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Author(s)	Pandian, Ganesh N; Nakano, Yusuke; Sato, Shinsuke; Morinaga, Hironobu; Bando, Toshikazu; Nagase, Hiroki; Sugiyama, Hiroshi
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# A synthetic small molecule for rapid induction of multiple pluripotency genes in mouse embryonic fibroblasts

Ganesh N. Pandian<sup>1</sup>, Yusuke Nakano<sup>2</sup>, Shinsuke Sato<sup>1</sup>, Hironobu Morinaga<sup>2</sup>, Toshikazu Bando<sup>2</sup>, Hiroki Nagase<sup>3,4</sup> & Hiroshi Sugiyama<sup>1,2</sup>

<sup>1</sup>Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Sakyo, Kyoto 606-8502, Japan, <sup>2</sup>Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8501, Japan, <sup>3</sup>Division of Cancer Genetics, Department of Advanced Medical Science, Nihon University School of Medicine, Tokyo 173-8610, Japan, <sup>4</sup>Division of Cancer Genetics, Chiba Cancer Center, Research Institute, 666-2 Nitona-cho, Chuo-ku, Chiba-shi, Chiba 260-8717, Japan.

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Correspondence and requests for materials should be addressed to H.S. (hs@kuchem.kyoto-u.ac.jp)

Cellular reprogramming involves profound alterations in genome-wide gene expression that is precisely controlled by a hypothetical epigenetic code. Small molecules have been shown to artificially induce epigenetic modifications in a sequence independent manner. Recently, we showed that specific DNA binding hairpin pyrrole-imidazole polyamides (PIPs) could be conjugated with chromatin modifying histone deacetylase inhibitors like SAHA to epigenetically activate certain pluripotent genes in mouse fibroblasts. In our steadfast progress to improve the efficiency of SAHA-PIPs, we identified a novel compound termed,  $\delta$  that could dramatically induce the endogenous expression of *Oct-3/4* and *Nanog*. Genome-wide gene analysis suggests that in just 24 h and at nM concentration,  $\delta$  induced multiple pluripotency-associated genes including *Rex1* and *Cdh1* by more than ten-fold.  $\delta$  treated MEFs also rapidly overcame the rate-limiting step of epithelial transition in cellular reprogramming by switching “ON” the complex transcriptional gene network.

Natural transcription factors precisely control genome-wide gene expression at various distinctive levels by switching “ON” and “OFF” the appropriate genes at the right place and time<sup>1</sup>. The eukaryotic genome is packaged into highly condensed chromatin and its functional unit nucleosomes are composed of histone proteins<sup>2</sup>. Histone tails protrude from the nucleosome structure and undergo precise covalent modifications to regulate chromatin dynamics and genome function<sup>3</sup>. Histone modifications regulate many genes in eukaryotic cells and control complex processes that govern cellular reprogramming<sup>4,5</sup>. It is known that histone-modifying enzymes including histone deacetylases (HDACs) are either rearranged and/or deleted in cancer cells and stem cells<sup>6,7</sup>. A recent study suggests that chromatin-modifying enzymes can act as both facilitators and barriers to epigenetic remodelling of differentiated cells to a stem cell configuration. Hence, selective chromatin modifiers could precisely modulate the complex transcriptional network with fewer exogenously introduced transcription factors and efficiently switch cellular identity<sup>8–10</sup>. In our lab, we have recently shown that it is possible to confer sequence-specificity to SAHA, a potent HDAC inhibitor by conjugating it with selective DNA-binding hairpin pyrrole-imidazole polyamides (PIPs)<sup>11</sup>. Consequently, we synthesized a new library of SAHA-PIPs with differential inducing ability termed, A to P and evaluated their effect on pluripotency genes in mouse embryonic fibroblasts (MEFs). We identified some SAHA-PIPs that could differentially upregulate the endogenous expression of distinctive iPSC factors by initiating epigenetic marks conferring to transcriptionally active chromatin in the promoter region of both *Oct-3/4* and *Nanog*<sup>12</sup>. Synthesis and screening of a series of derivatives of our hit SAHA-PIP E indicated that our programmable small DNA-binding SAHA-PIPs could be developed to induce the specific expression of core pluripotent genes<sup>13</sup>. However, the induction values were very low when compared to embryonic stem (ES) cells<sup>12,13</sup>. Our hypothesis is that the sequence-specific recognition ability of our first library of SAHA-PIPs is not sufficient enough to effectively induce gene expression. In this report, we show the synthesis, screening and identification of a new SAHA-PIP with improved recognition ability. Through genome-wide gene analysis we report for the first time about a novel small molecule that can rapidly activate multiple pluripotency genes, which are usually expressed at the later stages of reprogramming.



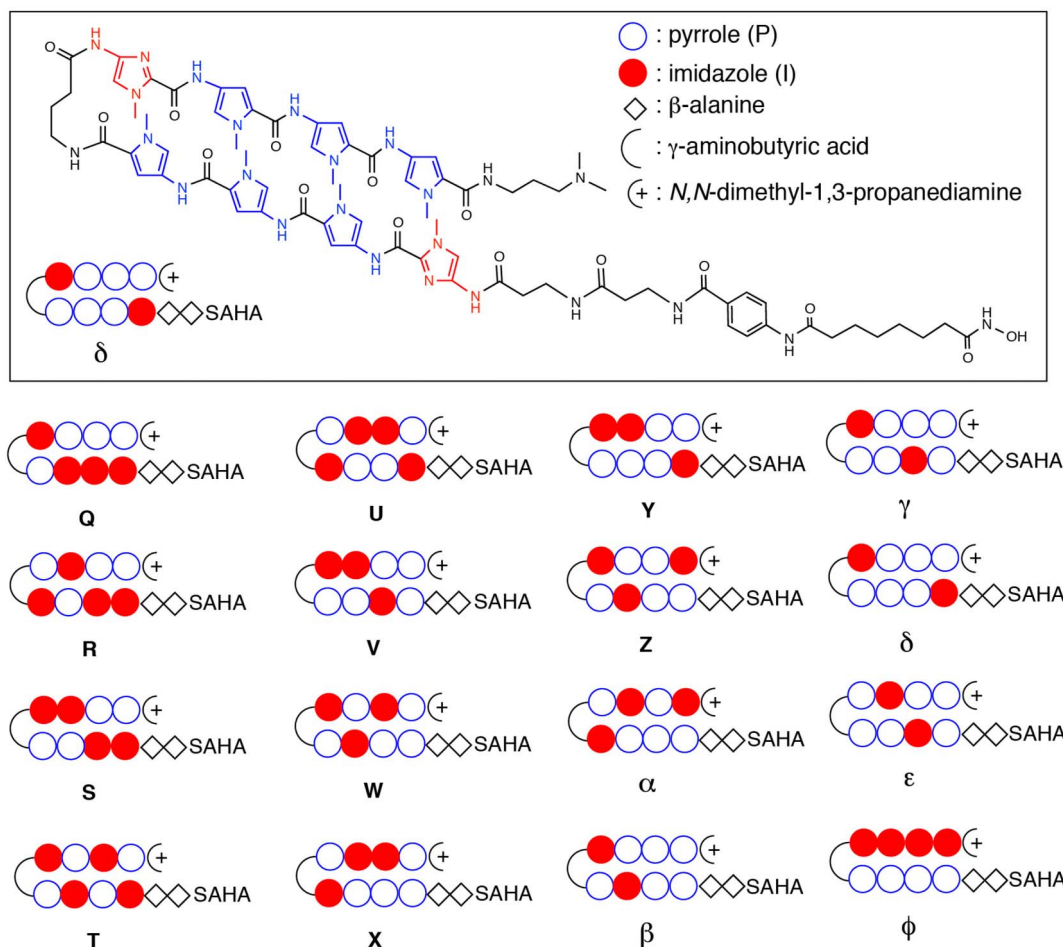
## Results

**Synthesis of novel SAHA-PIP conjugates with improved sequence-recognition ability and their characterization.** In this study, we designed 16 new SAHA-PIP conjugates that could differentially target a six-base-pair sequence according to the binding rule for PI polyamides. In these newly designed SAHA-PIP conjugates that were termed (Q to  $\Phi$ ), we placed imidazole at various positions in the top arm of the SAHA-PIPs (Figure 1). Our notion is that by placing imidazole at different positions, the selective inducing ability of SAHA-PIPs will be superior to our previous study owing to the improved recognition of GC rich sequences. The SAHA moiety was conjugated with a double  $\beta$ -alanine linker at the N-tail of the hairpin PI polyamides as mentioned before<sup>12,13</sup> and all our SAHA-PIPs were expected to have high binding affinities (nM order) towards specific DNA sequences. We synthesized PI polyamides possessing (8-methoxy-8-oxooctanamido) benzoic acid moiety as a SAHA precursor through Fmoc solid-phase synthesis, using an oxime resin and subsequent 3-(dimethylamino)-1-propylamine treatment as mentioned before<sup>12</sup>. Consistent with the previous report, each of the synthesized precursors was converted to SAHA-PIPs (Q to  $\Phi$ ) by 50% (v/v)  $\text{NH}_2\text{OH}$ . Q to  $\Phi$  was then purified (Figure S1) and their Purity was confirmed by HPLC and ESI-TOF MS as described previously<sup>12,13</sup>.

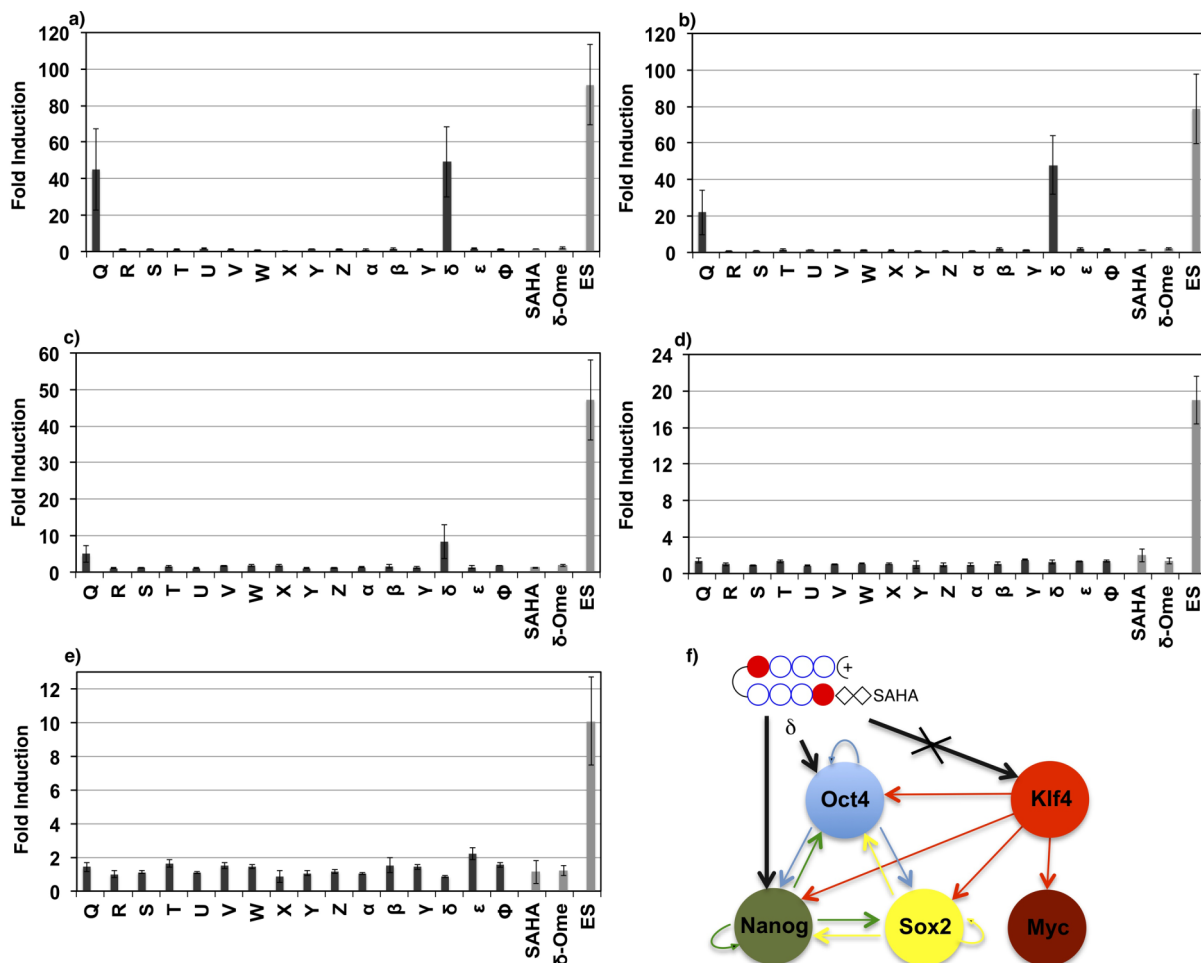
**Identification of a novel SAHA-PIP that could dramatically induce iPSC reprogramming factors in mouse embryonic fibroblasts.** Screening studies to check the effect of our new library of 16 SAHA-polyamides (Q- $\Phi$ ) on the endogenous expression of the

standard reprogramming factors was done as mentioned before<sup>12,13</sup>. Q and  $\delta$  dramatically induced the endogenous expression of *Oct-3/4* by about 45 to 50-fold (Figure 2a, Bars Q and  $\delta$ ). Interestingly,  $\delta$  also induced *Nanog* by about 47-fold; while Q treated MEFs displayed about 20-fold increase in the endogenous expression of *Nanog* (Figure 2b, Bars Q and  $\delta$ ). In the case of  $\delta$  treated MEFs, *Sox2* that belongs to the same core transcriptional network conferring to pluripotency showed about 8-fold increase in their endogenous gene expression, while Q treated MEFs showed about 4-fold induction (Figure 2c). Occasionally the induction values of *Oct-3/4* and *Nanog* in  $\delta$  treated MEFs were comparable to those in ES cells (Figure 2a and 2b, Bars  $\delta$  and ES). It is important to note here that our hit SAHA-PIPs remarkably induce these critical pluripotency genes in just 24 h. Although *Klf4* and *c-Myc* were not induced by any of the 16 SAHA-PIPs, it is known that their pathway<sup>14</sup> is different from the core pluripotency gene network (Figure 2d and 2e). Based on our induction values, either *Oct-3/4* or *Nanog* is suggested to be the direct target of  $\delta$ , while the *Klf4* pathway remains unaffected (Figure 2f).

SAHA with out PIP showed little or no induction of any iPSC factors suggesting that the PIP moiety conjugated with SAHA is the reason behind selective gene expression. To verify if the PIP  $\delta$  moiety alone could induce endogenous gene expression, we synthesized a non-functional SAHA-PIP  $\delta$  conjugate that possesses a methyl ester in the functional group of SAHA that causes HDAC inhibition ( $\delta$ -OMe). Employment of the same concentration of  $\delta$ -OMe did not have any effect on the endogenous expression of any iPSC factors, which suggests that SAHA moiety in  $\delta$  is required for epigenetic activation of pluripotent genes (Figure 2a-e).  $\delta$  displayed no



**Figure 1** | Chemical structures of the synthetic SAHA-pyrrole (P)-imidazole (I) polyamide conjugates (PIPs) Q- $\Phi$ . PIPs were designed by placing imidazole at various positions in their top arm for improved recognition of GC-rich sequences.



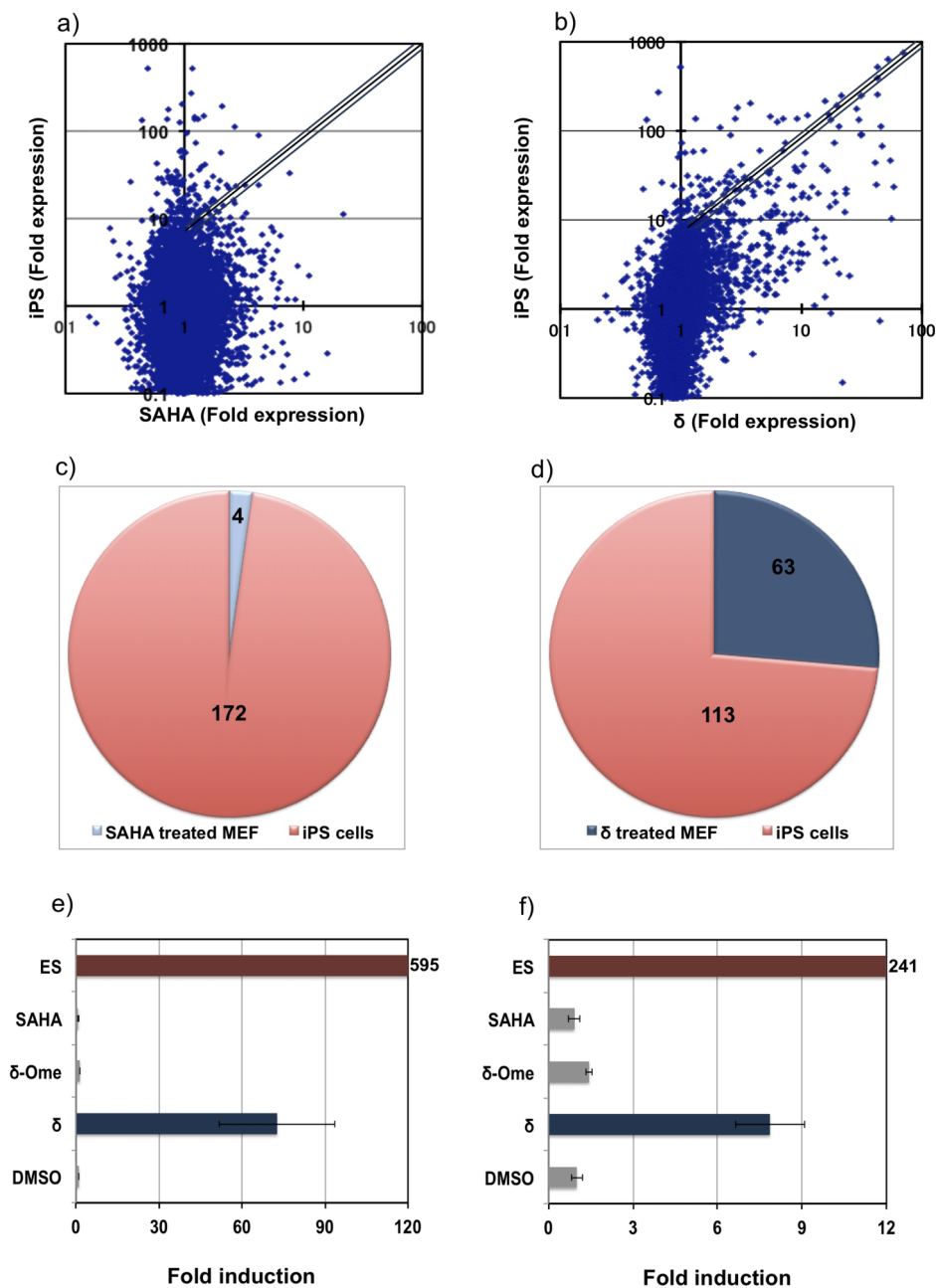
**Figure 2 | Identification of novel SAHA-PIP that is specific to core transcriptional network: qRT-PCR analysis of the expression level of the iPSC factors a) *Oct-3/4*, b) *Nanog*, c) *Sox2*, d) *Klf4*, e) *c-Myc*.** Dark gray bars represent the expression profile of the endogenous genes with 100 nM of 16 individual SAHA-PIPs (Q-Φ). Light gray bar represent the control samples. ES cells were used as the positive control. SAHA with out PIP (100 nM) was used as the control to corroborate sequence-specificity. While, the SAHA-PIP  $\delta$  conjugate that has a methyl ester in the functional group of SAHA ( $\delta$ -OMe) was employed at the same concentration as a negative control to validate the importance of HDAC activity in endogenous gene expression. Each analysis was done at least three times in triplicate. Each bar represents mean  $\pm$  SD from 18 well plates. f) Proposed mechanism of  $\delta$  targeting *Oct4* and *Nanog* based on the induction values but not the *Klf4* that belongs to different transcriptional network.

cytotoxic effect on MEFs even at 1  $\mu$ M, which implies that the cytotoxic effect is not correlated with gene inducing ability (Figure S2). The effective concentration of  $\delta$  is just 100 nM, which is relatively lower than that of other small molecules used in cellular reprogramming<sup>12</sup>.

**Genome-wide gene analysis of  $\delta$  reveals induction of several important pluripotency-associated genes.** Cellular phenotype is modulated through a gradual epigenetic process that resets the transcriptional network<sup>15</sup>. Although Q and  $\delta$ , notably induced *Oct-3/4*, we chose to evaluate the effect of  $\delta$  on genome-wide gene expression as it also induced *Nanog* and hence, could have a remarkable effect on their downstream genes. Based on our previous report, we kept a three-fold increase and a two-fold decrease as the notable effect on the up-regulation and down-regulation, respectively<sup>12</sup>. In SAHA treated MEFs, out of 21,672 detected genes, about 150 genes were up-regulated and in  $\delta$  treated MEFs out of 22,229 detected genes, about 305 genes were induced by more than three-fold (Tables S1 and S2). It is important to note here that  $\delta$  treated MEFs up-regulated about twice the number of genes than SAHA treated MEFs, and down-regulated only 200 genes in comparison with 400 genes, which were down-regulated in SAHA treated MEFs (Tables S1 and S2). While screening for the genes that

were induced at least by 10-fold, about 36% of the genes that were up regulated in  $\delta$  treated MEFs were also observed in induced pluripotent stem cells (iPSCs)<sup>16</sup> (Figure 3a–d). SAHA activated only 4% of the pluripotency-associated genes, which implies the role of the PIP in directing SAHA to genes conferring to pluripotency (Figure 3c).  $\delta$  activated *Rex1* and *Dppa4*, which are the critical pluripotency genes<sup>17</sup> by about 70-fold and 7-fold, respectively (Figure 3e and f, Blue bars). The expression level of *Rex1* and *Dppa4* in ES cells was about 595-fold and 241-fold, respectively (Figure 3e and f, Red bars). SAHA only and the non-functional SAHA-PIP  $\delta$  again did not affect the induction of these two genes (Figure 3e and f, Gray bars).

**SAHA-PIP  $\delta$  can rapidly initiate the critical mesenchymal epithelial transition stage to switch cell fate to pluripotency.** Genome-wide gene analysis suggested that  $\delta$  shifts the transcriptional network from fibroblasts to a de-differentiated state. MET (mesenchymal epithelial transition) is an important rate-limiting step during the de-differentiation of the somatic genome. The acquisition of an epithelial fate during cellular reprogramming appears to be closely associated with the pluripotent state, which reflects the need for cell-cell interactions that initiate and sustain pluripotency<sup>18</sup>. Several important pluripotent genes associated with cellular reprogramming that were rapidly induced by  $\delta$  include the epithelial gene,



**Figure 3 | Effect of  $\delta$  on genome-wide gene expression in MEFs.** Microarray scatter plots showing the global gene expression profile of a) SAHA and b)  $\delta$  treated MEFs. The horizontal and vertical axis represents the expression profile of MEFs treated with either  $\delta$  or SAHA and iPS cells, respectively. Ten-fold induction is kept as the cut-off for remarkable effect c) SAHA Vs iPS showed only 2% of the genes and d)  $\delta$  Vs iPS showed about 36% of the genes that were shared with iPS. Analysis was done in duplicate. qRT-PCR analysis to check the effect of  $\delta$  (Blue bars) on the endogenous expression of e) *Rex1* and f) *Dppa4* with DMSO, SAHA and non-functional SAHA-PIP  $\delta$  ( $\delta$ -Ome) as the negative control (Gray bars). ES cells were used as positive control (Red bars). Experiments were done in triplicate. The concentration of effectors (100 nM) and mean  $\pm$  SD is from 12 wells.

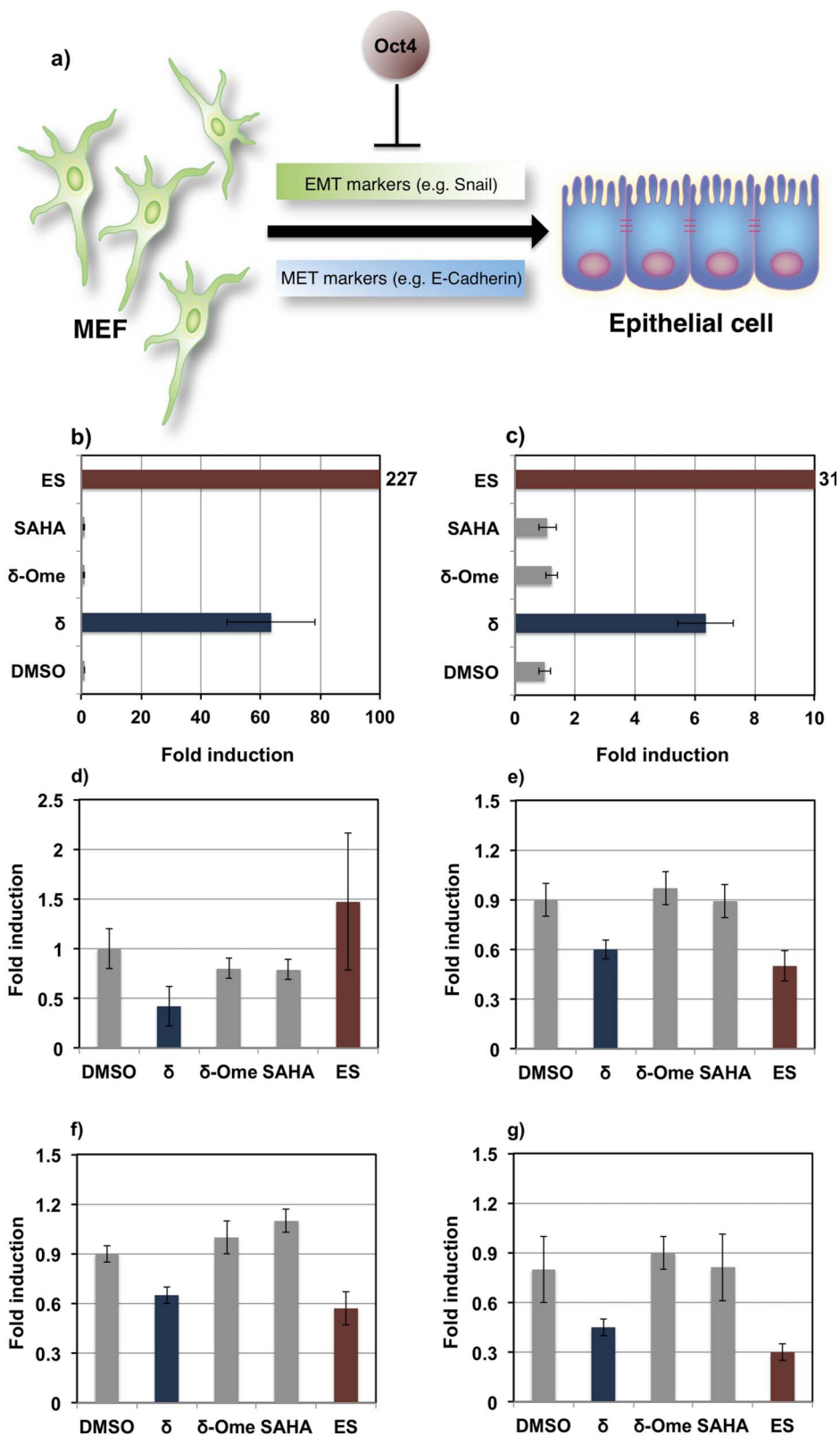
*Cdh1*, which suggests that  $\delta$ -treated MEFs could overcome MET stage that corresponds to initiation of cellular reprogramming<sup>19</sup>. To verify our micro array data, the endogenous expression of MET markers and EMT (Epithelial mesenchymal transition) markers were analyzed<sup>18</sup>. The critical up-regulated MET marker *Cdh1* was dramatically induced by about 70-fold, which is remarkable as it transpires in just 24 h (Figure 4b, Blue bar) and with out the activation of *Klf4*, which is its upstream gene. Another up-regulated marker that is associated with MET, *Cldn-7* was also notably induced by about 7-fold (Figure 4c, Blue bar). *Cdh1* and *Cldn-7* was induced by about 227 and 31-fold, respectively in ES cells (Figure 4b-c, Red bars).

*Fgf5* and some of the EMT markers including *Snai1*, *Snai2* and *Zeb2* were also down regulated by about 2-fold only in the case of  $\delta$ -treated MEFs (Figure 4d-g, Blue bars).

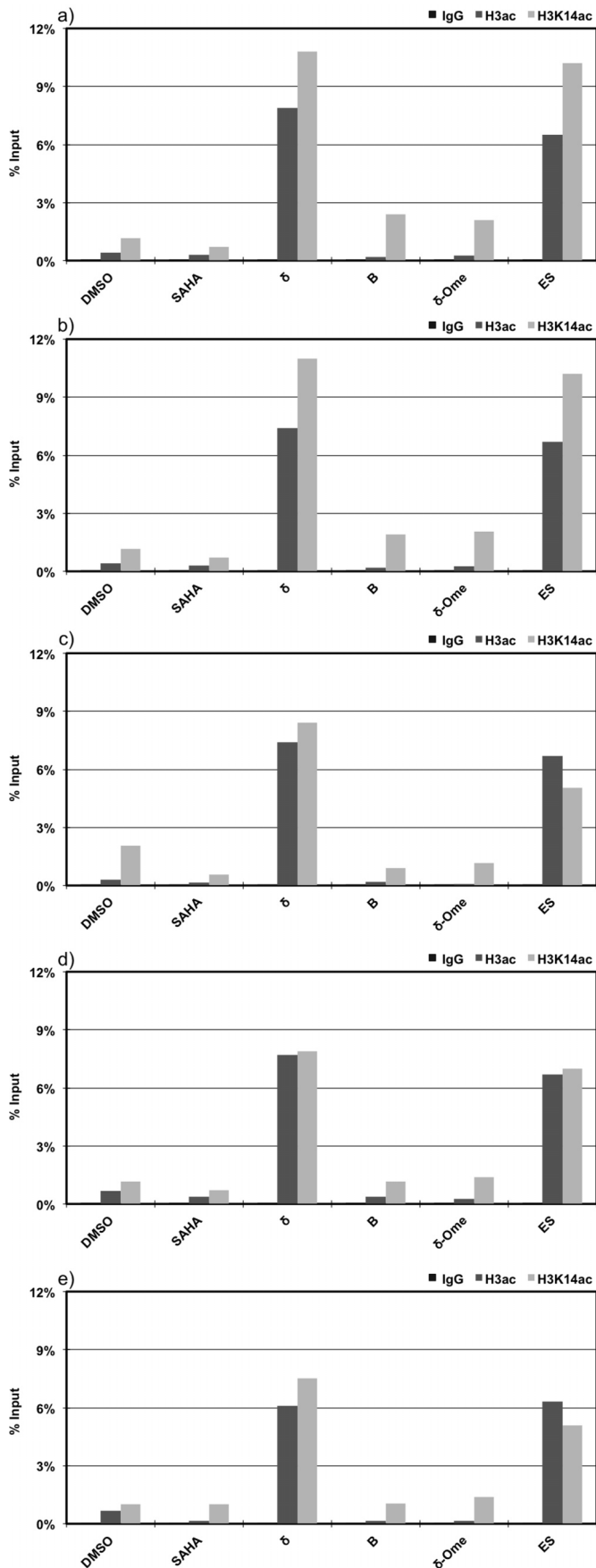
No such effect was observed in control samples (4d-g, Gray bars). The expression pattern of the EMT markers was comparable to ES cells (Figure 4e-g, Red bars). Taken together, these results suggest that  $\delta$  could rapidly overcome epithelial transition phase *via* specific initiation of pluripotency-related gene regulatory network.

**Chromatin immunoprecipitation assay reveal hyper acetylation in the promoter region of core pluripotency genes.** Consistent with the endogenous expression, the SAHA-PIP  $\delta$  markedly induced the





**Figure 4** | Effect of  $\delta$  on the endogenous expression of pluripotent genes associated with initiation of cellular reprogramming. **a)** Initiation of cellular reprogramming from fibroblasts to epithelial cell intermediate involves the down-regulation of EMT (epithelial mesenchymal transition) and up-regulation of MET (mesenchymal epithelial transition) markers. Expression level of **b)** *Cdh1*, **c)** *Cldn7*, **d)** *Fgf5*, **e)** *Zeb2*, **f)** *Snai1* and **g)** *Snai2* in 100 nM of  $\delta$  (Blue bars),  $\delta$ -OME, SAHA and DMSO treated MEFs (Gray bars). ES cells were used as positive control (Red bars). Experiments were done in triplicate and carried out twice. Each bar represents mean  $\pm$  SD from 12 wells.



**Figure 5 | Both SAHA and PIP  $\delta$  is essential for rapid induction of pluripotency genes.** MEFs were individually treated for 24 h with 100 nM of  $\delta$ ,  $\delta$ -Ome, control SAHA-PIP (B), SAHA only and 0.1% DMSO. Mouse ES cells were used as positive control. After immunoprecipitation with H3ac and H3K14ac antibodies, the amount of promoter sequence of (a) *Oct-3/4*, (b) *Nanog*, (c) *Dppa4*, (d) *Cdh1* and (e) *Rex1* in the co-precipitated DNAs was determined by qPCR. Control samples including SAHA only and  $\delta$ -Ome displayed no effect. Percent input is calculated by normalizing the data against input DNA, the enrichment with IgG antibody and with internal control primers. The primer sets are shown in Table S5. This experiment includes four treatments, each done in duplicate. Each bar represents the average of 9 wells.

acetylation of histone H3 in the promoter region of *Oct-3/4* and *Nanog* (Figure 5a and 5b, Bars H3ac). Increased acetylation of H3K14 reinstated that the inhibition of histone deacetylase occurs in the promoter region of these genes<sup>20</sup> (Figure 5a and 5b, Bars H3K14ac). It is important to note here that sequence-specificity is corroborated again as SAHA only and the negative control B, did not induce acetylation of both histone H3 and H3K14 (Figure 5a and 5b, Bars B). The importance of HDAC activity is also substantiated, as the non-functional SAHA-PIP lacking HDAC activity did not have notable effect on acetylation of histone H3 in MEFs (Figure 5a and b, Bars  $\delta$ -Ome), which suggests that both PIP  $\delta$  and SAHA are essential for gene activation.

A similar pattern was observed with *Dppa4*, *Rex1* and *Cdh1* where hyper acetylation of the histone H3 and H3K14 occurred in the promoter region of these pluripotency-associated genes (Figures 5c–e). Based on these results, it is reasonable to suggest that our hit SAHA-PIP  $\delta$ -mediated gene induction occurs through the hyper acetylation of histone H3 caused by the HDAC activity of SAHA. Acetylation level of histone H3 and H3K14 in  $\delta$  treated MEFs were comparable and occasionally surpass than that in ES cells (Figures 5a–e, Bars ES and  $\delta$ ).

## Discussion

Chromatin modifications orchestrate the activation and suppression of the transcriptional machinery, which in turn controls the specification of cell type<sup>15,21,22</sup>. The code that characterizes the epigenetic modifications still remains elusive and hence deciphering their mode of functions represents a key-challenge in cell biology<sup>23,24</sup>. Since artificial chromatin modifiers are known to improve efficiency of cellular reprogramming by gene activation<sup>25–27</sup>, complementing them with site-specificity can enhance their efficacy. Previously we have shown that certain SAHA-PIPs can induce the expression level of both *Oct-3/4* and *Nanog* with an added advantage of sequence-specific chromatin remodelling<sup>12</sup>. Although the induction effect of iPSC reprogramming factors by our hit SAHA-PIPs is notable, they were only three-fold. Among the newly developed SAHA-PIPs that possesses improved recognition ability,  $\delta$  dramatically induced the multiple pluripotency genes to overcome the important MET phase, which has been a huge barrier of somatic cell reprogramming<sup>18</sup>. Epithelial cells are more flexible to reprogramming as acquisition of pre-existing features of pluripotent cells suggests that only fewer changes are required to attain pluripotency<sup>19</sup>. Recent studies indicate that epigenetic errors or cultural conditions could switch the cells to be prone to tumor formation<sup>28,29</sup>.

A recent report implied that neural differentiation from partially reprogrammed cells display superior and rapid gliogenic competency than those differentiated from either iPSCs or directly from somatic cells<sup>30</sup>.  $\delta$  treated MEFs rapidly induce multiple pluripotency genes belonging to all three phases (Initiation, maturation and stabilization) of reprogramming (Table S3). We have already shown that it is possible to tailor cell-permeating PIPs to achieve improved induction values<sup>13</sup>. Hence,  $\delta$  could be developed to rapidly trigger partially or completely reprogrammed configuration in somatic cells, which may eventually lead to superior differentiation. In  $\delta$  treated



MEFs, the induction values of some pluripotency-associated genes were similar to ES cells (Figure 2a, 2b and 4e–g). In some pluripotency genes, relatively lower induction values (5–10%) was observed in  $\delta$  treated MEFs when compared to ES cells (Figures 3e, 3f and 4b–g). It is possible that the induction values in  $\delta$  treated MEFs gets normalized by the MEFs that are elite or stochastically resistant to reprogramming. Hence, the induction values in  $\delta$  treated MEFs are still remarkable as it could be obtained even with out the purification of the MEFs, which may be non-responsive to the treatment. Also, the induction values are notably higher when compared to the control samples treated with SAHA only, DMSO and PIP. Although, the actual six base pair binding site of  $\delta$  is yet to be clarified, it should relatively bind to fewer matching sites owing to their palindromic architecture. We hypothesize that combinatorial binding of PIP to conserved sequences effect this rapid induction of pluripotency genes. PIPs gain the advantage over other natural mimics as they could perturb the architecture of the packed chromatin<sup>31</sup>. Studies on the specificity landscapes of DNA binding molecules revealed PIPs are relatively superior to the natural DNA binding proteins<sup>32</sup>. Therefore, strategies to expand our tunable SAHA-PIPs could create an epoch-making approach in regenerative medicine to modulate the desired genes<sup>33,34</sup>.

## Methods

**Synthesis of SAHA-PIP  $\delta$  (SAHA- $\beta$ PIPPP $\gamma$ I PPPDp).** 4-(8-methoxy-8-oxo-octanamide) benzoic acid was synthesized and characterized as mentioned before<sup>11</sup>. PSSM-8 peptide synthesizer (Shimadzu, Kyoto) with a computer-assisted operating system using Fmoc chemistry was used for all polyamide syntheses with 45 mg of oxime resin (ca. 0.2 mmol/g, 200–400 mesh). As mentioned previously<sup>12</sup>, coupling and reaction steps were carried out to obtain the resin, which was then cleaved with *N,N*-dimethylaminopropylamine. The reaction mixture was filtered, triturated from Et<sub>2</sub>O, to yield 4-(8-methoxy-8-oxooctanamide) benzoyl PIP  $\delta$  as a yellow crude powder. This pre SAHA-PIP (about 10 mg) was dissolved in DMF (0.5 ml) and was added to an aqueous solution of 50% (v/v) NH<sub>2</sub>OH (0.5 ml). The reaction mixture was stirred for 8 h at room temperature. After the reaction, hydroxylamine was quenched with acetic acid (0.5 ml) at 0°C. The mixture was purified by flash column chromatography. The purity was checked by HPLC (elution with trifluoroacetic acid and a 0–100% acetonitrile linear gradient (0–40 min) at a flow rate of 1.0 mL min<sup>-1</sup> under 254 nm) to yield SAHA-PIP  $\delta$  as a white powder (2.0 mg, 14%). ESI-TOF-MS (positive) m/z calcd for C<sub>76</sub>H<sub>97</sub>N<sub>25</sub>O<sub>15</sub> 799.87; found 799.89. <sup>1</sup>H NMR (600 MHz, [D<sub>6</sub>] DMSO):  $\delta$  = 10.32 (s, 1H), 10.27 (s, 1H), 10.25 (s, 1H), 10.06 (s, 1H), 9.97 (s, 1H), 9.96 (s, 1H), 9.94 (s, 2H), 9.89 (s, 2H), 9.23 (brs, 1H), 8.35 (brt, 1H), 8.15 (brt, 1H), 8.03 (brt, 1H), 8.00 (brt, 1H), 7.76 (d, J = 8.9Hz, 2H), 7.64 (d, J = 8.9Hz, 2H), 7.46 (s, 1H), 7.43 (s, 1H), 7.27 (s, 2H), 7.22 (s, 2H), 7.17 (s, 2H), 7.16 (s, 2H), 7.07 (s, 2H), 6.95 (d, J = 2.1Hz, 1H), 6.91 (d, J = 2.1Hz, 1H), 3.96 (s, 3H), 3.95 (s, 3H), 3.86 (s, 6H), 3.85 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.43 (m, 2H), 3.33 (m, 2H), 3.24 (m, 2H), 3.21 (m, 2H), 3.07 (m, 2H), 2.79 (s, 3H), 2.78 (s, 3H), 2.52 (m, 2H), 2.47 (m, 2H), 2.37 (t, J = 7.6Hz, 2H), 2.35 (t, J = 7.6Hz, 2H), 2.31 (t, J = 7.6Hz, 2H), 1.93 (t, J = 7.6Hz, 2H), 1.84 (m, 2H), 1.79 (m, 2H), 1.57 (m, 2H), 1.48 (m, 2H), 1.26 (m, 2H).

A procedure similar to that used for the preparation of compound  $\delta$  was adopted to prepare all the other SAHA-PIPs as shown below.

SAHA-PIP (Q, R, S, T, U, V, W, X, Y, Z,  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\epsilon$ ,  $\phi$ )

SAHA-PIP Q (SAHA- $\beta$ IIIP $\gamma$ I PPPDp), SAHA-PIP R (SAHA- $\beta$ IIPI $\gamma$ I PPPDp), SAHA-PIP S (SAHA- $\beta$ IIIP $\gamma$ I PPPDp), SAHA-PIP T (SAHA- $\beta$ IIPI $\gamma$ I PPPDp), SAHA-PIP U (SAHA- $\beta$ IIPI $\gamma$ I PPPDp), SAHA-PIP V (SAHA- $\beta$ PIPP $\gamma$ I PPPDp), SAHA-PIP W (SAHA- $\beta$ PIPP $\gamma$ I PPPDp), SAHA-PIP X (SAHA- $\beta$ PIPP $\gamma$ I PPPDp), SAHA-PIP Y (SAHA- $\beta$ PIPP $\gamma$ I PPPDp), SAHA-PIP Z (SAHA- $\beta$ PIPP $\gamma$ I PPPDp), SAHA-PIP  $\alpha$  (SAHA- $\beta$ PIPP $\gamma$ I PPPDp), SAHA-PIP  $\beta$  (SAHA- $\beta$ PIPP $\gamma$ I PPPDp), SAHA-PIP  $\gamma$  (SAHA- $\beta$ PIPP $\gamma$ I PPPDp) and SAHA-PIP  $\epsilon$  (SAHA- $\beta$ PIPP $\gamma$ I PPPDp) and SAHA-PIP  $\phi$  (SAHA- $\beta$ PIPP $\gamma$ I PPPDp)

Other experimental details are submitted as supplementary information along with this manuscript.

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### Author contributions

Experiments were designed by H.S., T.B., H.N. and G.N.P. G.N.P., S.S., Y.N. and G.N.P. performed research. H.S., G.N.P., Y.N., S.S. and H.M. analyzed the data. The manuscript was written by H.S., G.N.P. and T.B.

### Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

**Competing financial interests:** The authors declare no competing financial interests.

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