K cells/well of a 12-well dish) were infected overnight with viral supernatant containing equal portions of each transduced factor (Klf4/c-Myc or OKM) plus 6 µg/mL polybrene (day -1). Media was changed 24 hours later (day 0) and Doxycycline (2 µg/mL) was added to induce Oct4 expression from the collagen locus of O4NR-MEFs, where appropriate. Compounds and VPA (1 mM in water) were added from days 2-12. Media and compound (or vehicle control; 0.1% DMSO, v/v) were changed every 3 days. At day 12, selection was initiated by adding G418 (300 µg/mL) to the culture media. At day 15, colonies were either fixed, AP-stained and counted or passaged onto irradiated MEFs in ES cell growth media. NL- and O4G MEFs were infected with lentiviral supernatant (OKM) and cultured as above, with the exception that neomycin was not added to the culture media.

Teratoma Assay: iPY-derived iPS cells were trypsinized, washed and resuspended in 300 µl mouse ES cell medium and injected subcutaneously into SCID mice (Taconic). 3 weeks after injection tumors

were removed from euthanized mice and fixed in formalin. Samples were paraffin-embedded, sectioned and analyzed on the basis of hematoxylin and eosin staining.

Blastocyst Injections: Injections of iPY-derived iPS cells into BALB/C host blastocysts were carried out as previously reported.[16]

Immunostaining: The immunostaining was performed as described previously.[12] Cells were imaged using a Nikon Eclipse TE2000-U microscope.

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Table S1: Tyrosine kinases inhibited by iPY. Data are represented as percent kinase inhibition (% i) in the presence of 5 µM iPY. Highlighted fields represent potential kinase targets that were analyzed further in colony forming assays; those in red are potently inhibited by iPY and those in yellow are members of the Src family of kinases.

Kinase	%i
Abl	74
AurA	35
AurB	19
AXL	30
BMX	51
BTK	58
CDK2	-9
CKIT	-2
cRaf	15
CSK	83
DYRK1a	13
EGFR	45
EphA3	12
EphB3	9
ERBB4	47
FAK2	21
FGFR3	44
FLT3	48
FMS	64
FYN	71
GSK-3β	6
IGF1R	8
IKKb	7
INSR	4
IRAK4	26
JAK2	55
JNK2	5
VEGFR2	91
LCK	59
LYNa	26
MapK1	-4
MAPK13	-7
MapK14	5
Mek1	13
MET	23
NEK2	14
p70s6k	16
PDGFRa	7
PDGFRb	29
Pim2	11
PKCA	-11
Plk1	4
RET	83
ROCK1	1
SGK1	14
Src	44
SYK	23
TRKA	36
TTK	13
TYRO3	5
ZAP70	-4

Table S2: Commercially available inhibitors directed against iPY candidate kinase targets that were applied in the Sox2 replacement assays. Compounds highlighted in yellow are able to replace Sox2 in the colony forming assay. Check marks indicate that the specified kinase is inhibited in biochemical assays (*in vitro*) at the concentration used in the colony forming assay (*in vivo*) in Figure 2.

	Drug [µM]	pan-SFK	Abl	RET	VEGFR2	CSK	TGFBR1 (ALK5)	ACVR1B (ALK4)
JK239[11]	10					✓		
iPY	10	✓	✓	✓	✓	✓		
PP1[4, 17]	10	✓	✓	✓		✓	✓	✓
Dasatinib[6, 18]	0.5	✓	✓	✓		✓		
Sunitinib[7, 18b]	1				✓			
VEGFRiII[2]	1				✓			
RETi[3]	10			✓				
ABLi/GNF-4[8]	1		✓					
Imatinib[9, 18b, 19]	2		✓					
SB-431542[5, 18b]	20						✓	✓
LY-364947[10]	1						✓	✓

Figure S1: iPS cells derived from *Oct4*-GFP MEFs with OKM-transduction and iPY- (10 μM) or Dasatinib-treatment (0.5 μM) reactivate endogenous *Oct4* expression. GFP is expressed from the *Oct4* locus (Oct4-IRES-GFP). Images were collected at 400× magnification.