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Running Title: dCas9 activators in hPSCs and hMSCs

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Summary

Human pluripotent stem cells (hPSCs) and mesenchymal stromal/stem cells (hMSCs) are clinically-relevant sources for cellular therapies and for modeling human development and disease. Many stem cell-based applications rely on the ability to activate several endogenous genes simultaneously to modify cell fate. However, genetic intervention of these cells remains challenging. Several catalytically-dead Cas9 (dCas9) proteins fused to distinct activation domains can modulate gene expression when directed to their regulatory regions by a specific single guide RNA (sgRNA). Here, we have compared the ability of the first-generation dCas9-VP64 activator and the second-generation systems, dCas9-SAM and dCas9-SunTag, to induce gene expression in hPSCs and hMSCs. Several stem cell lines were lentivirally-transduced with each activator, selected using antibiotic resistance or flow cytometry, and tested for single and multiplexed gene activation. When the activation of several genes was compared, all three systems induced specific and potent gene expression in both single and multiplexed settings, but the dCas9-SAM and dCas9-SunTag systems resulted in the highest and most consistent level of gene expression. Simultaneous targeting of the same gene with multiple sgRNAs did not result in additive levels of gene expression in hPSCs nor hMSCs. We demonstrate the robustness and specificity of second-generation dCas9 activators as tools to simultaneously activate several endogenous genes in clinically-relevant human stem cells.

Significance Statement

Different dCas9-based transcriptional activators induce potent and reliable gene activation, both in single and multiplexed approaches, in clinically-relevant stem cells (hPSCs and hMSCs). The second generation systems, dCas9-SAM and dCas9-SunTag, resulted in the highest level of gene expression, and simultaneous targeting of the same gene with multiple sgRNAs did not result in additive levels of gene expression. Dead-Cas9 activators represent robust and specific genome-editing tools to activate endogenous gene expression in clinically-relevant human stem cells.

Introduction

Human pluripotent stem cells (hPSC) including both human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) have the unique ability to self-renew indefinitely and to differentiate, potentially, into all cell types of the human body [1, 2]. They provide an unprecedented system to interrogate early human development and to provide a potential clinically-relevant cell source for regenerative medicine [3, 4]. Moreover, hiPSCs offer a unique platform for the *in vitro* generation of patient-specific differentiated cells for personalized therapies, disease modelling and drug screening [3, 4]. Similarly, mesenchymal stem cells (MSCs) are self-renewing multipotent cells present in a wide range of tissues that are capable of differentiating into various tissues of mesodermal origin and display unique immunosuppressive properties [5]. MSCs represent one of the most promising adult stem cells being used worldwide in a wide array of clinical applications involving autoimmunity, hemato-oncology, traumatology and cardiology [6-8].

The success of human stem cell-based applications often rely on methods to precisely edit the donor/patient-specific genome and/or to regulate gene expression. Manipulation of gene expression has generally involved the delivery of exogenous cDNA using expression or viral vectors. However in recent years, we have witnessed the development of strategies to activate the expression of endogenous gene expression using an adapted version of the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 system. This system was originally developed as a powerful and versatile tool for genome editing relying on the endonuclease (Cas9) being directed to a specific genomic site by a short guide RNA (sgRNA), resulting in a precise break in the target DNA [9, 10]. Mutations in residues involved in DNA catalysis have generated Cas9 proteins that lack nuclease activity while preserving DNA binding [11, 12]. When fused to effector/activation domains such as VP64, VPR or p65 and directed to regulatory region of a

genomic locus using sgRNAs, these nuclease-null, catalytically-dead Cas9 (dCas9) variants have been shown to modulate endogenous gene expression [11, 13-15]. Recently, Chavez et al. compared the first-generation dCas9-VP64 activator with various second-generation dCas9 activators including dCas9-VPR, dCas9-SAM, and dCas9-SunTag, in several human, mouse and fly cell lines and demonstrated the robustness and versatility of each system [16]. Here, we set out to compare single and multiplexed gene activation of several endogenous genes in clinically-relevant hPSCs and hMSCs using first-generation dCas9-VP64 activator and the second-generation activators dCas9-SAM and dCas9-SunTag. Our data demonstrate that all dCas9 systems can induce specific and potent gene expression but the second generation systems result in the highest and most consistent level of gene expression in these cell types. We provide guidance for laboratories wishing to adopt dCas9 activator technology to modulate gene expression in human stem cells.

Results

dCas9-VP64 is a fusion between the dCas9 protein and a VP64 transactivating domain [17]. dCas9-SAM represents a modified dCas9-VP64 system using a sgRNA that incorporates two protein-binding aptamers (MS2) capable of recruiting the transcriptional activators p65 and HSF1 (MCP-p65-HSF1) to the targeted promoter [18]. The dCas9-SunTag system is a fusion between the dCas9 protein to a tail of GCN4 peptides that can recruit up to ten copies of scFV-VP64, that amplifies the activation signal [19] (Figure 1A). We set out to compare these three dCas9 activators in clinically-relevant hPSCs and hMSCs. Two different hPSC lines were used: the hESC line H9 and a hiPSC line previously generated and characterized in our laboratory [20]. Two distinct hMSC lines were used, one derived from bone marrow (BM) and another from adipose tissue (Ad). Human PSCs and MSCs were lentivirally infected with each dCas9 system at identical multiplicity of infection (MOI) of 6 to normalize for integration events, and transduced cells were subsequently selected using antibiotic resistance or flow cytometry (Figure 1B). Stable expression of either dCas9 activator system did not alter the homeostasis and potency of either hPSCs or hMSCs (Figure 1 and 2). Antibiotic/FACS-selected modified hPSC and hMSCs were maintained for >20 and >10 passages, respectively, and retained normal morphology (Figure 1C and 2A). Each transgenic stem cell line expressed comparable levels of Cas9, and also showed appropriate expression of the corresponding activator components (VP64, MCP and scFV) (Figure 1D and 2B). All transgenic hPSCs retained the expression of pluripotency-associated transcription factors (Figure 1E), the surface marker, SSEA4 (Figure 1F), and the formation of teratomas in NSG mice which comprised tissues representing all three germ layers (Figure 1G) Similarly, transgenic hMSCs retained typical MSC immunophenotype (CD45-CD73+CD90+CD105+, Figure 2C) and differentiated equally well towards adipogenic and osteogenic lineages (Figure 2D).

The performance of dCas-VP64, dCas-SAM and dCas9-SunTag was compared across several endogenous genes representative of ectoderm (*NEUROD1*), endoderm (*FOXA2*) and mesoderm (*CXCR4*) germ layers. Three different sgRNAs were designed in the proximal promoter (up to 250 base pairs (bp) upstream of the transcription start site) for each target gene, cloned in a lentiviral vector containing a Puromycin-resistance cassette and transduced into the transgenic hPSC/hMSC lines. Gene expression was then analysed in puromycin-selected cells (Figure 3A). All three dCas9 activator systems demonstrated the ability to induce robust gene expression regardless of the locus targeted, with the second-generation systems, dCas9-SAM and/or dCas9-SunTag, resulting in higher levels of gene expression more consistently in both hPSCs (Figure 3B) and hMSCs (Figure 3C). Overall, the dCas9-SAM and dCas9-SunTag systems activated gene expression to comparable levels in hPSCs but the dCas9-SunTag tended to activate gene expression to a slightly higher level than dCas9-SAM in hMSCs. These data indicate that intrinsic differences in the cellular and (epi)genetic nature of hPSCs and hMSCs may impact on the performance of dCas9 activator systems. There was a slight ($p>0.05$) variation in gene activation between the different sgRNAs that were used to target the same locus but no correlation was observed between the promoter distal-proximal region targeted by each sgRNA and the levels of gene expression (Figure 3B and C). Consistent with previous reports, we noticed a negative correlation between the basal gene expression state and the magnitude of activation of a given gene [16, 18, 21, 22]. To analyse the effect of recruiting multiple dCas9 activators complexes to the same locus, we transduced the cells with a mixture of the three different sgRNAs directed against distinct promoter regions. Simultaneous targeting of the same gene with multiple sgRNAs did not lead to additive levels of gene expression in either hPSCs or hMSCs, suggesting that the most efficient individual sgRNA marks a gene activation plateau for each dCas9 activator (Figure 3B and C).

Human stem cell-based applications rely on the ability to simultaneously activate several endogenous genes with the potential to modify cell fate. Dead Cas9-based transcriptional activators are especially suitable to target multiple loci, requiring only the provision of one sgRNA for each gene to be targeted. We next analysed the feasibility to multiplex gene activation in both hPSCs and hMSCs. The three dCas9 activator systems performed well upon multiplexing gene activation, although the second-generation dCas9-SAM and dCas9-SunTag systems appeared superior to the dCa9-VP64 system at least in two out of the three genes targeted (Figure 4A and B). We next analyzed the top *in silico*-predicted off-targets of *FOXA2* sgRNA (*MEXA3, FUT11* and *BTBD17*), *NEUROD1* sgRNA (*CCDC88C, CLSTN1* and *DUSP27*) and *CXCR4* sgRNA (*TUSC5, CHRFAM7A* and *ADRA2B*) and found them all consistently unaltered, demonstrating the high specificity of all dCas9 activators in both hPSCs (Figure 4C) and hMSCs (Figure 4D). Taken together, our study demonstrate the robustness and specificity of dCas9 activators as a mean to simultaneously activate multiple transcription factors in human clinically-relevant stem cells.

Discussion

Human PSCs and MSCs are relevant cell sources which hold great promise in both basic and clinical research [4]. These applications largely rely on robust methods to precisely control gene expression and to simultaneously activate multiple endogenous genes with the potential to modify cell fate and/or cell function. Unfortunately, long-term *in vitro* maintenance of stemness and precise genetic manipulation using exogenous overexpression systems have long remained daunting tasks. Recently, the CRISPR/Cas9 system has arisen as a unique, powerful and versatile tool for genome editing in a wide range of cell types including stem cells. More recently, dCas9 variants lacking nuclease activity while preserving DNA binding have been reported [11, 12]. When fused to activation domains such as VP64, VPR, or p65 they can precisely modulate endogenous gene expression in any given locus within the genome when directed to their regulatory regions by a specific sgRNA, thus representing a versatile tool to regulate locus-specific gene expression [11, 13-15].

Here, we have compared the first-generation dCas9-VP64 activator with the second-generation systems dCas9-SAM and dCas9-SunTag for the first time in clinically relevant human stem cells. Importantly, we report for the first time that the stable expression of each of the three dCas9 activators is compatible with stem cell homeostasis and potency, which is a critical requisite for any downstream stem cell application. The three activator systems show the ability to induce potent gene expression in both single and multiplexed gene activation settings. Multiplexed gene activation in stem cells is highly desired because a simultaneously activation of several master transcription factors is key to modulate complex transcriptional networks dictating stem cell fate. Despite initial work suggesting a general decrease in gene activation upon multiplexing [18, 23], our data confirms the robustness of these systems as tools to actuate complex transcriptional multiplexed regulation [16]. In line with previous studies, the second generation systems dCas9-

SAM and dCas9-SunTag delivered higher and more consistent levels of gene expression than dCas9-VP64. Interestingly, the dCas9-SAM and dCas9-SunTag systems performed at a comparable level in hPSCs whereas the dCas9-SunTag was slightly superior in hMSCs. This indicates that intrinsic cellular and (epi)genetic differences between hPSCs and hMSCs may impact the performance of dCas9 activator systems. In fact, differences in chromatin accessibility between hESCs and hESC-derived MSCs have been reported [24]. We have also demonstrated that simultaneous targeting of the same gene with multiple sgRNAs did not lead to additive levels of gene expression in neither human PSCs nor MSCs suggesting that the most efficient individual sgRNA marks a gene activation plateau for each dCas9 activator. This is in contrast to that described by Chavez et al [16] for other cell lines that did not include stem cells. This could either reflect the different nature of the cells targeted or differences in the DNA delivery methods or both. It might also be the fact that each system depends upon the recruitment of different downstream transcriptional effectors, so the availability of these within the cells may favour certain systems. In summary, our work reveals the robustness, specificity and versatility of all dCas9 activators in single and multiplexed gene activation systems in clinically-relevant human stem cells and demonstrated the superior levels of gene activation by the second-generation systems.

Experimental Procedures

Contact for reagent and resource sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Pablo Menendez (pmenendez@carrerasresearch.org).

hPSC and hMSC culture

Two hPSC lines were used: the hESC line H9 obtained from Wicell (Madison, WI), and a hiPSC line previously generated in our laboratory from B-cell progenitors [20]. Both hPSCs were maintained on Matrigel (BD)-coated plates in hESC medium as extensively reported by our group [25-27]. hESC media was changed daily and hPSC cultures were split weekly. Bone marrow (BM)- and adipose tissue (Ad)-derived human MSC cultures were maintained in Advanced DMEM (Gibco) supplemented with 10% fetal bovine serum (Sigma), L-glutamine, and penicillin-streptomycin-amphotericin B (Gibco), as previously reported by our group [28]. hMSC cultures were assessed daily for changes in growth rates and morphology and split every 8-10 days. Approval for hESC/hiPSC work was obtained from the Spanish National Embryo Ethical Committee (26/2013).

dCAS9 and sgRNAs lentivectors

All the vectors used in this study were obtained from Addgene: dCAS9-VP64 (#61425), MCP-p65-HSF1 (#61426), dCAS9-10xGCN4 (#60903) and scFv-VP64 (#60904). The final constructs used to generate hPSC/hMSCs stably expressing the different transcriptional activators are: VP64 (dCAS9-VP64), SunTag (dCAS9-10xGCN4 + scFv-VP64) and SAM (dCAS9-VP64 + MCP-p65-HSF1) (Figure 1A). For sgRNA delivery, both the Lentiguide-Puro (#52963) or sgRNA(MS2)-puro (#73795) backbone was Golden-Gate cloned with all the guide variants according to the established protocol [18]. NEUROD1 and CXCR4 sgRNAs sequences were taken from Chavez et al (2016). Best scored FOXA2 sgRNAs sequences were defined with the CRISPRa/i sgRNA

designer tool from the BROAD institute. The individual sgRNAs targeting *FOXA2* at bp -42, *NEUROD1* at bp -221 and *CXCR4* at bp -162 were used in multiplexing gene activations experiments.

Virus production and transduction of hPSC/hMSC

A second generation lentiviral production system was used to produce viral particles in HEK293T cells. The psPAX2 packaging plasmid, pMD2.G envelope and the lentiviral transfer vector were co-transfected using PEI (Polysciences) as previously detailed [29]. Virus-containing supernatants were harvested 48-72h post-transfection, concentrated by ultracentrifugation and titered in 293T cells. For transduction, hPSC/hMSC were split 48h before exposure to viral supernatants, MOI of 6. Infected cells were expanded in the presence of blasticidine (3 μg/ml for VP64) and blasticidine plus hygromycin (3 μg/ml and 60 μg/ml for SAM). SunTag-infected cells were FACS-sorted using GFP and BFP reporters, thus generating hPSC/hMSCs stably expressing the different transcriptional activators (Figure 3B). To select for integration of gRNAs construct, puromycin (0.3 μg/ml) was added to the cultures

Quantitative RT-PCR

Total RNA was extracted with Maxwell® RSC simplyRNA cells kit (Promega) and subsequently incubated with TurboDNAse (Ambion) to remove potential genomic contamination. Reverse transcription was performed with ~500ng RNA using Superscript III and random hexamers primers (Invitrogen). cDNA was diluted 1:4 and 1 ul was used for each 10ul reaction. Real-time PCR was performed with Power up SYBR green master mix (Applied biosystems) in triplicate on a Biorad CFX384 real-time system. All primer pairs were designed with Primer-Blast software and validated by gel electrophoresis to amplify specific single products. A standard curve with serial dilution of cDNA was always performed to guarantee correct amplification of primer pairs. GAPDH was used

as a housekeeping gene. Table S1 shows the sequences of all primers and gRNAs used in this study. For the off-targets analysis, the top three in silico-predicted (gRNA design checker, IDT) off-targets for FOXA2 -42 (MEXA3, FUT11 and BTBD17), NEUROD1 -221 (CCDC88C, CLSTN1 and DUSP27) and CXCR4 -162 (TUSC5, CHRFAM7A and ADRA2B) have been checked by Real-time PCR in all the systems multiplex experiments for both hMSCs and hPSCs.

Flow cytometry analysis

The following antibodies (Becton Dickinson, San Jose, CA) were used in FACS experiments: SSEA-4-V450, CD73-BV510, CD105-FITC, CD90-APC, and CD45 APC-Cy7. For staining, 200000 cells were resuspended in 200 ul PBS+2% FBS with 1:100 antibody dilution, for 20min at 4ºC. Cells were then washed twice with PBS and acquired on a FACS Canto-II flow cytometer equipped with FACS Diva analysis software (Becton Dickinson)

Adipogenic and osteogenic in vitro differentiation of hMSC cultures

Human MSC differentiation was assessed by growing hMSC in specific differentiation media for 2-3 weeks according to manufacturer's instructions (Lonza, Switzerland). The detailed differentiation procedure is described elsewhere [28]. Briefly, for adipogenic differentiation, cells were cultured in Adipogenic MSC Differentiation BulletKit (Lonza) and differentiated cells were stained with Oil Red-O (Sigma). For osteogenic differentiation, cells were cultured in Osteogenic MSC Differentiation BulletKit (Lonza) and differentiated cells were stained with Alizarin Red-S (Sigma) [28].

Teratoma formation assay

Undifferentiated hESC/hiPSC cultures at 80-90% confluence were collected through enzymatic dissociation using collagenase IV, and 2 million cells were re-suspended and injected with 250 µl DMEM and 50µl Matrigel subcutaneously in the back of the NSG mice [30]. Tumors generally

developed within 6-10 weeks. When tumours reached 1cm diameter, mice were sacrificed and the teratomas removed and fixed overnight in paraformaldehyde-containing solution. Teratomas were then embedded in paraffin, sectioned and stained for hematoxylin & eosin to assess the presence of cells representing the three germ layers [30]. Animal experimentation protocols was approved by the Animal Care Committee of the PRBB.

Author contribution

P.P: conceptualization, methodology, investigation, data analysis, and writing. R.T-R, A.F: conceptualization, methodology, investigation, and data analysis. H.R-H, F.G-A, J.C, S.R-P, R.DdlG, B.L-M, C.B: methodology and investigation. A.B and L.M.F: conceptualization. P.M: conceptualization, writing and financial support.

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Declaration of interest: authors have nothing to disclose.

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Figure Legends

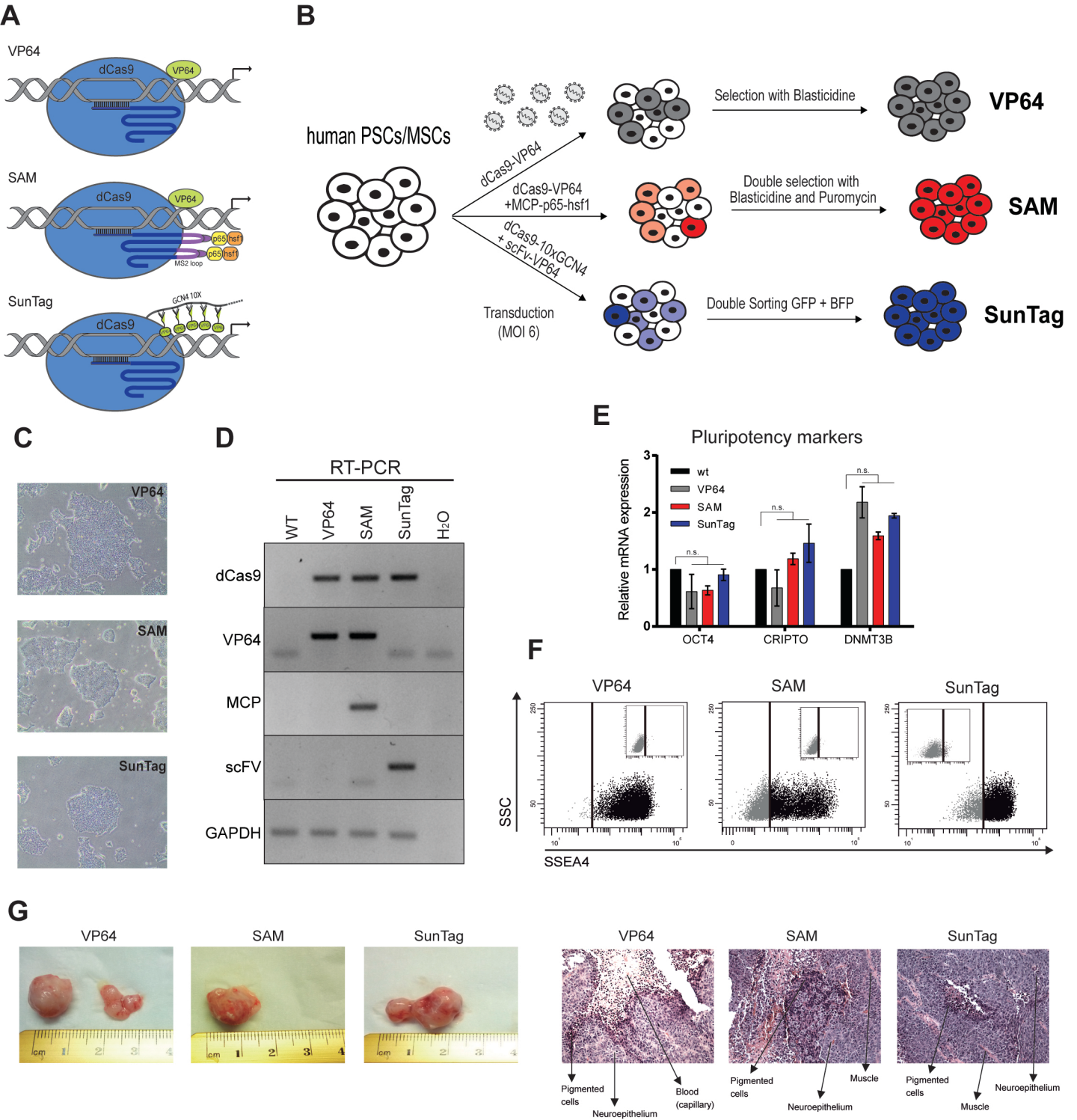
Figure 1. Transgenic hPSCs expressing dCas9 transcriptional activators remain pluripotent. (A) Schematic of the different dCas9 transcriptional activators used in this study, VP64, SAM and SunTag. (B) Schematic of the generation of stable transgenic hPSCs-VP64, hPSC-SAM and hPSC-SunTag lines. (C) hESC-like morphology of representative colonies from hPSCs-VP64, hPSC-SAM and hPSC-SunTag lines. (D) RT-PCR confirming expression of the different dCas9 system components in the different transgenic hPSC lines. (E) qRT-PCR expression of the indicated pluripotency genes in hPSCs-VP64, hPSC-SAM and hPSC-SunTag lines (n=2). (F) Representative FACS data confirming expression of the pluripotency surface maker SSEA-4 in hPSCs-VP64, hPSC-SAM and hPSC-SunTag lines. Insets represent unstained cells. (G) hPSCs-VP64, hPSC-SAM and hPSC-SunTag cells similarly formed teratomas in NSG mice. Left panels show macroscopic teratomas. Right panels show cellular structures representing the three germ layers.

Figure 2. Transgenic hMSC expressing dCas9 transcriptional activators remain multipotent. (A) Phase-contrast morphology of hMSC-VP64, hMSC-SAM and hMSC-SunTag cultures revealing normal fibroblastoid-like morphology. (B) RT-PCR confirming expression of the different dCas9 system components in transgenic hMSC cultures. (C) FACS analysis revealing *bona fide* MSC immunophenotype (CD45-CD73+CD90+CD105+) for hMSC-VP64, hMSC-SAM and hMSC-SunTag cultures. (D) hMSC-VP64, hMSC-SAM and hMSC-SunTag retained osteogenic (bottom panels show alizarin red staining) and adipogenic (upper panels show oil red staining) differentiation potential.

Figure 3. Direct comparison of the three dCas9 transcriptional activators in both hPSCs and hMSCs. (A) Schematic workflow for the dCas9-mediated transcriptional activation. (B, C) Gene

expression analysis by qRT-PCR for FOXA2 (endoderm), NEUROD1 (ectoderm) and CXCR4 (mesoderm) in hPSCs (n=4 independent experiments using 2 hESCs and 2 iPSC lines) (B) and hMSCs (n=4 independent experiments with 2 BM-MSC = and 2 Ad-MSC) (C). Three sgRNAs were tested for each gene. The genomic localization of each gRNA relative to the TSS is shown. sgRNAs were used individually or pooled. Gene expression is represented as fold change relative to non-transduced (NT) cells. Statistical significance was determined by Student's *t* test (two-tail). *p<0.05, **p<0.01.

Figure 4. Multiplexed activation of endogenous genes and off-target analyses in both hPSCs and hMSCs. Both hPSCs (A) and hMSCs (B) were simultaneously transduced with three sgRNAs, one for each gene. Gene expression is shown as fold change relative to NT cells. (C, D) Analysis of the top *in silico*-predicted off-targets of *FOXA2* sgRNA (*MEXA3, FUT11* and *BTBD17*), *NEUROD1* sgRNA (*CCDC88C, CLSTN1* and *DUSP27*) and *CXCR4* sgRNA (*TUSC5, CHRFAM7A* and *ADRA2B*) in hPSCs (B) and hMSCs (C). Statistical significance was determined by Student's *t* test (two-tail). *p<0.05, **p<0.01. n=4 independent experiments.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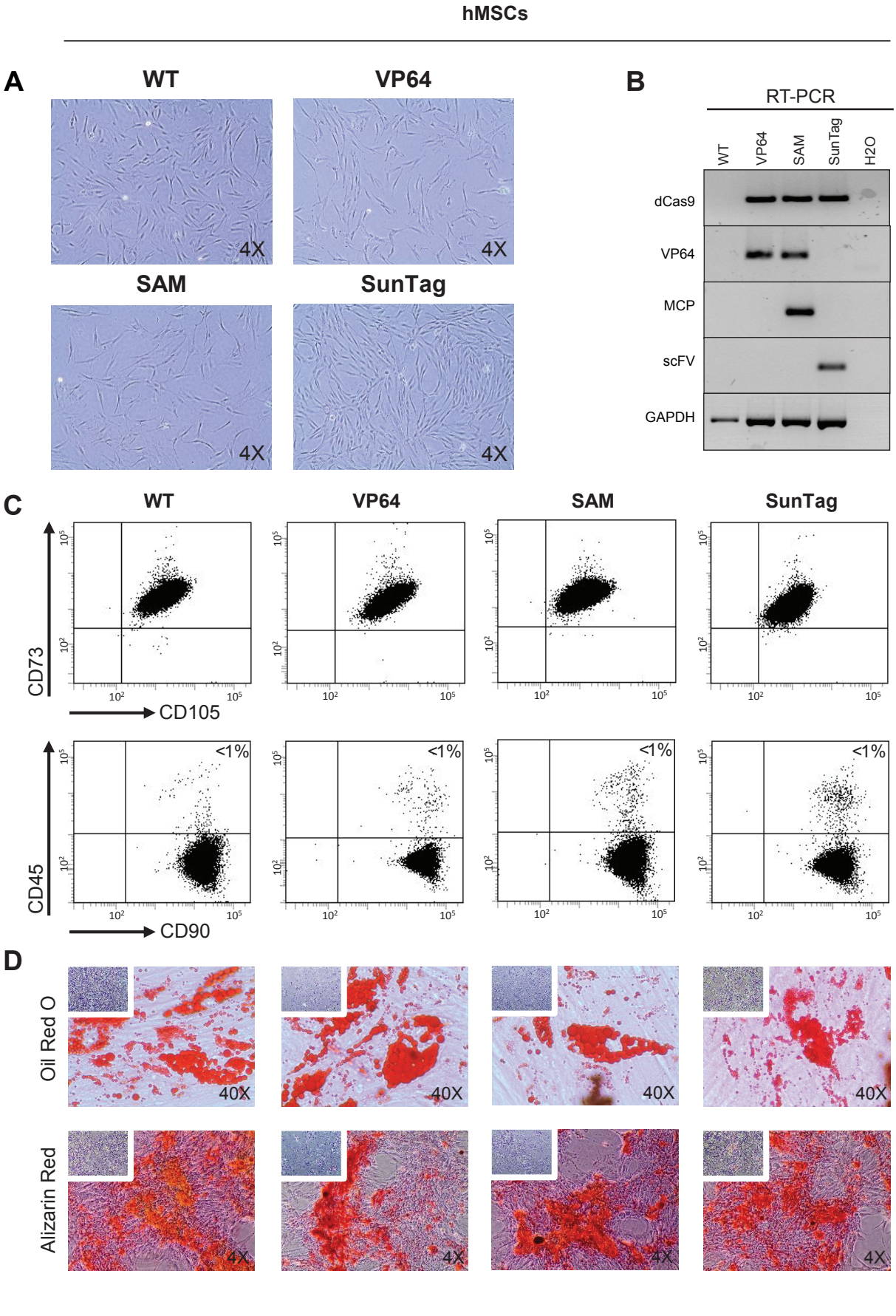
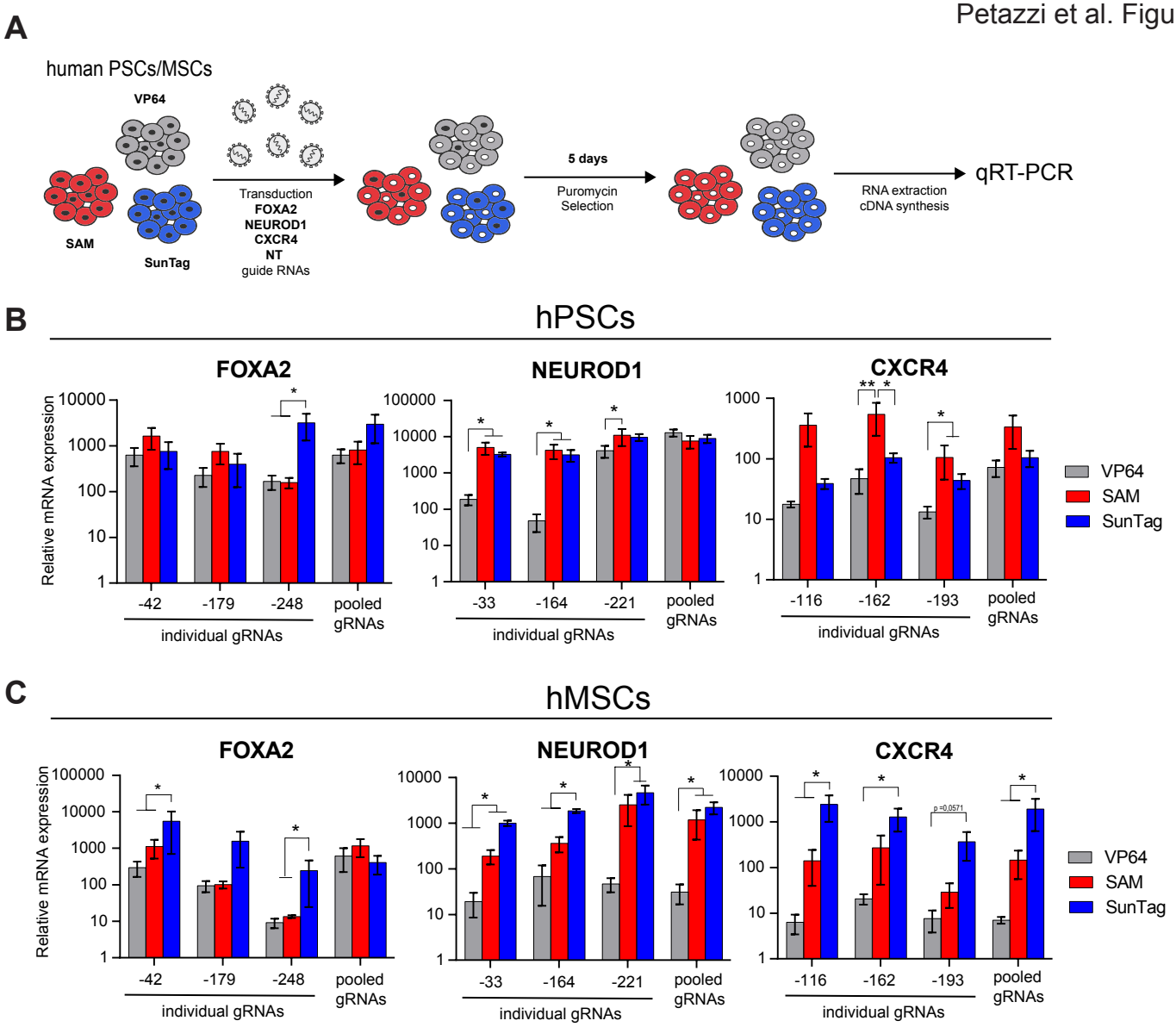
SAM
Double Sorting GFP + BFP
SunTag
C
VP64
SAM
SunTag
D
RT-PCR
WT
VP64
SAM
SunTag
H2O
dCas9
VP64
MCP
scFV
GAPDH
E
Pluripotency markers
Relative mRNA expression
wt
VP64
SAM
SunTag
n.s.
n.s.
n.s.
OCT4
CRIPTO
DNMT3B
F
VP64
SAM
SunTag
SSC
SSEA4
G
VP64
SAM
SunTag
VP64
SAM
SunTag
Pigmented cells
Neuroepithelium
Blood (capillary)
Pigmented cells
Muscle
Neuroepithelium
Pigmented cells
Neuroepithelium
Muscle


hMSCs
A
WT
VP64
SAM
SunTag
4X
B
RT-PCR
WT
VP64
SAM
SunTag
H2O
dCas9
VP64
MCP
scFV
GAPDH
C
WT
VP64
SAM
SunTag
CD73
CD105
CD45
CD90
<1%
D
Oil Red O
Alizarin Red
40X
4X


A
human PSCs/MSCs
VP64
SAM
SunTag
Transduction
FOXA2
NEUROD1
CXCR4
NT
guide RNAs
5 days
Puromycin
Selection
RNA extraction
cDNA synthesis
qRT-PCR
B
hPSCs
FOXA2
NEUROD1
CXCR4
Relative mRNA expression
-42
-179
-248
pooled gRNAs
individual gRNAs
-33
-164
-221
-116
-162
-193
VP64
SAM
SunTag
C
hMSCs
p =0.0571

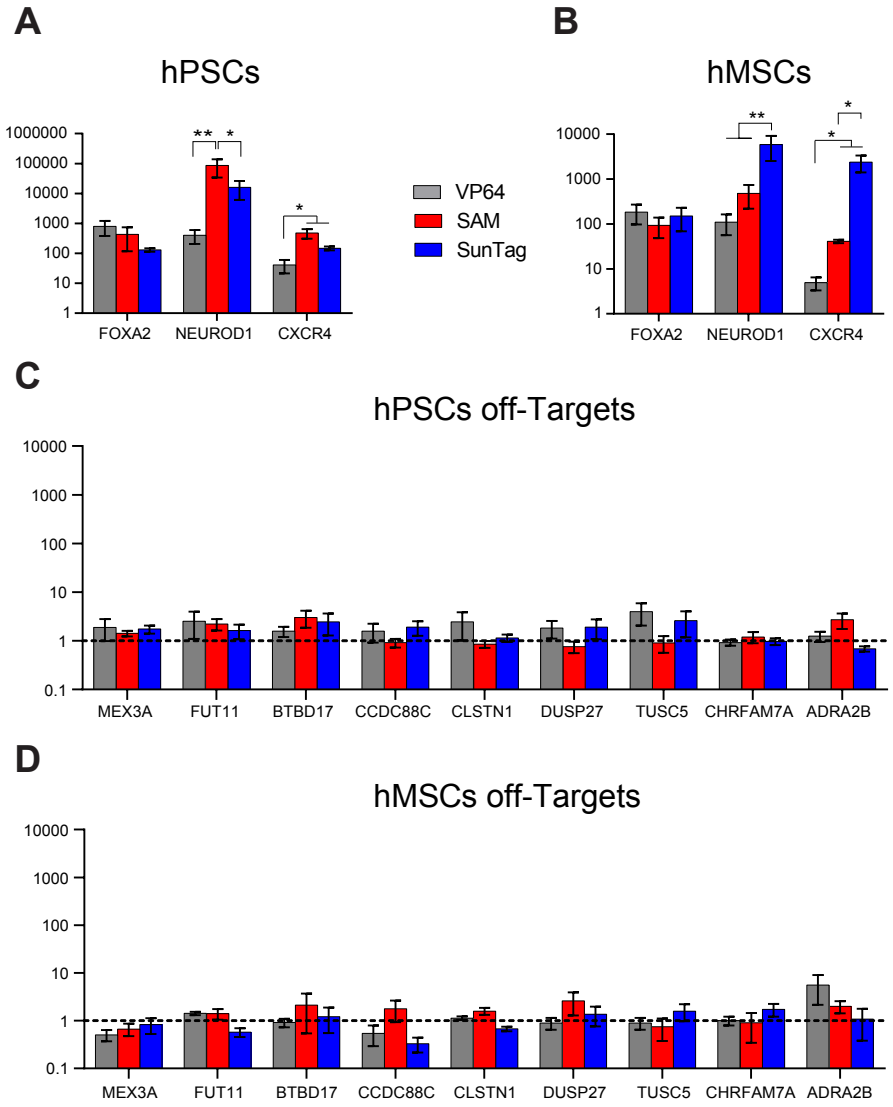

A
hPSCs
1000000
100000
10000
1000
100
10
1
**
*
*
FOXA2
NEUROD1
CXCR4
VP64
SAM
SunTag
B
hMSCs
10000
1000
100
10
1
**
*
*
FOXA2
NEUROD1
CXCR4
C
hPSCs off-Targets
10000
1000
100
10
1
0.1
MEX3A
FUT11
BTBD17
CCDC88C
CLSTN1
DUSP27
TUSC5
CHRFAM7A
ADRA2B
D
hMSCs off-Targets
10000
1000
100
10
1
0.1
MEX3A
FUT11
BTBD17
CCDC88C
CLSTN1
DUSP27
TUSC5
CHRFAM7A
ADRA2B

Table S1. List of gRNAs and primers used in this study

gRNAs	Sequence
FOXA2 -42	ggcgggtgctccctaccgcg
FOXA2 -179	ccacttccaactaccgcctc
FOXA2 -248	tgattgctggtcgtttgttg
NEUROD1 -33	aggtccgcggagtctctaac
NEUROD1 -164	acctgcccatttgtatgccg
NEUROD1 -221	aggggagcggttgtcggagg
CXCR4 -116	gcagacgcgaggaaggagggcgc
CXCR4 -162	ccgaccacccgcaaacagca
CXCR4 -193	gcctctgggaggtcctgtccggctc
NT	cggaggctaagcgtcgcaac
Primers	Sequence
Cas9_F	agcacgtggcacagatcctgg
Cas9_R	ggaaatccttccggaaatcgg
VP64_F	aaaagaggaaggtggcggcc
VP64_R	cgtcactgccgagcatgtcg
MS2_F	aaggtgacatgcagcgtcagg
MS2_R	ccatgttcaggtaggacctcc
scFV_F	tgatcggcgacaaggccacc
scFV_R	gcgcttcagctccaccttgg
GAPDH_F	gcaccgtcaaggctgagaac
GAPDH_R	agggatctcgctcctggaa
OCT4_F	gggtttttgggattaagttcttca
OCT4_R	gcccccacccttttgtgtt
CRIPTO_F	cggaactgtgagcacgatgt
CRIPTO_R	gggcagccaggtgtcatg
DNMT3B_F	gctcacagggcccgatactt
DNMT3B_R	gcagtcctgcagctcgagttta
FOXA2_F	cacgagccgtccgactggag
FOXA2_R	atggcggccgccgacatgc
NEUROD1_F	ggatgacgatcaaaagcccaa
NEUROD1_R	gcgtcttagaatagcaaggca
CXCR4_F	actacaccgaggaaatgggct
CXCR4_R	cccacaatgccagttaagaaga