SUPPORTING INFORMATION

Identification of a small molecule that turns `ON` the pluripotency gene circuitry in human fibroblasts

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Methods

General.

HDF from 54-year-old Caucasian female were purchased from Cell Applications, Inc. Unless specified, all the reagents and solvents used in this work were purchased from standard suppliers and used without further purification. Abbreviations used: P, N-methylpyrrole; I, N-methylimidazole; AF, ammonium formate; Fmoc, 9-fluorenylmethoxycarbonyl;HCTU,1-[bis(dimethylamino)methylene]-5-chloro-1H-benzothiazolium 3-oxide hexafluorophosphate; DMSO, dimethylsulfoxide; DIEA, N,N-diisopropylethylamine; β, β□alanine; γ, γ□aminobutyric acid; SAHA, suberoylanilide hydroxamic acid. ^{1}H NMR spectra were recorded on a JEOL JNM-FX 400 model NMR spectrometer. HPLC analysis was performed with a 4.6 x 150 mm column on a JASCO PU-2089 plus pump model with UV-2075 plus HPLC UV/VIS detector and a Chemcobond 5-ODS-H 10 x 150 mm column (Chemco Scientific Co., Ltd, Osaka, Japan) was used for the purification of SAHA-PIPs and their precursor. Electrospray Ionization Time-of-Flight mass (ESI-TOF-MS) was recorded on BioTOF II ESI-TOF Bruker Daltonics Mass Spectrometer (Bremen, Germany). Flash column system was performed using Combi Flash Companion model (Teledyne Isco Inc., NE, USA). Teledyne Isco Redi Sep Rf column (40 g) was used for the purification of 4-(8-methoxy-8-oxooctanamido)benzoic acid.

Optimization of parameters for treatment of SAHA-PIP against HDF

HDF cells with in the passage P6 were trypsinized for 5 min at 37 °C, and were resuspended in the fresh HDF medium to a concentration of 1.5 x 10^5 cells/ml in a 35 mm plate and were grown for 24 h as mentioned before.[1, 2] The medium was then removed and replaced with 2 ml of fresh HDF medium followed by the addition of 1 mM of each individual SAHA-PIP (**A** to **ϕ**) to achieve a final polyamide concentration of 1 µM in 0.1% DMSO and then were incubated in a 5% CO_2 atmosphere at 37 °C for 48 h. 0.1% DMSO treated cells were used as the control.

Effective concentration of the SAHA-PIPs was standardized based on the initial optimization experiments and the treatment of HDF with various concentration of PI polyamide SAHA conjugates (100 nM, 500 nM, 1 µM and 10 µM). Also, variation of incubation time (24 h, 48 h and 72 h) suggested that 48 h is optimal to achieve consistent expression. Hence, 1 µM of effectors and 48 h incubation was employed for all studies. For q-RT-PCR analysis, about 9 biological replicates (36 well plates) were performed treated at various time points in cells of passages P6 to P12. Omitting the top 6 and bottom 6 values, the values obtained from 24 well plates were normalized. 201B7 human induced pluripotent stem cells (Riken Bioresource Center) were seeded on mitomycin C-treated MEF (Oriental Kobo) and maintained in DMEM/F12 (Invitrogen) supplemented with 20% KSR (Invitrogen),

2mM L-glutamine (Invitrogen), 1% NEAA (Invitrogen), 1% penicillin and streptomycin (Nacalai Tesque), and 4 ng/ml bFGF (Wako).

Cytotoxicity, gene expression and chromatin immunoprecipitation studies

Colorimetric assays were done as mentioned previously using WST-8 (Dojindo, Kumamoto, Japan) in 96-well plates with various concentrations of SAHA-PIPs **I**, Pre-**I**, **K** and SAHA as mentioned before.[1] Quantification of expression of marker genes in human dermal fibroblasts was carried out as described before using an RNeasy Mini Kit (Millipore) as mentioned previously.[3] cDNA was synthesized from 500 ng of total RNA using a ReverTra Ace qPCR RT kit (Toyobo, Japan) and used as a template for polymerase chain reaction (PCR). Primer pairs of endogenous expression of iPSC factors that were used for RT-PCR analysis is as shown in table S5 and as given.[4] The statistical significance was determined by the t-test and p values were obtained with the two-tailed method. The p values less than 0.05 reflect the significance of the difference and the values more than that are assigned as non-significant. Antibodies for acetylated histone H3 and normal rabbit IgG were purchased from Upstate Biotechnology Inc., USA and H3K14 was purchased from Abcam (ab10812, UK). After 48 h treatment of each of the cells individually with 1 μM of **I**, **K**, SAHA and 0.1 % DMSO, ChIP assay was performed according to the protocol described before[5] and that in the kit manual

(SimpleChIP™ Enzymatic Chromatin IP Kit, Cell Signaling Technology, USA). DNA fraction was then purified QIAquick PCR Purification Kit (Qiagen, USA) and analyzed with qRT-PCR as mentioned before. Details of the purchased primer pairs used for *OCT-3/4*, *SOX2* promoter and enhancer were shown in Table S5.

Microarray analysis

HDFs were treated with 1 μM of effectors and after 48 h incubation total RNA was isolated using RNeasy MINI Kit (Qiagen, CA, USA) according to the manufacturer's instructions. Microarray studies were then carried out as mentioned before[1] using SurePrint G3 Human GE v2 8x60K Microarray (Agilent Technologies, USA) or Human Gene 2.1 ST Array (Affymetrix, USA). The raw data and associated sample information were processed by GeneSpring GX v12.1.0 (Agilent Technologies, USA). As mentioned before, the interpretation of the microarray data is carried out by Ingenuity pathway analysis, which uses the dataset containing the gene identifiers and its respective fold change values. The significantly regulated pathways are identified from the library of canonical pathways in the Ingenuity pathway knowledge base. Fischer's exact test is employed to measure the p-value that determines the association between the genes in the dataset and the canonical pathway. In network analysis, the genes in the submitted dataset were mapped to its corresponding gene

objects called focus genes in the Ingenuity pathway knowledge base. Unsupervised clustering analysis was performed using Cluster 3.0 and the results were visualized using Java Treeview.

Long-term treatment and alkaline phosphatase (AP) staining

HDFs were seeded at 4,000 cells in 96-well plates. Next day compounds, SAHA-PIP **I**, A-03-01 (Wako, Japan), PS48 (Stemgent), Pifithrin–α (Wako, Japan), CHIR99021(Wako, Japan) were added at a concentration of 1 μM with a combination. [6, 7] Treatment was continued for three weeks with every two days medium replacement. After 10 days, Fibroblast Basal Medium was replaced for human ES cell medium (DMEM/F12 containing 20% KSR, 2 mM L-glutamine, 1 x 10^{-4} M non essential amino acids, 1 x 10^{-4} M 2-mercaptoethanol, 50 units and 50 mg/mL penicillin and streptomycin and 0.2 mL (500 mL of medium) of 10 mg/mL bFGF) Alkaline phosphatase staining was performed according to manufacture's instructions using Alkaline Phosphatase Kit II (Stemgent). Images were obtained using optical microscope (BZ-9000, Keyence, Japan).

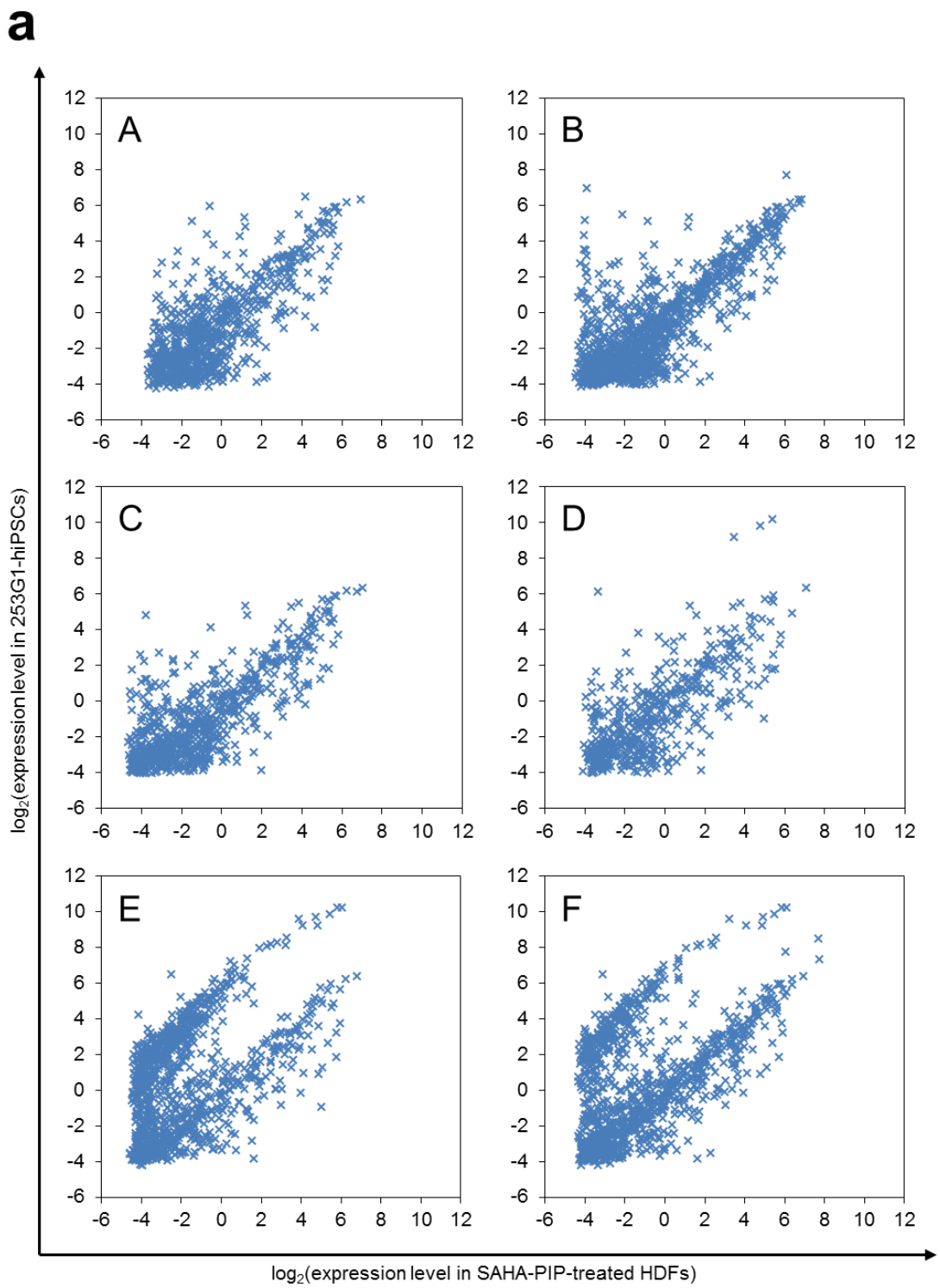
Chromatin immunoprecipitation sequencing (ChIP-Seq) analysis

ChIP-Seq experiments with an H3K14Ac-specific antibody were conducted to gain better

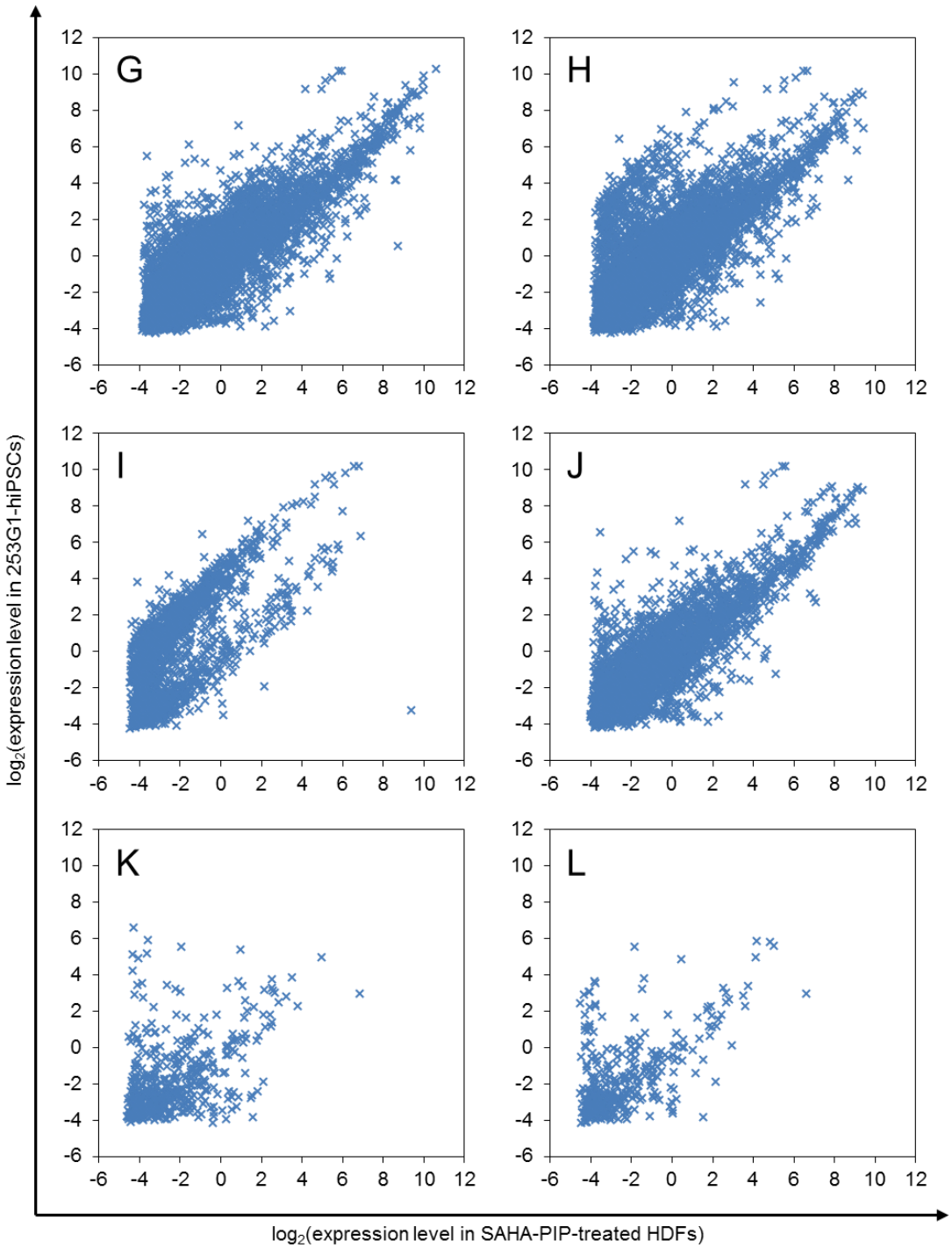
understanding of **I** - mediated acetylation. The highest acetylation of the proximal promoters of the known iPSC factors was observed after 48 h treatment. This time point was therefore chosen for ChIP-Seq experiments. ChIP samples were combined to yield a total of 50–100 ng DNA. ChIP-Seq libraries were prepared by using standard Ion Xpress™ Plus gDNA Fragment library preparation reagents and protocols (Life techologies, USA). The libraries were subjected to quality and quantity check with Agilent DNA High sensitivity BioAnalyzer kit (Agilent technologies, USA). The qualified libraries were used for template preparation using Ion PGMTM template OT2 200 kit in Ion one touch2 system. The templates were then enriched using Ion one touch ES. The enriched libraries were sequenced as single read with the read length of 200 nt was performed with Ion PGM sequencer using Ion PGMTM sequencing 200 kit v2 and 316/318 chip (Life technologies, USA) by following the manufacturer's instructions, typically producing 9.5–10 million post filtered reads per library. Employing standard program packages in the Ion torrent suit 3.4.2, we processed the data. Sequencing reads were trimmed from adapters and then mapped against the human chromosomes (excluding all random chromosomes) using the hg19 version of the human genome as a reference. Torrent Mapping Alignment Program 3.4.3-1 (TMAP) was used for aligning reads, ChIP-seq peaks was called using MACS 1.4.2.[8]

SUPPLEMENTARY FIGURES

Figure 1



b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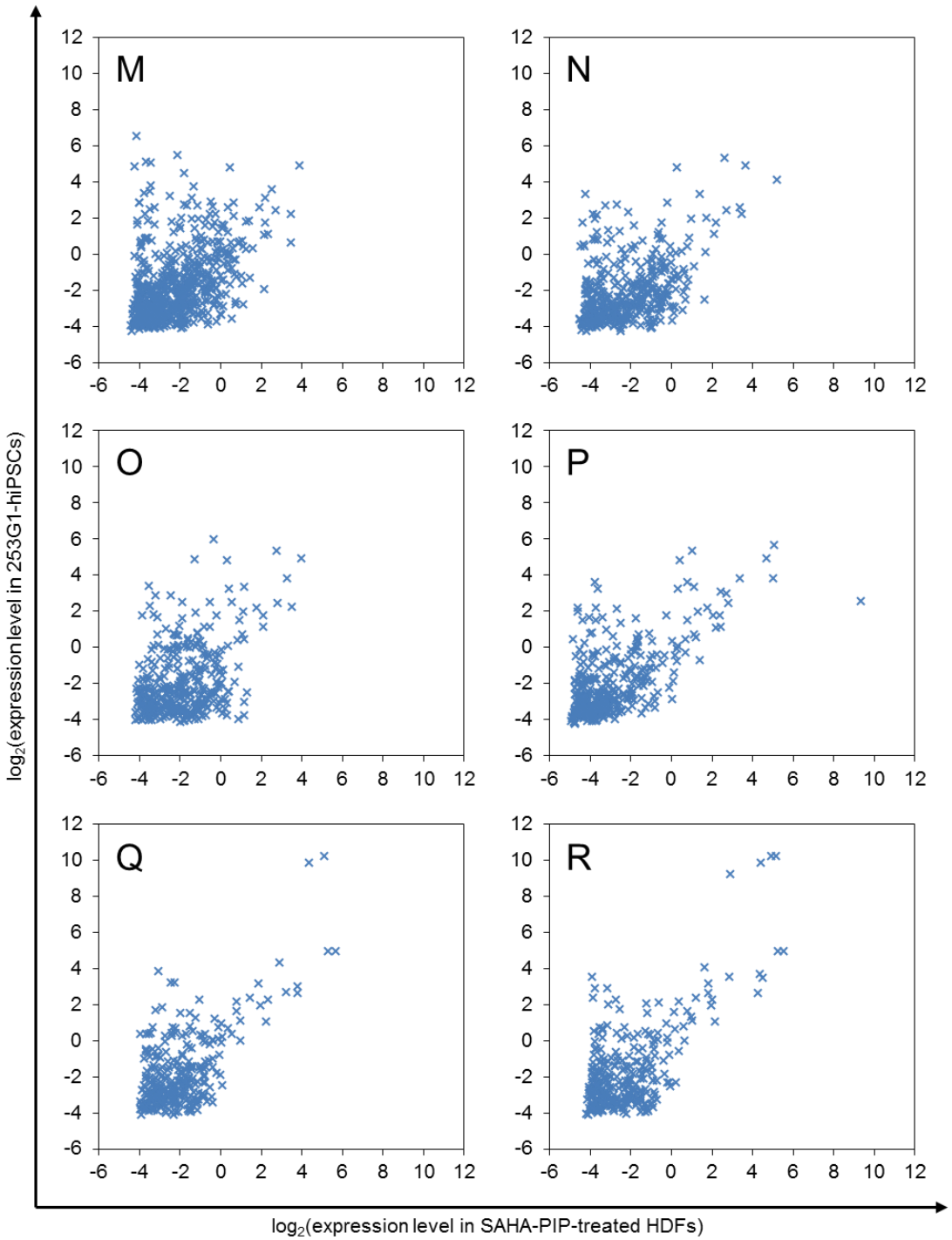
log2(expression level in 253G1-hiPSCs)
log2(expression level in SAHA-PIP-treated HDFs)

c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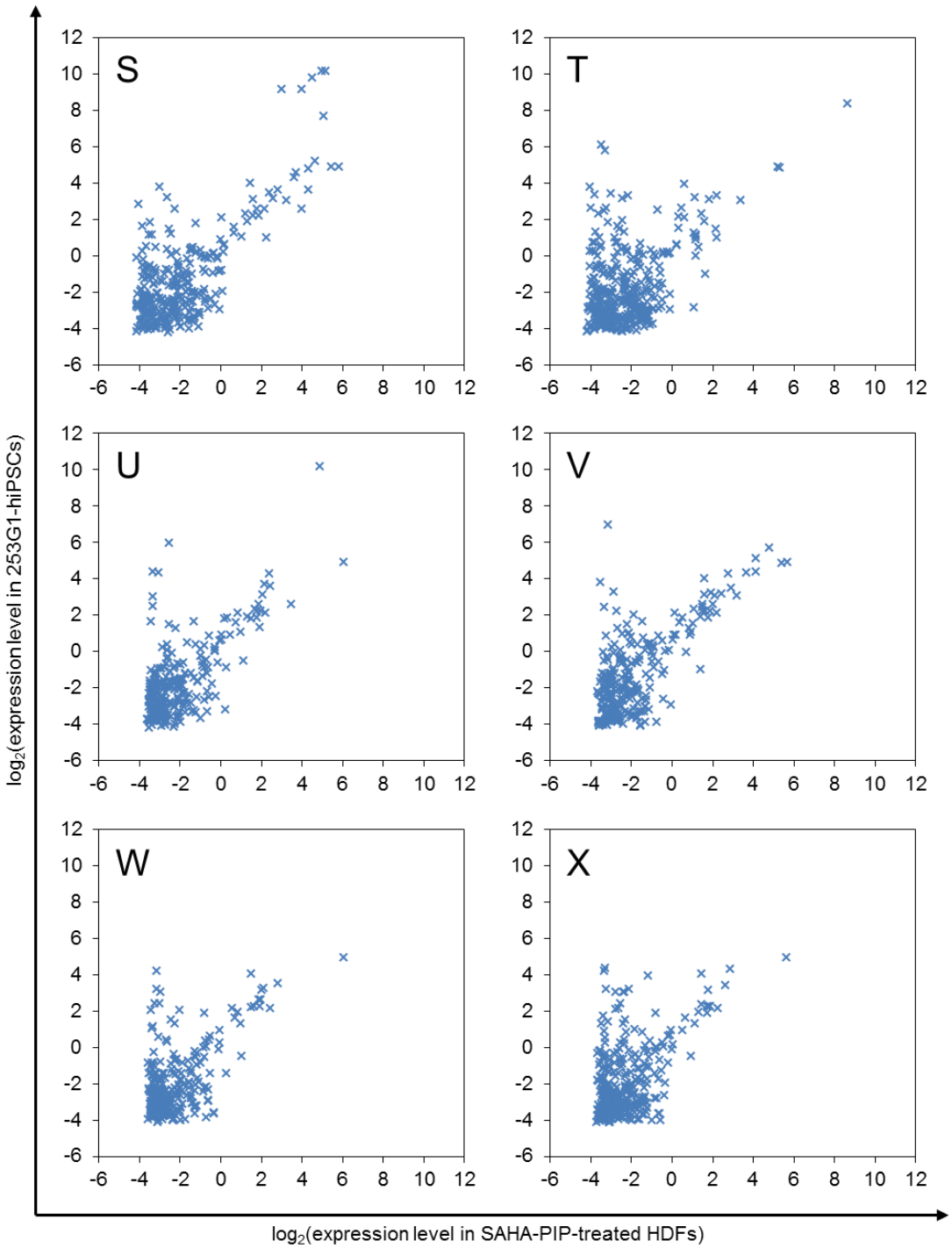
log2(expression level in 253G1-hiPSCs)
log2(expression level in SAHA-PIP-treated HDFs)

d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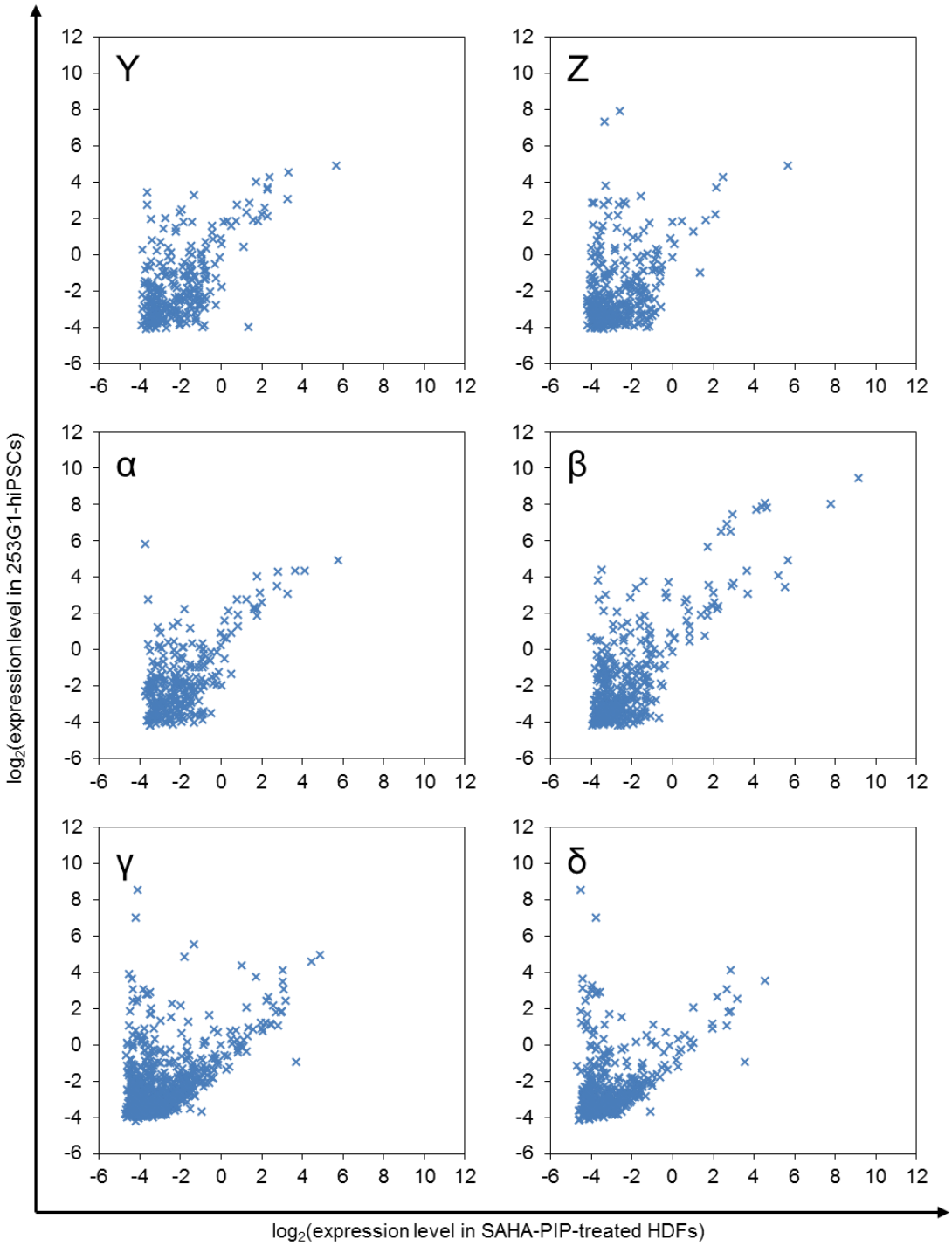
log2(expression level in 253G1-hiPSCs)
log2(expression level in SAHA-PIP-treated HDFs)

e


Y
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log2(expression level in 253G1-hiPSCs)
log2(expression level in SAHA-PIP-treated HDFs)

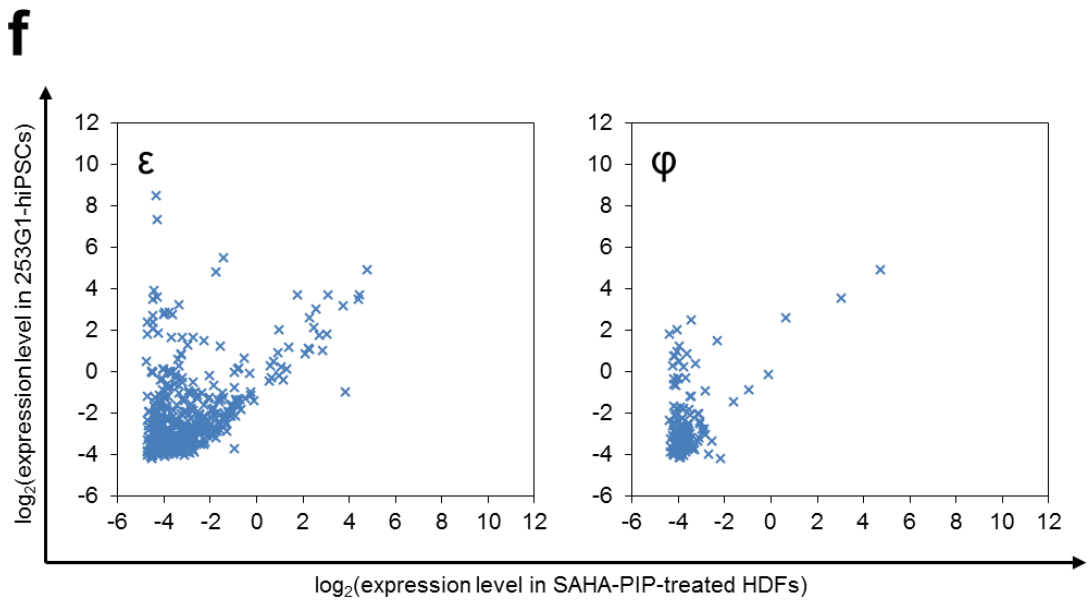


Figure 1. Scatter plots of >2 fold up/down-regulated genes. Genes which were >2 fold up/down-regulated by each SAHA-PIP were extracted and plotted based on expression level in the SAHA-PIP-treated human dermal fibroblasts (HDFs) and in 253G1 human induced pluripotent stem cells (253G1-hiPSCs). (a) SAHA-PIP **A-F**, (b) **G-L**, (c) **M-R**, (d) **S-X**, (e) **Y-δ** and (f) **ε-φ**.

Figure 2.

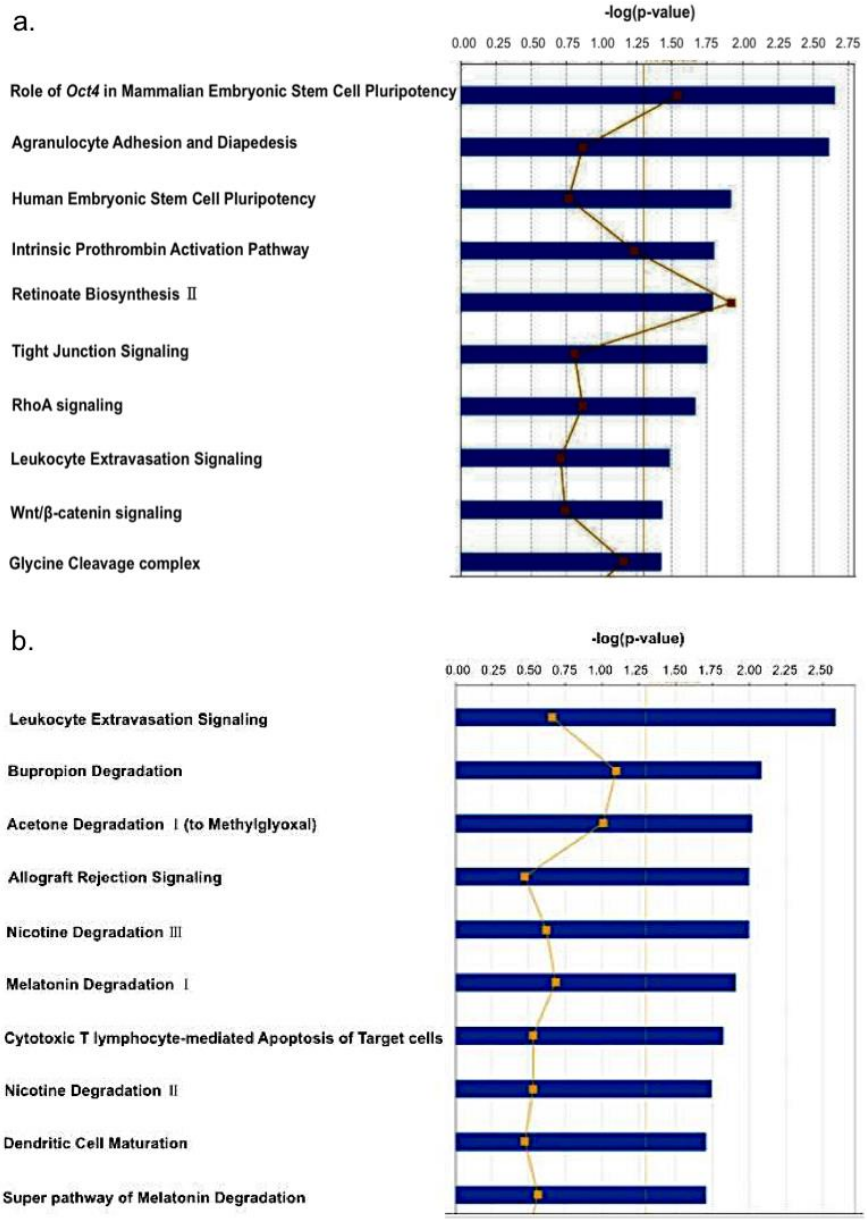
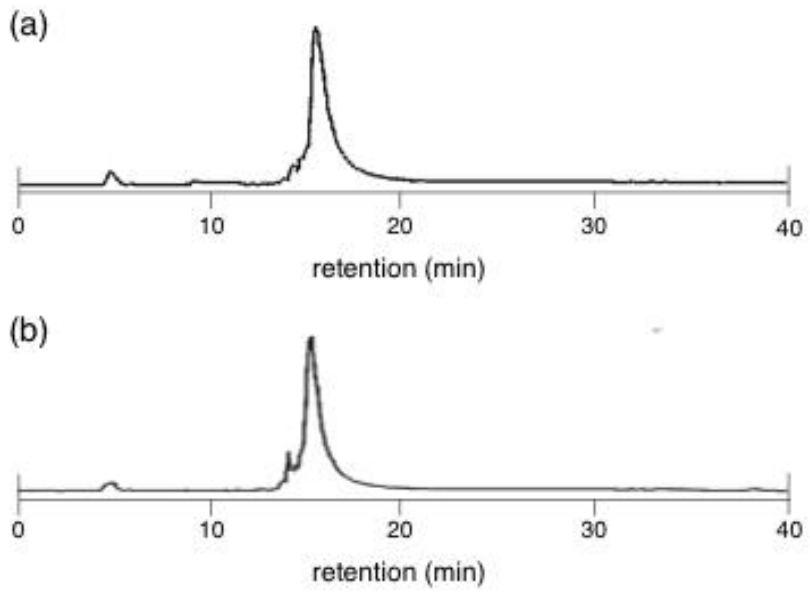


Figure 2. Top ten significantly enriched pathways in (a) SAHA-PIP I and (b) SAHA-treated HDFs generated by IPA (Ingenuity pathway analysis) out of 2-fold up or down regulated genes. Y-axis is log-# transformed significance=0.05 (Yellow line).

Figure 3. Chromatogram of SAHA-PIP `**I**` and `**K**`



Retention time: (a) 15.3 min, (b) 15.1 min

Figure 3. HPLC chromatogram of I (a) and K (b). The purities of the **I** and **K** were checked by HPLC (elution with 0.1% trifluoroacetic acid and a 0-100% acetonitrile linear gradient (0-40 min) at a flow rate of 1.0 mL min^{-1} under 254 nm).

Figure 4

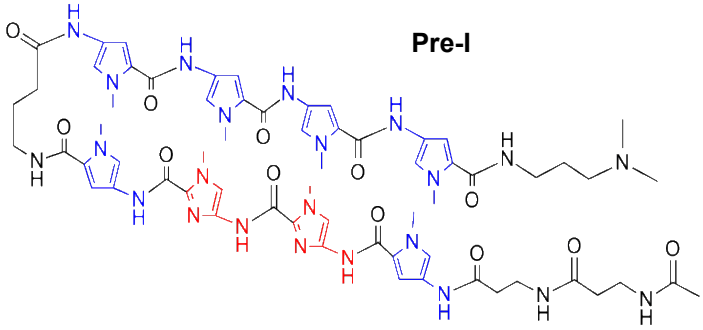


Figure 4. Chemical structure of PIP **I** lacking SAHA moiety termed Pre-**I**.

Figure 5

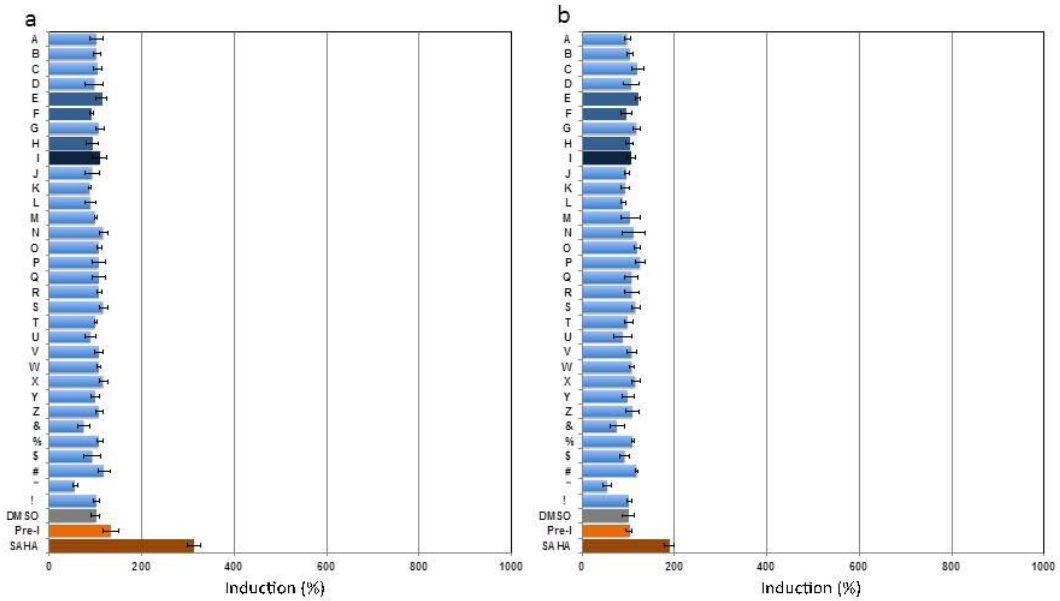


Figure 5. Expression profile of (a) *KLF4* and (b) *c-MYC* in SAHA-PIP treated HDF. All bars represent the expression profile of the endogenous genes with 1 μM of SAHA-PIPs (**A-Φ**). Light blue bars represent unsuccessful SAHA-PIPs, Blue bars indicates SAHA-PIPs that have moderate effect and Dark blue bar represent expression profile of **I**, which is our hit SAHA-PIP. Orange and red bars pre-**I** and SAHA that were employed with the same concentration as other SAHA-PIPs. Gray bar represent the expression profile of 0.1% DMSO treated cells, which is taken as internal standard that corresponds to 100%. Each bar represents mean ± SD from 24 wells.

Figure 6

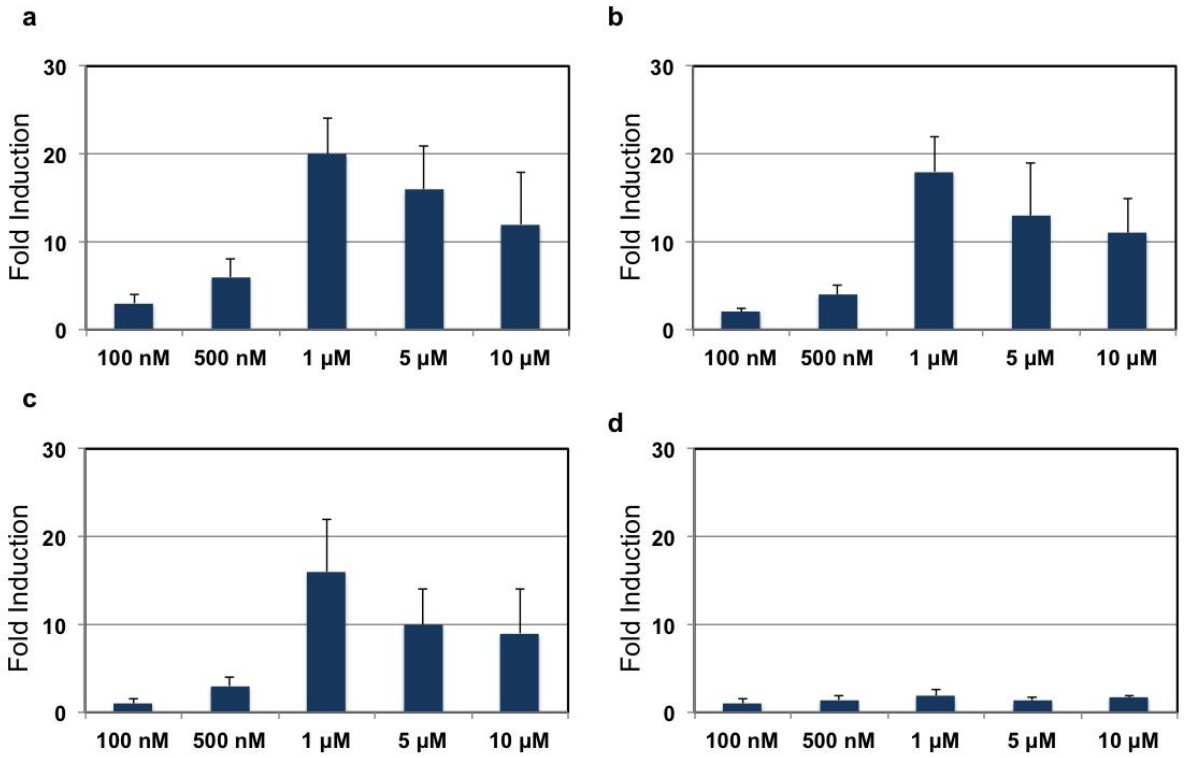


Figure 6. Standardization studies to identify optimal concentration required for effective gene induction of (a) *OCT-3/4*, (b) *SOX2*, (c) *NANOG* and (d) *KLF4* in HDFs. 0.1% DMSO treated cells is taken as internal standard that corresponds to 1-fold. Each bar represents mean ± SD from 24 wells.

Figure 7

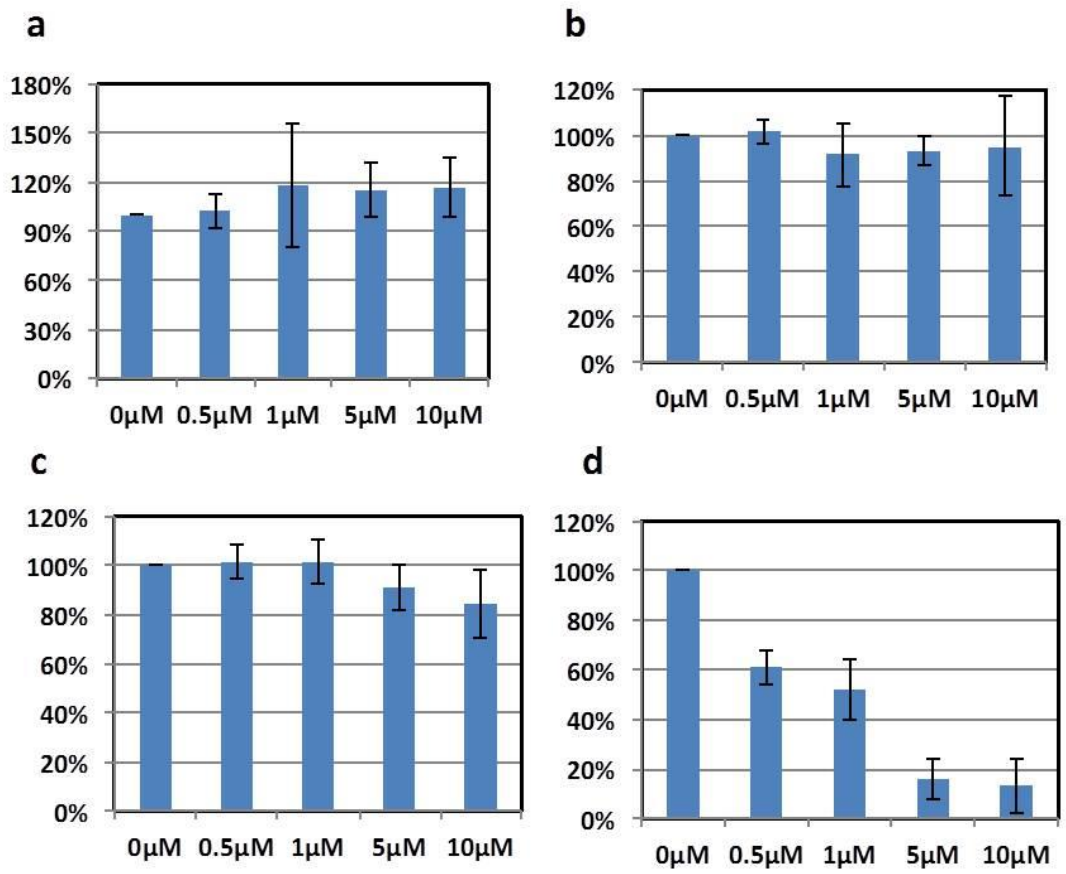
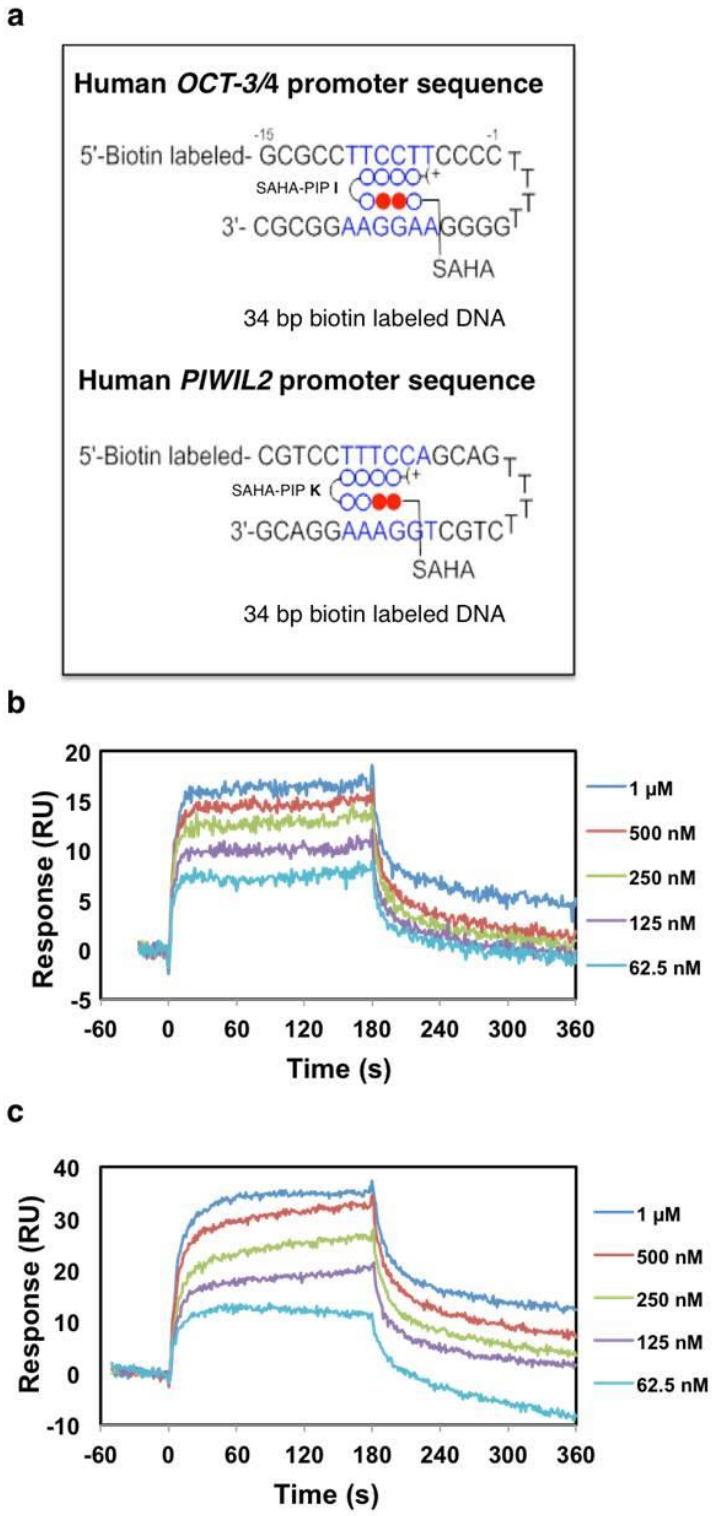


Figure 7. Cytotoxicity assay of (a) SAHA-PIP I, (b) K, (c) pre-I and (d) SAHA on human dermal fibroblasts. Cell viability of HDF was measured after 48 h treatment of the above effectors with various concentrations. Each bar represents mean ± SD from 12 wells.

Figure 8


a
Human OCT-3/4 promoter sequence
5'-Biotin labeled- GCGCCTTCCTTCCCC
SAHA-PIP I
3'- CGCGGAAGGAAGGGG
SAHA
34 bp biotin labeled DNA
Human PIWIL2 promoter sequence
5'-Biotin labeled- CGTCCTTTCCAGCAG
SAHA-PIP K
3'-GCAGGAAAGGTCGTC
SAHA
34 bp biotin labeled DNA
b
Response (RU)
Time (s)
1 μM
500 nM
250 nM
125 nM
62.5 nM
c
Response (RU)
Time (s)
1 μM
500 nM
250 nM
125 nM
62.5 nM

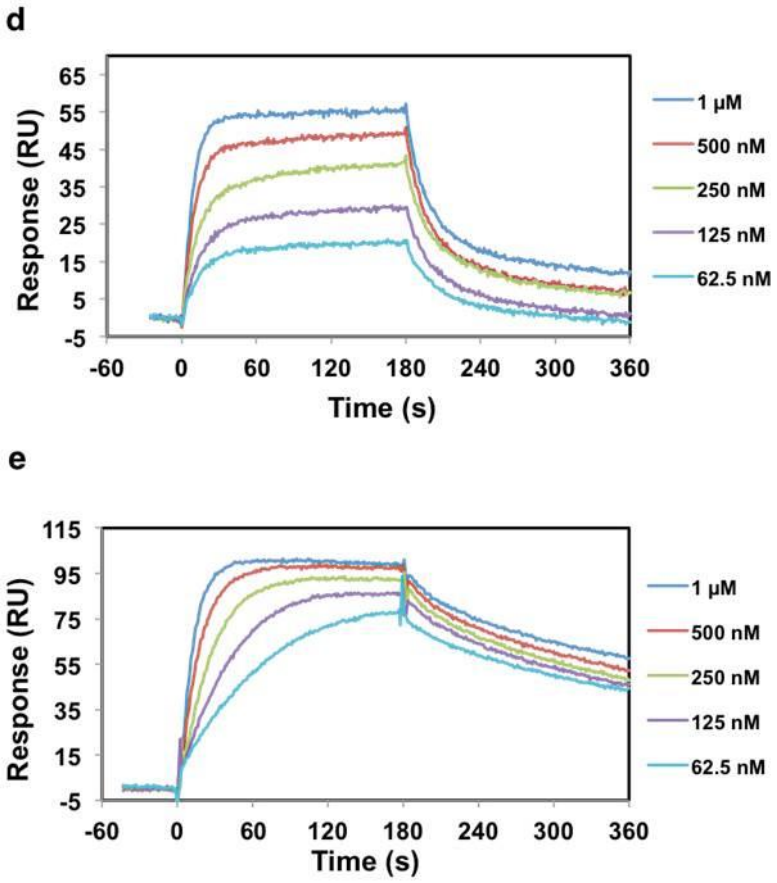


Figure 8. (a) The matching sequence (blue) for SAHA-PIP **I**, and SAHA-PIP **K** in human *OCT-3/4* and *PIWIL2* promoter region. SPR sensogram for the interactions of SAHA-PIP with its match and mismatch binding sequences: SAHA-PIP **I** binding with (b) *OCT-3/4* promoter sequence and (c) *PIWIL2* promoter sequence. SAHA-PIP **K** binding with (d) *OCT-3/4* promoter sequence and (e) *PIWIL2* promoter sequence is also shown.

Figure 9

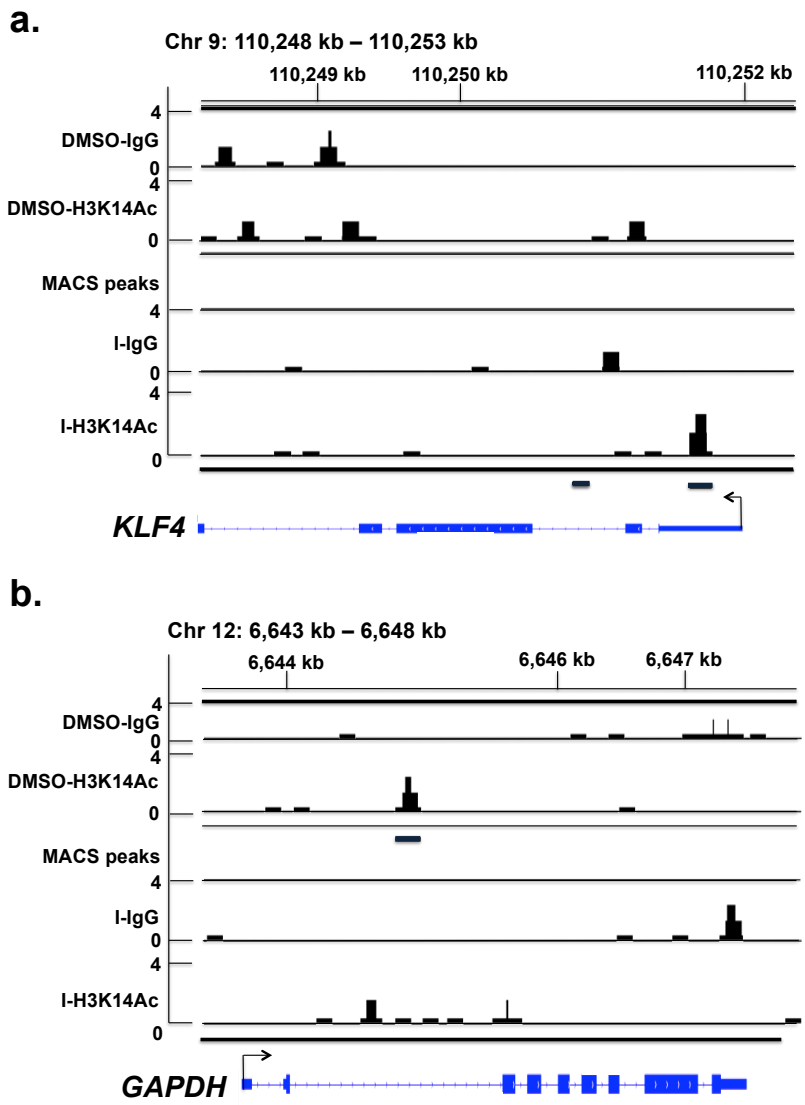


Figure 9. ChIP-Seq analysis of (a) *KLF4* and (b) *GAPDH* regions was performed as mentioned in methods given in supporting information

Figure 10

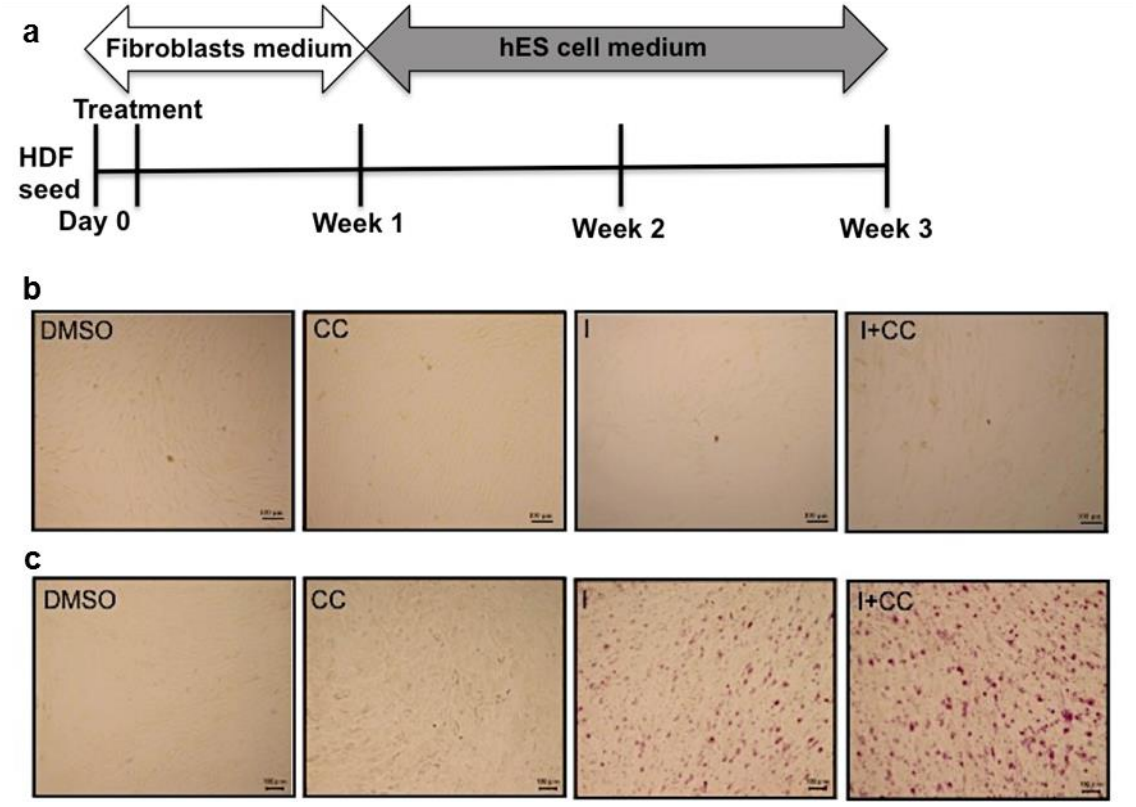


Figure 10. Effect of SAHA-PIP on the morphology of HDFs. (a) Timeline of the treatment of our effectors, which were treated every 48 h from Day 1 for four weeks. HDFs seeded at 4000 cells/ well were treated with either SAHA-PIP **I** (I) or chemical cocktail (CC) or combination of **I** and chemical cocktail (I + CC). 0.1% DMSO treated cells were used as control. Chemical cocktail encompasses A-83-01 (TGF-β inhibitor), CHIR99021 (GSK3 inhibitor), Pifithrin-α (p53 inhibitor) and PS48 (PDK1 activator). All compounds were treated at the effective concentration. Representative phase contrast microscopic image of effector treated HDF that were stained after (b) two weeks and (c) three weeks.

Figure 11

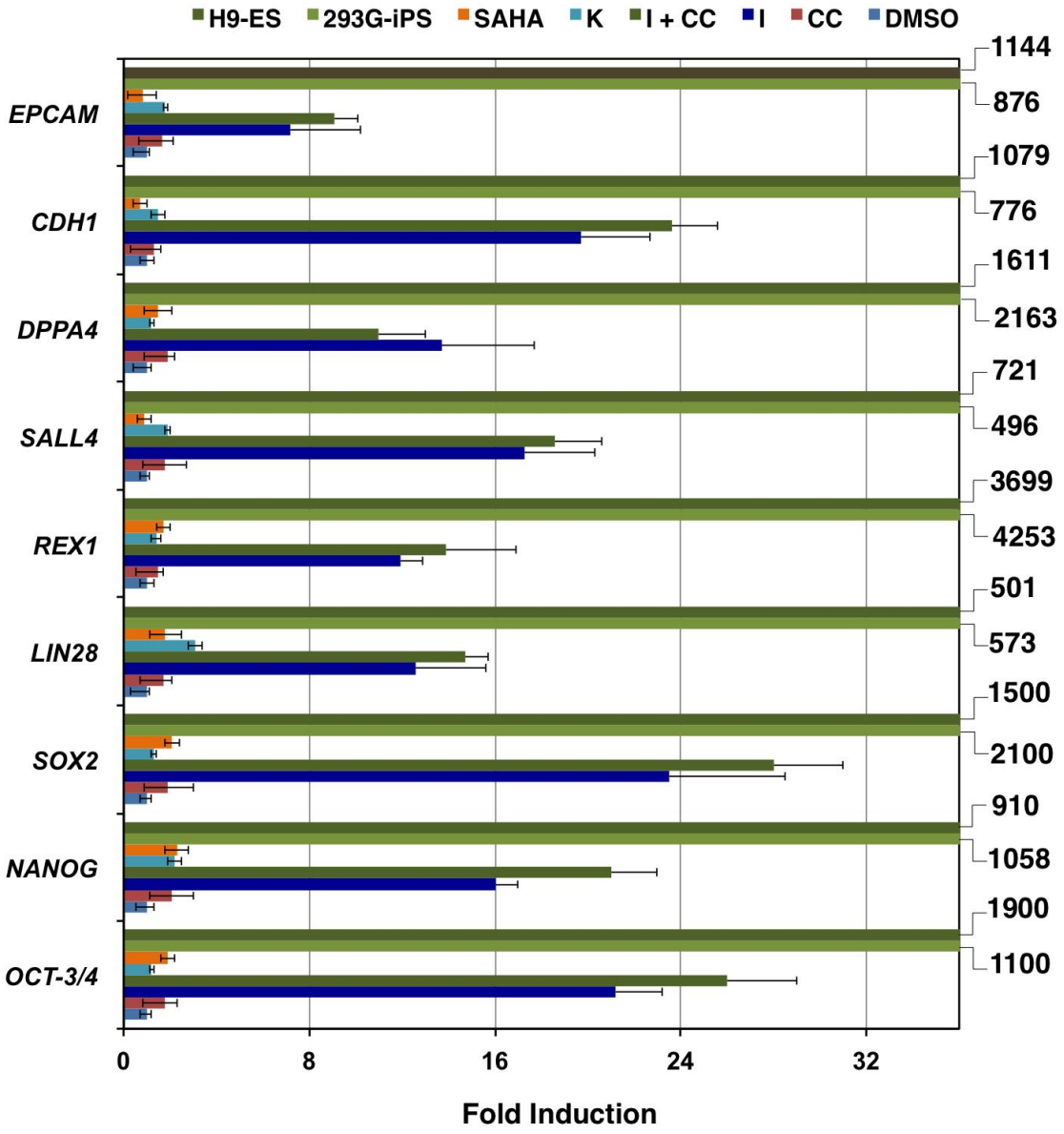


Figure 11. Expression profile of pluripotency genes in HDFs after 3 weeks treatment with effectors mentioned in **Figure 2**.

SUPPLEMENTARY TABLES

Table 1| Top 5 genetic networks in I and SAHA-treated HDFs

Analysis	Network ID	Molecules in network	P-Score	Focus Genes	Top Functions
I	1	Adaptor protein 2,**C3AR1**↑, **CALCRL***↓, **CCKBR**↑, **CELSR1**↑, **CELSR2**↑, **CYSLTR1**↑, **DRD5**↓,**EMR1**↑,**FPR3**↓,**FZD5**↑ **GABBR1**↑, GNRH, Gpcr, **GPR19**↑, **GPR20**↑,**GPR32**↑, **GPR50**↓, **GPR61**↓, **GPR64**↑,**GPR75**↓,**GPR143**↑,**GPR160**↑, **GPRC5C**↑, **GRM4**↑,**GRM7**↑, **GRPR**↑,**HTR1D**↑, **LPAR4**↑,**MC5R**↓, **NPBWR1**↓, **NTSR2**↑,**RGR**↑,**SSTR3**↑,Trk Receptor.	43	31	Cell-To-Cell Signaling and interaction, Nervous system development and function, Cell signaling.
	2	Amylase,**ARHGEF16**↑,**BCL11A**↑,**BHLHA15**↑,**C1orf106**↑, **CBR1**↓,**CDH1**↑,**CORO2A**↑,Ctbp,**DNMT3B**↑, **DSC3**↑, **DSG2**↑, **ELF3**↑,**ESRP2**↑, **FAM189A2**↑, **FLT1**↑, Focal Adhesion Kinase,**JAKMIP2***↑,**KIAA1524**↑, **KRT8**↑,**KRT18***↑, **KRT19**↑, **MALAT1**↑, **MAT1A**↑, Pias,**PKP2**↑, **PKP3**↑, **RAB39B**↑, Secretase gamma, **SFN**↑,**SGK223**↑, **SH2D3A**↑, **SPG7**↓,**ST5**↓, TCF	39	29	Cell Morphology, Cellular Assembly and Organization, Cellular compromise.
	3	**ACVR2B**↑,Akt,**ARID3B**↑,**CNKSR1**↑,**DPPA4**↑, **FOXD3**↑, **GPC3***↑,**HIF3A**↑,**IGSF1***↑, **KLK1**↑,**L1TD1**↑,**LEFTY2**↑, **MST1**↓, **NODAL**↑,Nodal Receptor, **NR6A1**↑, **POU5F1**↑, **PRODH***↑,**RHPN1**↑, **RHPN2**↑,**SALL2**↑, **SALL4**↑, Smad2/3, **SOX2**↑,SOX2-OCT4, SOX2-OCT4-NANOG,**SPRR3**↑, **TCL1A**↑, **TDGF1**↑,**TET1**↑, **VGF**↑, **VRTN**↑,**YY1AP1**↓, **ZFP42**↑, **ZIC3***↑	38	30	Cellular function and Maintenance, Cellular development, Embryonic development.
	4	**ABL2**↓,ADRB,**ALS2**↑,**ARHGAP6**↓, **ARHGAP8/PRR5-ARHGAP8**↑,**CCL21**↑, **CITED1**	34	27	Cell Morphology, Cellular Assembly

		↓,**CRB3**↑, **EGFL6**↑, ERK, Erm, **GNAO1**↑, GTPase, **HOTAIR**↑,**ICA1**↑, **KYNU**↓, **LLGL2**↑,**MATK**↑,**MUC8**↑,Par6, **PARD6A**↑,**PARD6B**↑,**PCDHB5**↑, Pde, **PDE4D**↓, **PDE6A**↑,**PDK4**↓, **PLCB1**↓, Proinsulin, **RASGRF2**↑,**RGS5**↑,RhoGap,**SPINT2**↑, **TACSTD2**↑, **ZFP36L1**↑			and Organization, Hereditary disorder.
	5	**ALOX15**↑, **ARHGAP4**↑,**ATP1A2**↑,**B3GAT1**↑, **BMP7**↑, **CHN2***↓, **COCH**↑,**COL11A2***↑, **COL1A2**↑,**COL2A1**↑,Collagen, Collagen Type II, Collagen Type III, **CTSL2**↑,**EPHA1**↑, ERK1/2, **F11R**↑, **FGF7**↓,**FGF13**↑, Fibrin, **GPRIN2**↑, **HAS3**↑, **LINGO1***↑, **NELL2**↑,**NR2E3**↑, **OLFM1**↑, **PDE6C**↑, **PEBP4**↑,PKC alpha/beta, **RTN4R**↑,**SLC29A2**↑, SMAD1/5,**SULF1**↓, **SULF2**↑, **WISP1**↓	34	28	Carbohydrate Metabolism, Small molecule Biochemistry, Connective Tissue Disorders.
SAHA	1	**ABI3BP**↓,**AQP11**↑,**BCKDHB**↓,**CLPB**↑,**DHX37**↑, **DNPH1**↑, **DPP3**↑, Enolase, **ETHE1**↓, **FBXO2**↓, **FXYD1**↓, **GBAS**↑, **GCHFR**↓, **GJB2**↑, **IPO4**↑, **KEAP1**↑, **LIN28B**↑, **MTHFD1L***↑, **MTHFR**↑, **MYC**↓, **NUP188**↑, **PRKCDBP**↑, **PTP4A3**↑, **RARRES1**↓, **RBM23***↑, **RIN3**↑, **RTN2**↓,**SCPEP1***↓, **SIMC1**↓, **SLC25A19**↑,**TOR2A**↑,**TP53I3***↑, **TUBA4A***↓, **TYMS**↑, **UBE3C**↑	41	34	Amino Acid Metabolism, Small Molecule Biochemistry, Vitamin and Mineral Metabolism.
	2	**ASPM***↑,**AURKB**↑,**B3GNT2**↓,**BUB1**↑,**BUB1B**↑,C8, **C8G**↑, **CASC5**↑, **DOK5**↓, ERK1/2, **FAM160B2**↑, **FAM83D**↑, **FBLN2**↓, **FIGF**↑, **FXN***↑, **HMMR**↑, **IL27RA**↑, **IQGAP3**↑, **KIF15**↑, **LAMC3**↓, **NDC80**↑, **NID2**↓, **NTN4**↓, **NUF2**↑, PKC alpha/beta, **PMF1**↑, **PRELP**↓, **RTN4R**↑,**SBSN**↑,**SHC4**↓, **SKA1**↑, **SKA3**↑, **SPC25**↑, **TMEM176B**↓,**TPX2**↑	37	32	Cell cycle, Cellular Assembly and Organization, DNA Replication, Recombination and Repair
	3	**ADD2**↑,**ANK1**↑,**ANK3**↑,**BCL2L12**↑, **BRPF3**↑, cannabinoid receptor, **CENPV**↑, **CNTN1**↓,**CSMD2**↓, **DNM*3**↓, **DOM3Z**↑, **EPB49**↑, **EXOC6**↑, **FLOT2**↑,	37	32	Hematological disease, Organismal Injury

	FYN↓, **GAB3**↓, **GAD1**↓,GAD,**HCN2**↓, **ITPKB**↑, **NBEA**↓,**NCAM1**↓, **NEK8**↑, **NFASC**↓, **OBSCN**↑, **OBSL1***↓, **PRICKLE3**↓, **SHROOM2**↑, **SLC4A1**↓, Spectrin, **SPTBN1**↓, **ST8SIA4**↓, **TSGA10**↑, **TUBA3C/TUBA3D**↓,**VPS13A**↓			and Abnormalities, Cell Morphology
4	**ADRA2C**↑,**ADRBK2**↓,**BAI2***↓,Beta Arrestin, **CCKBR**↑,**CCRL1**↑, **CELSR3**↓, **EDNRA***↑, **F2RL2**↓, **FPR1**↓, **FZD10**↑, **GABBR2**↓, Gpcr, **GPER***↑,**GPR4**↓, **GPR39**↑, **GPR50**↓, **GPR56**↑, **GPR68**↑, **GPR113**↑, **GPR116**↓, **GPR123**↑, **GPR149**↓, **GPR155***↓, **GPR137C**↓, **LPHN1**↓, **MC1R**↑, **NTSR1**↑, **OXTR**↑,**PIK3R5**↓, **PTGER1**↑, **PTGFR**↓, **PTH1R**↓, Relaxin, **TAS1R1**↑	37	32	Cell-To –Cell Signaling and Interaction, Carbohydrate Metabolism, Molecular Transport
5	**ADAM11***↓,**APBA2**↑,**APC2**↑,**APLP1**↓,**ATE1**↑, atypical protein kinase C, **BEGAIN**↑, **CATSPER1**↑, **CATSPER3**↑, **CEP55***↑, **CEP170***↑, **DEF6**↑, **GRASP**↓, **HMGN2***↑, **JAM2***↓,JAM, **KIAA1967**↓ ,**KIF2C**↑, **KIF4A***↑, **LGI4**↓, **MAPRE3**↓, **NCAPG**↑,Par6, **PARD6G**↓,Pkc(s), **PNMA2**↓, **PNMAL1**↑,**PRC1**↑, **PRUNE2***↓, **SLC6A9**↓,**SMC4**↑, **SPAG5**↑, **STC2**↓, **TROAP**↑, **WDR62**↑	35	31	DNA Replication, Recombination , and Repair, Cellular Assembly and Organization, cellular Movement

*Bold proteins were those differentially expressed in relative to DMSO control. Other proteins were either not on the array or not significantly changed. Up/down arrows stand for up/down regulation.

Table 2| Induction of core pluripotency genes				
Gene Symbol	**Fold change[I]**	**Regulation [I]**	**Fold change [SAHA]**	**Regulation [SAHA]**
BAMBI	9.97	up	1.01	down
BMP7	10.48	up	1.05	up
CA2	8.57	up	-	-
CTGF	1.08	down	6.66	up
DHRS3	11.90	up	7.06	up
DLX5	11.20	up	-	-
DPPA4	218.38	up	-	-
EPHA1	6.15	up	-	-
FGFR2	12.14	up	1.18	up
FOXD3	26.44	up	-	-
GALNT3	14.10	up	-	-
GRHL2	7.98	up	-	-
KCNN2	5.57	up	1.43	up
LEFTY2	27.18	up	-	-
LINGO1	38.26	up	-	-
LRRN1	60.78	up	-	-
NANOG	98.15	up	-	-
NR6A1	9.97	up	1.31	up
OBSL1	1.78	up	7.83	up
ORC1	1.86	up	-	-
POU5F1	229.71	up	1.32	down
PPP1R17	7.92	up	-	-
PRDM14	9.96	up	-	-
SOX2	401.26	up	-	-
TDGF1	164.29	up	-	-
USP44	10.81	up	2.03	up
WDR86	31.83	up	-	-
ZIC3	234.31	up	-	-
ZIC5	19.16	up	-	-

Table 3| Comparison of top 50 non-coding RNAs highly expressed in human ES (H9) and 253G1-iPS cell lines with those expressed in SAHA, K and I treated HDFs.

Probe Name	Log^2ratio (vs DMSO)				
	I	K	SAHA	253G1-iPS	H9-ES
A_33_P3843873	10.6	#N/A	#N/A	14.8	13.3
A_21_P0014207	7.3	#N/A	#N/A	11.9	10.8
A_21_P0005250	6.4	#N/A	#N/A	10.9	9.8
A_21_P0000821	7.5	#N/A	#N/A	10.6	10.0
A_33_P3501721	5.2	#N/A	#N/A	9.5	7.4
A_19_P00319155	5.3	#N/A	#N/A	9.5	7.5
A_19_P00319154	5.9	#N/A	#N/A	9.5	7.5
A_21_P0005718	5.8	#N/A	#N/A	9.4	8.9
A_21_P0013285	4.0	#N/A	#N/A	9.3	4.3
A_21_P0012675	4.6	#N/A	#N/A	9.3	7.9
A_32_P232559	1.8	0.2	-1.6	9.3	7.6
A_33_P3412262	3.9	#N/A	#N/A	9.0	9.8
A_21_P0002403	#N/A	#N/A	#N/A	8.9	-0.3
A_21_P0007477	4.0	-0.3	0.3	8.7	7.3
A_21_P0010095	#N/A	#N/A	#N/A	8.7	-2.5
A_19_P00322183	3.4	#N/A	#N/A	8.6	7.8
A_23_P373708	5.3	0.9	0.5	8.3	8.2
A_19_P00317287	5.3	#N/A	#N/A	8.3	8.2
A_21_P0014913	3.0	#N/A	1.5	8.2	7.1
A_21_P0000505	2.5	0.4	0.2	8.1	8.5
A_21_P0000491	2.1	0.5	2.1	7.9	7.3
A_19_P00315625	4.2	#N/A	#N/A	7.9	7.5
A_21_P0010434	4.0	#N/A	#N/A	7.9	6.3
A_19_P00315631	4.3	#N/A	#N/A	7.8	2.7

A_21_P0011254	3.1	#N/A	#N/A	7.7	6.9
A_19_P00807053	4.7	#N/A	#N/A	7.7	8.2
A_32_P34826	3.9	#N/A	#N/A	7.7	7.9
A_21_P0004707	4.1	#N/A	#N/A	7.7	8.5
A_19_P00800681	1.9	-0.1	1.1	7.6	7.4
A_19_P00803019	1.7	-0.2	0.9	7.6	7.4
A_21_P0012630	4.2	#N/A	#N/A	7.6	5.9
A_19_P00319534	0.7	#N/A	1.0	7.5	7.2
A_33_P3382296	3.0	#N/A	#N/A	7.5	6.1
A_33_P3240747	#N/A	#N/A	#N/A	7.5	8.6
A_21_P0003854	3.7	0.6	0.3	7.5	7.1
A_21_P0013869	3.8	#N/A	#N/A	7.4	7.7
A_32_P86578	3.6	#N/A	#N/A	7.3	7.1
A_19_P00319369	3.0	#N/A	#N/A	7.3	6.7
A_21_P0011685	3.6	#N/A	#N/A	7.3	7.5
A_21_P0009595	3.7	#N/A	#N/A	7.2	8.3
A_19_P00321221	2.2	#N/A	#N/A	7.2	7.3
A_21_P0000492	2.2	0.5	2.2	7.2	6.7
A_21_P0008067	#N/A	#N/A	#N/A	7.2	4.6
A_21_P0007460	3.6	#N/A	#N/A	7.2	7.1
A_21_P0000229	1.2	0.3	0.8	7.2	6.7
A_21_P0013554	2.9	#N/A	#N/A	7.0	7.2
A_19_P00320101	4.6	#N/A	#N/A	7.0	7.0
A_33_P3385371	#N/A	#N/A	#N/A	7.0	5.2
A_32_P195719	#N/A	#N/A	#N/A	7.0	7.9
A_21_P0000813	8.3	3.3	4.0	7.0	5.4

Each result is the summary of analysis of data derived from two individual culture plates.

Table 4| Binding affinities of PI polyamide with its match and mismatch sequences

	OCT-3/4	*PIWIL2*	Specificity mode
I	K_a ($M^{-1}s^{-1}$) - $5.04x10^5$ K_d (s^{-1}) - 0.0287 K_D (M) - $5.7x10^{-8}$	K_a ($M^{-1}s^{-1}$) - $1.4x10^5$ K_d (s^{-1}) - 0.0203 K_D (M) - $1.5x10^{-7}$	*OCT-3/4 > PIWIL2*
K	K_a ($M^{-1}s^{-1}$) - $1.56x10^5$ K_d (s^{-1}) - 0.0166 K_D (M) - $1.1x10^{-7}$	K_a ($M^{-1}s^{-1}$) - $1.23x10^5$ K_d (s^{-1}) - $3.2x10^{-3}$ K_D (M) - $2.6x10^{-8}$	*PIWIL2 > OCT-3/4*

Table 5| Primer sequences for ChIP and endogenous expression		
Primer	**Sequence 5'→3'**	**Application**
***Oct3/4* F**	GAC AGG GGG AGG GGA GGA GCT AGG	RT-PCR
***Oct3/4* R**	CTT CCC TCC AAC CAG TTG CCC CAA AC	
***Sox2* F**	GGG AAA TGG GAG GGG TGC AAA AGA GG	
***Sox2* R**	TTG CGT GAG TGT GGA TGG GAT TGG TG	
***Nanog* F**	CAG CCC TGA TTC TTC CAC CAG TCC C	
***Nanog* R**	TGG AAG GTT CCC AGT CGG GTT CAC C	
***c-Myc* F**	GCG TCC TGG GAA GGG AGA TCC GGA G	
***c-Myc* R**	TTG AGG GGC ATC GTC GCG GGA GGC TG	
***Klf4* F**	GAT TAC GCG GGC TGC GGC AAA ACC TAC ACA	
***Klf4* R**	TGA TTG TAG TGC TTT CTG GCT GGG CTC C	
***β-Actin* F**	CAA TGT GGC CGA GGA CTT TG	
***β-Actin* R**	CAT TCT CCT TAG AGA GAA GTG G	
***Oct3/4* proximal promoter F**	AGT CTG GGC AAC AAA GTG AGA	ChIP PCR
***Oct3/4* proximal promoter R**	AGA AAC TGA GGC GAA GGA TG	
***Sox2* promoter F**	GAG AAG GGC GTG AGA GAG TG	
***Sox2* promoter R**	AAA CAG CCA GTG CAG GAG TT	
***Sox2* enhancer F**	GGA TAA CAT TGT ACT GGG AAG GGA CA	
***Sox2* enhancer R**	CAA AGT TTC TTT TAT TCG TAT GTG TGA GCA	
***Nanog* promoter F**	GTT CTG TTG CTC GGT TTT CT	
***Nanog* promoter R**	TCC CGT CTA CCA GTC TCA CC	
***GAPDH* promoter F**	CTG AGC AGA CCG GTG TCA CAT C	
***GAPDH* promoter R**	GAG GAC TTT GGG AAC GAC TGA G	

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