

# Robustness of Catalytically Dead Cas9 Activators in Human Pluripotent and Mesenchymal Stem Cells

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**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). In this study, 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 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.**

## INTRODUCTION

Human pluripotent stem cells (hPSCs), including both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), have the unique ability to self-renew indefinitely and to differentiate, potentially, into all cell types of the human body.<sup>1,2</sup> They provide an unprecedented system to interrogate early human development and to provide a potential clinically relevant cell source for regenerative medicine.<sup>3,4</sup> Moreover, hiPSCs offer a unique platform for the *in vitro* generation of patient-specific differentiated cells for personalized therapies, disease modeling, and drug screening.<sup>3,4</sup>

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.<sup>5</sup> MSCs represent one of the most promising adult stem cells being used worldwide in a wide array of clinical applications involving autoimmunity, hematology-oncology, traumatology, and cardiology.<sup>6–8</sup>

The success of human stem cell-based applications often relies 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 single-guide RNA (sgRNA), resulting in a precise break in the target DNA.<sup>9,10</sup> Mutations in residues involved in DNA catalysis have generated Cas9 proteins that lack nuclease activity while preserving DNA binding.<sup>11,12</sup> When fused to effector/activation domains such as VP64, VPR, or p65 and directed to regulatory regions of a genomic locus using sgRNAs, these nuclease

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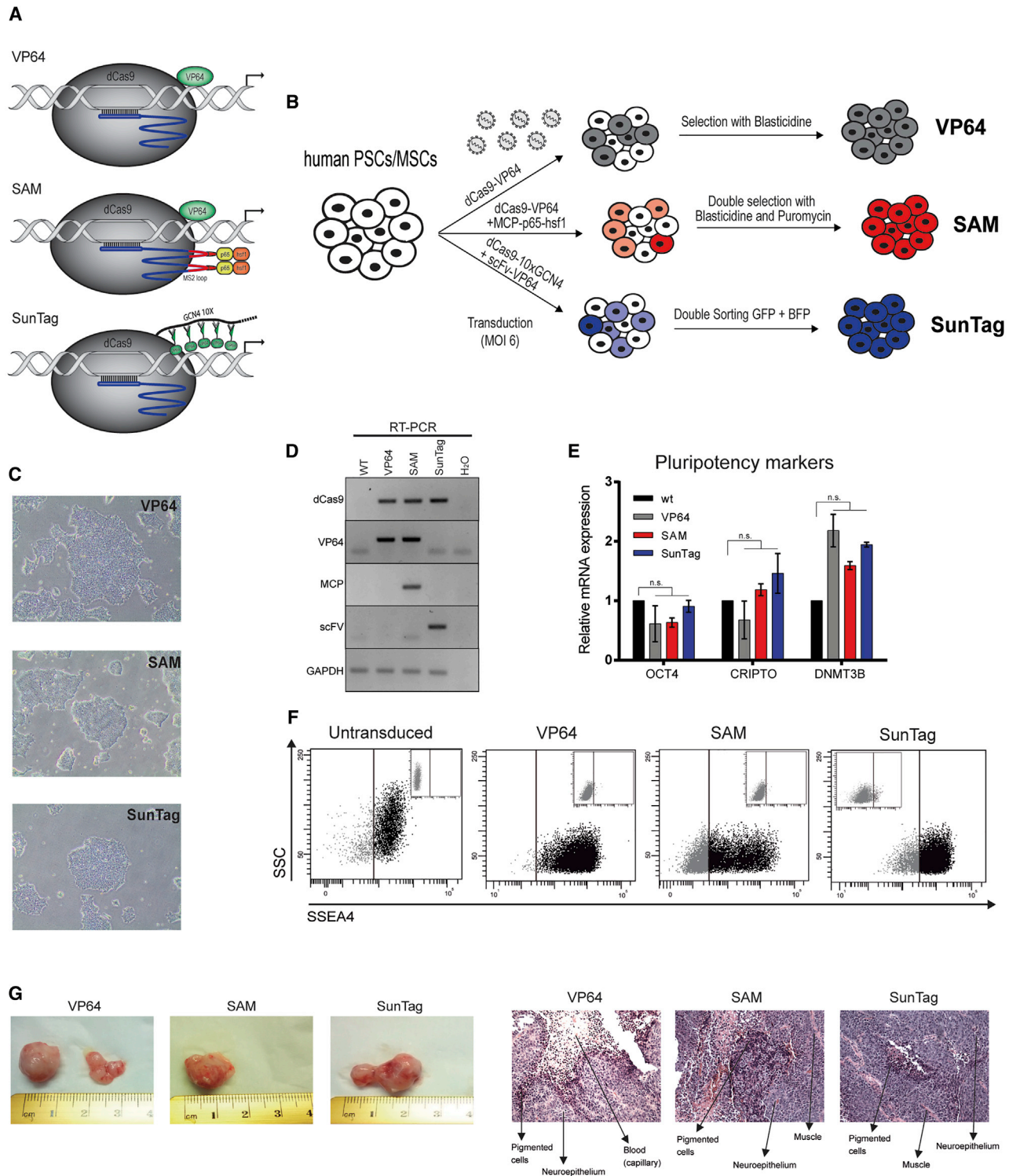
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**Figure 1. Transgenic hPSCs Expressing dCas9 Transcriptional Activators Remain Pluripotent**

(A) Schematic of the different dCas9 transcriptional activators used in this study, VP64, SAM, and SunTag. (B) Schematic of the generation of stable transgenic hPSC-VP64, hPSC-SAM, and hPSC-SunTag lines. (C) hESC-like morphology of representative colonies from hPSC-VP64, hPSC-SAM, and hPSC-SunTag lines. (D) RT-PCR confirming

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null, catalytically dead Cas9 (dCas9) variants have been shown to modulate endogenous gene expression.<sup>11,13–15</sup> Recently, Chavez et al.<sup>16</sup> 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. In this study, we set out to compare single and multiplexed gene activation of several endogenous genes in clinically relevant hPSCs and human MSCs (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 wanting to adopt dCas9 activator technology to modulate gene expression in clinically relevant human stem cells.

## RESULTS

dCas9-VP64 is a fusion between the dCas9 protein and a VP64 transactivating domain.<sup>17</sup> 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.<sup>18</sup> The dCas9-SunTag system is a fusion between the dCas9 protein to a tail of GCN4 peptides that can recruit up to 10 copies of scFV-VP64, which amplifies the activation signal<sup>19</sup> (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.<sup>20</sup> 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 (dCas9-VP64 and dCas9-SAM systems) or flow cytometry (dCas9-SunTag system) (Figure 1B). Stable expression of either dCas9 activator system did not alter the homeostasis and potency of either hPSCs or hMSCs (Figures 1 and 2). Antibiotic/fluorescence-activated cell sorting (FACS)-selected modified hPSCs and hMSCs were maintained for >20 and >10 passages, respectively, and retained normal morphology (Figures 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) (Figures 1D and 2B). All transgenic hPSCs retained the expression of pluripotency-associated transcription factors (Figure 1E) and the surface marker SSEA4 (Figure 1F), and they formed teratomas in NSG mice comprised of tissues representing all three germ layers (Figure 1G). Similarly, transgenic hMSCs retained

the typical MSC immunophenotype (CD45<sup>-</sup>CD73<sup>+</sup>CD90<sup>+</sup>CD105<sup>+</sup>, Figure 2C) and differentiated equally well toward adipogenic and osteogenic lineages (Figure 2D).

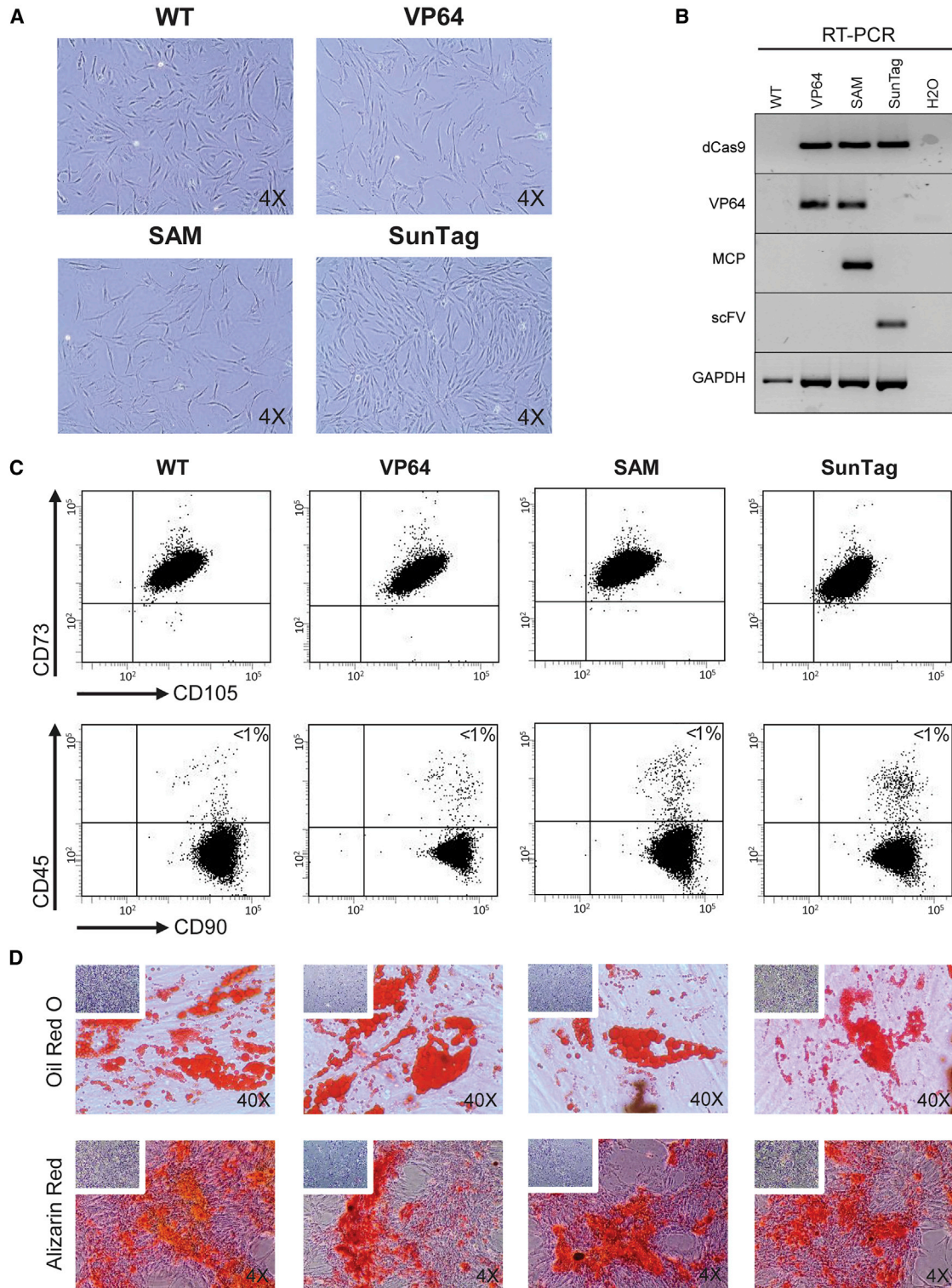
The performance of dCas9-VP64, dCas9-SAM, and dCas9-SunTag was compared across several endogenous genes representative of ectoderm (*NEUROD1*), endoderm (*FOXA2*), and mesoderm (*CXCR4*) germ layers. Three different sgRNAs were designed in the proximal promoter (up to 250 bp upstream of the transcription 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 analyzed 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 dCas9-SunTag, resulting in consistently higher levels of gene expression 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 higher level than did dCas9-SAM in hMSCs. These data indicate that intrinsic differences in the cellular and (epi)genetic nature of hPSCs and hMSCs may impact 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 (Figures 3B and 3C). Consistent with previous reports, our data show a negative correlation between the basal gene expression state and the magnitude of activation of a given gene in both hPSCs and hMSCs (Figure 3D).<sup>16,18,21,22</sup> To analyze the effect of recruiting multiple dCas9 activator complexes to the same locus, we co-transduced the cells with 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 (Figures 3B and 3C). Of note, all three systems displayed comparable and relatively few integration events in both hPSCs (between 5 and 10) and hMSCs (between 4 and 11) (Figure 3E).

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 analyzed 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

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expression of the different dCas9 system components in the different transgenic hPSC lines. (E) qRT-PCR expression of the indicated pluripotency genes in hPSC-VP64, hPSC-SAM, and hPSC-SunTag lines ( $n = 2$ ). Error bars indicate SEM values. (F) Representative FACS data confirming expression of the pluripotency surface marker SSEA-4 in untransduced hPSCs, hPSC-VP64, hPSC-SAM, and hPSC-SunTag lines. Insets represent unstained cells. (G) hPSC-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.

hMSCs



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dCas9-SunTag systems appeared superior to the dCas9-VP64 system at least in two out of the three genes targeted (Figures 4A and 4B). 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, CHRFA7A, 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 demonstrates the robustness and specificity of dCas9 activators as a means to simultaneously activate multiple transcription factors in clinically relevant human stem cells.

## DISCUSSION

Human PSCs and MSCs are relevant cell sources that hold great promise in both basic and clinical research.<sup>4</sup> 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 has 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.<sup>11,12</sup> 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.<sup>11,13–15,23</sup>

In this study, 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. Of note, the stable expression of each of the three dCas9 activators is compatible with stem cell homeostasis and potency, which is a critical prerequisite 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 simultaneous activation of several master transcription factors is key to modulate complex transcriptional networks that dictate stem cell fate. Despite initial work suggesting a general decrease in gene activation upon multiplexing,<sup>18,24</sup> our data confirm the robustness of these systems as tools to activate complex transcriptional multiplexed regulation.<sup>16</sup> In line with previous studies, the second-generation systems dCas9-SAM and dCas9-SunTag delivered higher and more consistent levels of gene expression than did 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.<sup>25</sup> 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 dictates a gene activation plateau for each dCas9 activator. This is in contrast to that described by Chavez et al.<sup>16</sup> for other non-stem cell lines. This could either reflect the different nature of the cells targeted or differences in the DNA delivery methods or a combination both. It is also plausible that each system depends on the recruitment of different downstream transcriptional effectors, so the availability of these within the cells may favor 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 demonstrates the superior levels of gene activation by the second-generation systems.

## MATERIALS AND METHODS

### Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to, and will be fulfilled by, Pablo Menéndez ([pmenendez@carrerasresearch.org](mailto:pmenendez@carrerasresearch.org)).

### hPSC and hMSC Cultures

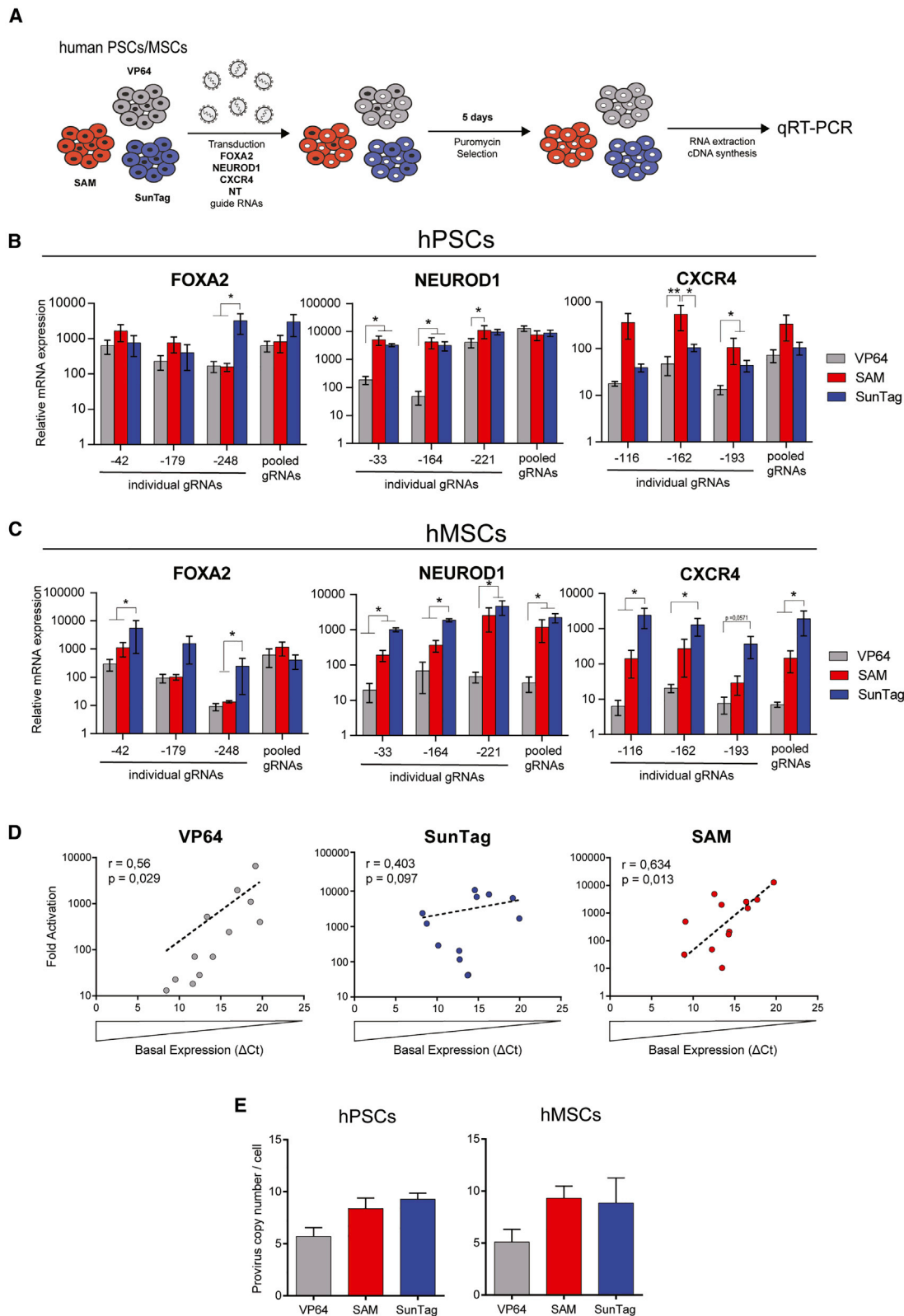
Two hPSC lines were used: the hESC line H9 obtained from WiCell (Madison, WI, USA), and a hiPSC line previously generated in our laboratory from B cell progenitors.<sup>20</sup> Both hPSCs were maintained on Matrigel (BD Biosciences, San Diego, CA, USA)-coated plates in hESC medium as extensively reported by our group.<sup>26–28</sup> hESC media were changed daily, and hPSC cultures were split weekly. BM- and Ad-derived hMSC cultures were maintained in Advanced DMEM (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO, USA), L-Glutamine, and penicillin-streptomycin-amphotericin B (Thermo Fisher Scientific), as previously reported by our group.<sup>29</sup> 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 ISCHII-Comisión Nacional de Garantías (26/2013).

### dCAS9 and sgRNA Lentivectors

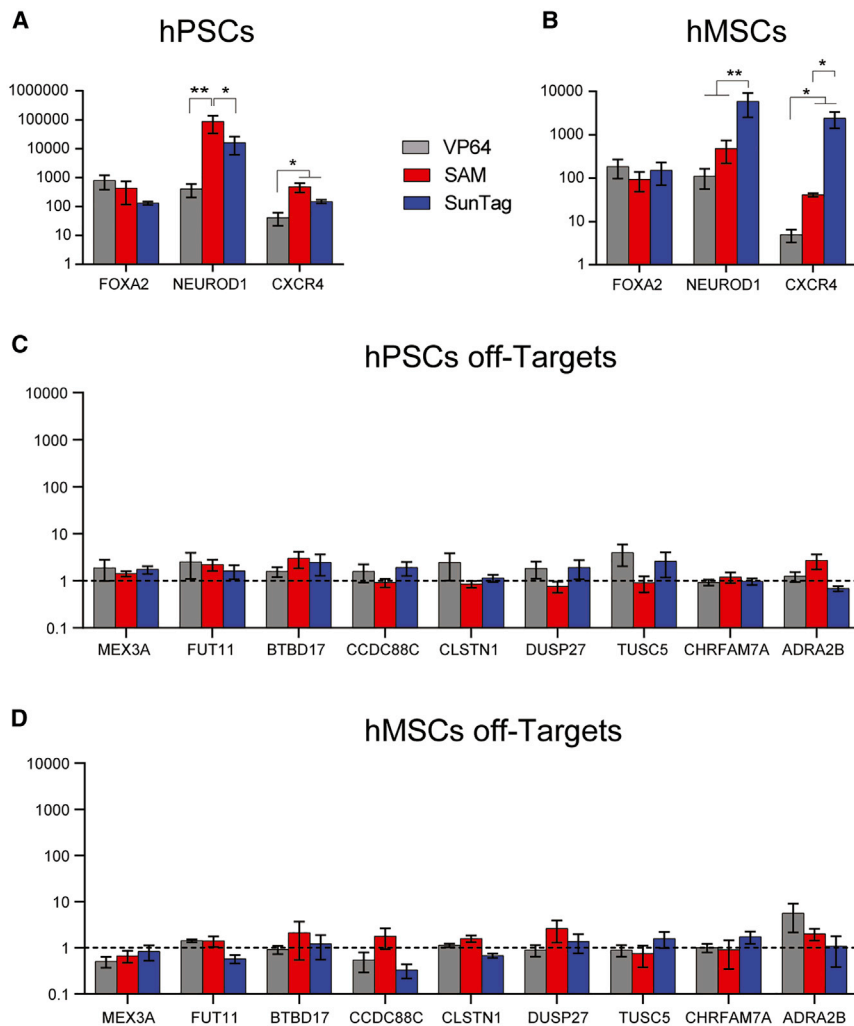
All vectors used in this study were obtained from Addgene (Cambridge, MA, USA), including dCAS9-VP64 (#61425), MCP-p65-HSF1 (#61426), dCAS9-10xG4 (#60903), and scFv-VP64 (#60904). The final constructs used to generate hPSC/hMSCs stably

### Figure 2. Transgenic hMSCs 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<sup>-</sup>CD73<sup>+</sup>CD90<sup>+</sup>CD105<sup>+</sup>) for hMSC-VP64, hMSC-SAM, and hMSC-SunTag cultures. (D) hMSC-VP64, hMSC-SAM, and hMSC-SunTag cultures retained osteogenic (bottom panels, alizarin red staining) and adipogenic (upper panels, oil red O staining) differentiation potential.



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expressing the different transcriptional activators are as follows: 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.<sup>18</sup> *NEUROD1* and *CXCR4* sgRNA sequences were taken from Chavez et al.<sup>16</sup> The best scored *FOXA2* sgRNA 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 activation experiments.

reporters, thus generating hPSCs/hMSCs stably expressing the different transcriptional activators (Figure 1B). Puromycin (0.3  $\mu$ g/mL) was added to Cas9 activator-expressing hPSCs/hMSCs to select for guide RNA (gRNA) integration.

#### Quantitative Real-Time PCR

Total RNA was extracted with a Maxwell RSC simplyRNA cells kit (Promega, Madison, WI, USA) and subsequently incubated with Turbo DNase (Thermo Fisher Scientific) to remove potential genomic contamination. Reverse transcription was performed with ~500 ng of RNA using SuperScript III and random hexamer primers (Thermo

### 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 and C) Gene expression analysis by qRT-PCR for *FOXA2* (endoderm), *NEUROD1* (ectoderm), and *CXCR4* (mesoderm) in hPSCs (n = 4 independent experiments using hESC and iPSC lines) (B) and hMSCs (n = 4 independent experiments with BM-MSC and Ad-MSC) (C). Three sgRNAs were tested for each gene. The genomic localization of each gRNA relative to the transcription start site (TSS) is shown. sgRNAs were used individually or pooled. Gene expression is represented as fold change relative to non-transduced (NT) cells. Error bars indicate SEM values. All statistical significance was determined by a Student's t test (two-tailed). \*p < 0.05, \*\*p < 0.01. (D) Inverse correlation between basal gene expression and output gene activation for the three genes in both hPSCs and hMSCs. A Pearson's test was used to determine the correlation index (r) and p value. (E) Analysis of integration events by genomic qPCR in hPSCs and hMSCs.

### 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 non-transduced (NT) cells. (C and 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 (C) and hMSCs (D). Error bars indicate SEM values. Statistical significance was determined by a Student's t test (two-tailed). \*p < 0.05, \*\*p < 0.01. n = 4 independent experiments.

### Virus Production and Transduction of hPSCs/hMSCs

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 polyethyleneimine (PEI) (Polysciences, Warrington, PA, USA) as previously detailed.<sup>30</sup> Virus-containing supernatants were harvested 48–72 h post-transfection, concentrated by ultracentrifugation and titered in 293T cells. For transduction, hPSCs/hMSCs were split 48 h before exposure to viral supernatants (MOI of 6). Infected cells were expanded in the presence of blasticidin (3  $\mu$ g/mL for VP64) and blasticidin plus hygromycin (3 and 60  $\mu$ g/mL for SAM). SunTag-infected cells (transduction efficiency, 1%–13%) were FACS sorted (>95% purity) using GFP and blue fluorescence protein (BFP) re-

Fisher Scientific). cDNA was diluted 1:4 and 1  $\mu$ L was used for each 10- $\mu$ L reaction. Real-time PCR was performed with PowerUp SYBR Green master mix (Thermo Fisher Scientific) in triplicate on a Bio-Rad (Hercules, CA, USA) 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, Coralville, IA, USA) off-targets for *FOXA2* –42 (*MEXA3*, *FUT11*, and *BTBD17*), *NEUROD1* –221 (*CCDC88C*, *CLSTN1*, and *DUSP27*) and *CXCR4* –162 (*TUSC5*, *CHRFAM7A*, and *ADRA2B*) were analyzed by real-time PCR in multiplexed experiments for both hMSCs and hPSCs.

#### Provirus Integration Analysis

Integrated lentivirus copy number analysis was performed on genomic DNA of transduced cells by using Lenti-X provirus quantitation kit (Takara, Japan) following the manufacturer's guidelines.

#### Flow Cytometry Analysis

The following antibodies (Becton Dickinson, San Jose, CA, USA) were used in FACS experiments: SSEA-4-V450, CD73-BV510, CD105-FITC, CD90-allophycocyanin (APC), and CD45 APC-Cy7. For staining, 200,000 cells were resuspended in 200  $\mu$ L of PBS + 2% FBS with 1:100 antibody dilution, for 20 min at 4°C. Cells were then washed twice with PBS and acquired on a FACSCanto II flow cytometer equipped with FACSDiva analysis software (Becton Dickinson, San Jose, CA, USA).

#### Adipogenic and Osteogenic *In Vitro* Differentiation of hMSC Cultures

hMSC differentiation was assessed by growing hMSCs in specific differentiation media for 2–3 weeks according to the manufacturer's instructions (Lonza, Basel, Switzerland). The detailed differentiation procedure is described elsewhere.<sup>30</sup> Briefly, for adipogenic differentiation, cells were cultured in an adipogenic MSC differentiation BulletKit (Lonza), and differentiated cells were stained with oil red O (Sigma). For osteogenic differentiation, cells were cultured in an osteogenic MSC differentiation BulletKit (Lonza) and differentiated cells were stained with alizarin red-S (Sigma).<sup>29</sup>

#### 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  $\mu$ L of DMEM and 50  $\mu$ L of Matrigel (BD Biosciences) subcutaneously in the back of the NSG mice.<sup>31</sup> Tumors generally developed within 6–10 weeks. When tumors reached 1-cm 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 and eosin to assess the presence of cells representing the three germ layers.<sup>31</sup> Animal

experimentation protocols were approved by the Animal Care Committee of the PRBB (Parc de Recerca Biomèdica de Barcelona).

#### SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.omtn.2020.02.009>.

#### AUTHOR CONTRIBUTIONS

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.D.d.I.G., B.L.-M., C.B.: methodology and investigation. A.B. and L.M.F.: conceptualization. P.M.: conceptualization, writing and financial support.

#### CONFLICTS OF INTEREST

The authors declare no competing interests.

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## **Supplemental Information**

### **Robustness of Catalytically Dead Cas9**

### **Activators in Human Pluripotent**

### **and Mesenchymal Stem Cells**

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

<b>gRNAs</b>	<b>Sequence</b>
FOXA2 -42	ggcgggtgctccctaccgcg
FOXA2 -179	ccactccaactaccgcctc
FOXA2 -248	tgattgctggtcgtttgtg
NEUROD1 -33	aggccgcggagtctctaac
NEUROD1 -164	acctgccattgtatgccg
NEUROD1 -221	aggggagcgggtgtcggagg
CXCR4 -116	gcagacgcgaggaaggaggcgc
CXCR4 -162	ccgaccaccgcaaacagca
CXCR4 -193	gcctctggaggctcctgtccggctc
NT	cggaggctaagcgtcgcaac
<b>Primers</b>	<b>Sequence</b>
Cas9_F	agcacgtggcacagatcctgg
Cas9_R	ggaaatcctccggaatcgg
VP64_F	aaaagaggaaggtggcggcc
VP64_R	cgtcactgccgagcatgtcg
MS2_F	aaggtgacatgcagcgtcagg
MS2_R	ccatgttcaggtaggacctcc
scFV_F	tgatcggcgacaaggccacc
scFV_R	gcgcttcagctccacctgg
GAPDH_F	gcaccgtcaaggctgagaac
GAPDH_R	agggatctcgctcctggaa
OCT4_F	gggttttgggattaagttctca
OCT4_R	gccccaccctttgtgtt
CRIPTO_F	cggaactgtgagcacgatgt
CRIPTO_R	gggcagccagggtgtcatg
DNMT3B_F	gctcacagggcccgatactt
DNMT3B_R	gcagtcctgcagctcgagtta
FOXA2_F	cacgagccgtccgactggag
FOXA2_R	atggcggccgccgacatgc
NEUROD1_F	ggatgacgatcaaagcccaa
NEUROD1_R	gcgtcttagaatagcaaggca
CXCR4_F	actacaccgaggaatgggct
CXCR4_R	cccacaatgccagttaagaaga