

**Modelling and drug targeting of a
myeloid neoplasm with atypical
3q26/*MECOM* rearrangement using
patient-specific iPSCs**

（患者特異的 iPS 細胞を用いた
非定型 3q26/*MECOM*再構成を有する
骨髓性腫瘍の新規病態モデルと
薬効評価系の構築）

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Modelling and drug targeting of a myeloid neoplasm with atypical 3q26/*MECOM* rearrangement using patient-specific iPSCs

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Abstract

Structural variations involving enhancer hijacking induce aberrant oncogene expression and cause tumorigenesis. A rare translocation, t(3;8)(q26.2;q24), is associated with *MECOM* and *MYC* rearrangement, causing myeloid neoplasms with a dismal prognosis. The most recent World Health Organization classification recognises myeloid neoplasms with *MECOM* rearrangement as acute myeloid leukaemia (AML) with defining genetic abnormalities. Recently, the increasing use of induced pluripotent stem cell (iPSC) technology has helped elucidate the pathogenic processes of haematologic malignancies. However, its utility for investigating enhancer hijacking in myeloid neoplasms remains unclear. In this study, we generated iPSC lines from patients with myelodysplastic syndromes (MDS) harbouring t(3;8)(q26.2;q24) and differentiated them into haematopoietic progenitor cells to model the pathophysiology of MDS with t(3;8)(q26.2;q24). Our iPSC model reproduced the primary patient's *MECOM* expression changes and histone H3 lysine 27 acetylation (H3K27ac) patterns in the *MECOM* promoter and *MYC* blood enhancer cluster (BENC). Furthermore, we revealed the apoptotic effects of the bromodomain and extra-terminal motif (BET) inhibitor on iPSC-derived MDS cells by suppressing activated *MECOM*. Our study demonstrates the usefulness of iPSC models for uncovering the precise mechanism of enhancer hijacking due to chromosomal structural changes and discovering potential therapeutic drug candidates for cancer treatment.

Introduction

Chromosomal structural variations may result in enhancer hijacking if an active enhancer is improperly positioned, causing it to regulate genes that are not its original targets. (1) This phenomenon has been described in some cancers, where an enhancer is relocated through chromosomal rearrangements to induce aberrant oncogene expression. (2) This has been revealed as the principal pathogenic mechanism in many haematologic malignancies, (3,4) including myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML), which harbour recurrent chromosome 3 rearrangements, such as *inv(3)(q21.3q26.2)/t(3;3)(q21.3;q26.2)*. Chromosomal rearrangements, such as *inv(3)/t(3;3)*, juxtapose a *GATA2* distal haematopoietic enhancer with the *EVII* promoter that causes aberrant *EVII* expression, which promotes proliferation and impairs the differentiation of myeloid precursor cells, leading to leukemogenesis. (5,6) Both MDS and AML with *inv(3)/t(3;3)* are associated with chemoresistance and have a 2-year survival rate lower than 10%. (7-9) A group of leukaemias with other 3q26/*MECOM* rearrangements, known as MDS/AML with atypical 3q26 rearrangements, often results in *MECOM* overexpression. (7,10) The rare translocation *t(3;8)(q26.2;q24)*, an atypical 3q26/*MECOM* rearrangement, has been identified in a limited number of patients with myeloid neoplasms and is associated with *MECOM* and *MYC* rearrangement, resulting in poor prognosis with a median survival of 6 months. (11-14) Recent reports showed that atypical 3q26/*MECOM* rearrangements, including *t(3;8)(q26.2;q24)*, also overexpress *MECOM* by repurposing enhancer elements from the translocation partners, similar to the enhancer hijacking of the *GATA2* distal enhancer in *inv(3)/t(3;3)*. (11,12) Based on these findings, the World Health Organization (WHO) designated a new category of AML with *MECOM* rearrangement in 2022. (15) Many haematological malignancies involve enhancer hijacking, (16,17) and specific disease models need to be developed for novel drug discovery and development.

Induced pluripotent stem cells (iPSCs) were generated from somatic differentiated cells by

Yamanaka and Takahashi. (18) Since their discovery, the application of iPSC technology has significantly contributed to our understanding of the pathophysiology of various inherited and acquired haematological disorders. (19,20) One of the key advantages of iPSCs in disease modelling is their comparability to isogenic controls, enabling the study of the cooperative effects of genomic alterations and their impact on disease progression via *in vitro* haematopoietic differentiation, colony-forming and replating assays, and cell signalling studies. Another advantage of iPSCs is their ability to create complex, large-scale chromosomal rearrangements, such as translocations, which are difficult to achieve with genetic tools, such as clustered regularly interspaced short palindromic repeats (CRISPR). Moreover, disease modelling using iPSCs has consistently uncovered new therapeutic strategies. (21) Aberrant histone methylation and acetylation have been implicated in cancer development. In a previous report of iPSCs derived from AML patients with *KMT2A* translocations, reprogrammed AML-iPSCs reset their leukemic DNA methylation profiles in the pluripotent state, thus effectively eliminating their leukemic potential. (22) However, haematopoietic differentiation allowed the reacquisition of the leukemic DNA methylation signature and phenotype, indicating that leukemic genomic alterations associated with histone and DNA methylation are sufficient to induce leukemogenesis. To date, there are no reports on how aberrant histone acetylation in MDS/AML changes upon reprogramming into iPSCs or subsequent redifferentiation into haematopoietic cells.

In this study, we generated iPSC lines from a patient with MDS harbouring t(3;8)(q26.2;q24) and differentiated them into haematopoietic progenitor cells (HPCs) to model the pathophysiology of MDS with t(3;8)(q26.2;q24). Using this platform, we found that the bromodomain and extra-terminal motif (BET) inhibitor JQ1 suppressed *MECOM* expression and induced apoptosis in MDS cells carrying t(3;8)(q26.2;q24). Our study demonstrates the usefulness of iPSC models for uncovering the precise mechanism of enhancer hijacking due to chromosomal structural changes and discovering potential therapeutic drug candidates for cancer treatment.

Materials and methods

Generation of iPSCs from a patient with t(3;8)(q26.2;q24)

We established iPSCs from a patient with MDS carrying t(3;8)(q26.2;q24) using episomal vectors as previously described. (23) Briefly, bone marrow or peripheral blood mononuclear cells from the patient were transfected with an episomal vector mix, including pCXLE-hOCT3/4-shp53, pCXLE-hSK, pCXLE-hUL, and pCXWB-EBNA1, and seeded onto mouse embryonic fibroblast feeder cells. Isogenic normal iPSCs were generated after T cell activation using Dynabeads Human T-Activator CD3/CD28 (Thermo Fisher Scientific) according to the manufacturer's instructions. MDS-iPSC lines were denoted as MDS-25-A3, MDS-25-B8, MDS-25-G1, MDS-25-J1, and MDS-25-J4 iPSCs, and isogenic normal iPSC lines were denoted as N-25-A1 and N-25-B1 iPSCs. We also used an iPSC line derived from a healthy donor (692D2) as a control. Chromosomal G-band analysis of the iPSC lines was performed at Nihon Gene Research Laboratories (Sendai, Japan). This study was approved by the Ethics Committees of the Graduate School of Medicine, Kyoto University, and Kyoto University Hospital and performed according to the Declaration of Helsinki. Written informed consent was obtained from the patient.

Statistical analyses

GraphPad Prism 10 (GraphPad Software) was utilised to conduct all statistical analyses. $P < 0.05$ was considered statistically significant.

Results

Translocation of 8q24/*MYC* to 3q26/*MECOM* causes aberrant *MECOM* expression in a patient with MDS harbouring t(3;8)(q26.2;q24)

We identified a patient with atypical 3q26/*MECOM*-rearranged MDS who succumbed to the disease within five months of diagnosis (Table 1). Bone marrow aspiration revealed a blast cell percentage of 19.6% (Figure 1A). Immunostaining of the bone marrow clot demonstrated high *MECOM* and low *MYC* expression (Figure 1B–C). Karyotype analysis revealed a translocation between chromosomes 3 and 8 (46, XY, t(3;8)(q26.2;q24), del(9)(q?)) (Figure 1D). Fluorescence *in situ* hybridisation analysis indicated that over 90% of the split signals for *MECOM* and *c-MYC* were positive (Supplemental Figure 1A–C). Targeted sequencing identified mutations in *ETNK1* (p.N155S), *PTPN11* (p.D61V, p.A72T, and p.E76K), and *EZH2* (c.2181-1G>C) (Supplemental Table 1). Copy number alteration confirmed a decrease in the total copy number on chromosome 9 and abnormalities in the allelic ratio on chromosomes 7 and 9 due to the deletion of chromosome 9 and uniparental diploidy on chromosome 7 (Supplemental Figure 2A). Whole-genome sequencing revealed that the breakpoints were 6.4 kb upstream of *MECOM* (6.4 kb upstream of the *MDS1-EVII* promoter and 523 kb upstream of the *EVII* promoter) and 200 kb upstream of blood enhancer cluster (BENC), which is known as the *MYC* super-enhancer (Figure 1E; Supplemental Figure 2B–D). This specific breakpoint location is consistent with those in previously reported cases of t(3;8) MDS/AML. (11,12) These findings suggest that the t(3;8)(q26.2;q24) translocation may be causally associated with the high *MECOM* expression and MDS development in this patient.

Generation of MDS-iPSC and isogenic normal iPSC lines from the patient with MDS harbouring t(3;8)(q26.2;q24)

We generated several iPSC lines derived from MDS clones and two iPSC lines derived from normal T cells of a patient with t(3;8)(q26.2;q24) (Figure 1F; Supplemental Figure 3A;

Supplemental Table 2). Karyotype analysis of MDS-iPSC lines revealed concordance with the patient karyotype (Figure 1F; Supplemental Figure 3B–D). Conversely, isogenic normal iPSC lines exhibited normal karyotypes (Figure 1F; Supplemental Figure 3E–F). Sanger sequencing showed that the MDS-iPSC lines had the same breakpoint as the patient (Supplemental Figure 3G; Supplemental Table 3). Gene expression of pluripotency markers, including *NANOG*, *OCT3/4*, and *SOX2*, was verified using quantitative RT-PCR (Supplemental Figure 3H; Supplemental Table 4). The MDS-iPSC lines displayed a characteristic morphology and expressed pluripotent stem cell markers at levels comparable to those of the isogenic normal iPSC lines (Supplemental Figure 3I). The mutations present in the starting bone marrow samples were assessed through Sanger sequencing of five generated MDS-iPSC lines, revealing *ETNK1* and *EZH2* mutations in all lines, with only one line harbouring the *PTPN11* (p.A72T) mutation (Figure 1F; Supplemental Tables 3 and 5). By contrast, the two isogenic normal iPSC lines revealed no mutations present in the patient (Figure 1F; Supplemental Tables 3 and 5). These findings demonstrated that MDS cells were successfully reprogrammed into iPSCs, retaining the original genomic abnormalities.

MDS-iPSCs exhibited reduced haematopoietic potential and decreased clonogenic capacity

Next, we differentiated MDS-iPSC, isogenic normal iPSC, and control iPSC lines into haematopoietic cells via embryoid body formation (Figure 2A). MDS-iPSC lines gave rise to CD45⁺ haematopoietic cells with efficiencies comparable to those of isogenic normal iPSC lines but retained CD34 expression until day 17 and beyond (Figure 2B). In addition, the MDS-iPSC lines did not produce CD235a⁺ erythroid cells (Figure 2C). Haematopoietic cells derived from MDS-iPSCs express the myeloid lineage marker CD33, which is known to be highly expressed in AML blasts (Supplemental Figure 4). FACS-sorted CD45⁺CD34⁺ HPCs from MDS-iPSC lines demonstrated leukemic blast morphology (Figure 2D) and showed markedly decreased myeloid colony-forming ability with no erythroid potential in contrast to those from the control and

isogenic normal iPSC lines (Figure 2E–F). These results demonstrated that MDS-iPSC-derived HPCs had differentiation defects in both the myeloid and erythroid lineages.

MDS-iPSC-derived HPCs exhibited an aberrant gene expression pattern similar to that of primary myeloid neoplasm cells

Next, we performed RNA sequencing (RNA-seq) on CD45⁺CD34⁺ HPCs derived from MDS-iPSCs and isogenic normal iPSCs (Figure 3A). Principal component analysis revealed that MDS-iPSCs were located on the positive side of principal component 1, accounting for 97% of the variance, whereas those derived from isogenic normal iPSCs were located on the negative side (Figure 3B). We compared the differentially expressed genes in MDS-25-G1 and N-25-A1 iPSC-derived HPCs and created a volcano plot, revealing the significant downregulation of 726 genes in MDS-25-G1 iPSC-derived HPCs, including critical factors expressed during haematopoietic differentiation, and the significant upregulation of 2018 genes in MDS-25-G1 iPSC-derived HPCs, including factors with previously described functions in haematopoietic stem cell (HSC) maintenance (Figure 3C). (24) Gene set enrichment analysis showed enrichment of gene expression signatures of primary AML cells in HPCs derived from MDS-iPSCs (Figure 3D–E; Supplemental Table 6). These transcriptome data support the myeloid neoplasm phenotype of MDS-iPSC-derived HPCs.

MDS-iPSC-derived HPCs showed high *MECOM* expression comparable to that in original MDS cells

We compared *MECOM* expression among iPSCs, iPSC-derived HPCs, K562 cells, and CD34⁺ HPCs from the patient. Gene expression analysis of the *MDS1-EVII* variant and total *MECOM* was performed using qRT-PCR with primers listed in Supplemental Table 4. Haematopoietic differentiation of iPSCs into HPCs increased *MECOM* expression (Figure 4A–B; Supplemental Figure 5A–B). MDS-iPSC-derived HPCs showed higher expression of the *MDS1-EVII* variant

and total *MECOM* than isogenic normal iPSC-derived HPCs. *MECOM* expression levels in MDS-iPSC-derived HPCs were comparable to those in HPCs from the patient (Figure 4C–D). Consistent with a previous report, (12) *MYC* expression in MDS-iPSC-derived HPCs was not significantly different from that in isogenic normal iPSC-derived HPCs (Figure 4E; Supplemental Table 4). These results suggest that the translocation caused high *MECOM* expression, especially of the *MDS1-EVII* variant, which may be involved in MDS pathogenesis in this patient.

***MECOM* promoter hyperactivation upon interaction with *MYC* super-enhancer in t(3;8) MDS**

We conducted H3K27ac ChIP sequencing (ChIP-seq) with iPSCs, iPSC-derived CD45⁺CD34⁺ HPCs, K562 cells, and CD34⁺ HPCs from the patient according to previous reports (Supplemental Tables 7–8). (12,25) ChIP-seq revealed BENC activity in both control iPSC- and MDS-iPSC-derived HPCs in a pattern similar to that of the patient CD34⁺ HPCs (Figure 5A). BENC is a cluster of multiple individual enhancer modules (A–I), which encompasses approximately 150 kb of chromatin enriched with the histone mark H3K27ac and is located 1.7 Mb downstream of *MYC*. This locus has been documented as essential for normal haematopoiesis. (25) H3K27ac levels, as determined via ChIP-seq, revealed both *MDS1-EVII* and *EVII* promoter activity in control iPSC- and MDS-iPSC-derived HPCs, and the patient HPCs (Figure 5B; Supplemental Figure 6A–D). However, *MDS1-EVII* promoter activity was higher in MDS-iPSC-derived HPCs and patient primary HPCs than in control iPSC-derived HPCs, consistent with the markedly increased expression of the *MDS1-EVII* variant in these MDS samples (Figure 4A). These data support the hypothesis that *MECOM* overexpression in this patient with t(3;8) MDS results from translocation of the *MYC* super-enhancer.

t(3;8) MDS cells exhibited H3K27 acetylation in the *MDS1-EVII* promoter region after reprogramming

To evaluate the effects of cell reprogramming and differentiation on histone modification, we compared H3K27ac activation between iPSCs and iPSC-derived HPCs. Initially, we examined the activity of genes specific to each cell type and made intriguing discoveries. Specifically, we observed the unique activation of *NANOG*, *POU5F1* (*OCT3/4*) and *SOX2* in iPSCs (Supplemental Figure 7A–C). By contrast, the *EVII* promoter and *GATA2* enhancer exhibited activity only in iPSC-derived HPCs (Figure 5B; Supplemental Figures 6A–B and 7D). Unexpectedly, active H3K27ac in the *MDS1-EVII* promoter region was detected at low levels in MDS-iPSCs as well as in iPSC-derived HPCs (Figure 5B; Supplemental Figure 6C–D). This result was consistent with the higher *MDS1-EVII* expression in MDS-iPSCs than in control iPSCs (Figure 4A; Supplemental Figure 5A).

Bromodomain-containing 4 (BRD4) inhibitor JQ1 suppressed *MECOM* expression and t(3;8) MDS cell growth

BRD4 is a member of the BET subfamily that abundantly accumulates at super-enhancers. (26) BRD4 inhibitors, such as JQ1, inhibit the function of super-enhancers, leading to the selective repression of oncogenes in haematological malignancies. (17,27) Since the BENC super-enhancer appeared to promote t(3;8) MDS development by activating *MECOM* expression, we examined whether JQ1 abrogated the proliferation of MDS-iPSC-derived HPCs *in vitro*. As expected, JQ1-treated MDS-iPSC-derived HPCs had significantly lower expression levels of *MECOM* than DMSO-treated HPCs (Figure 6A–B). Conversely, control iPSC-derived HPCs did not differ significantly in *MECOM* expression between the JQ1-treated and untreated groups (Supplemental Figure 8A–B). Next, we assessed JQ1-induced apoptosis using Annexin V/7-AAD staining and flow cytometry. JQ1 markedly increased the fraction of apoptotic cells in a dose-dependent manner in MDS-iPSC-derived HPCs, but not in control and N-25-A1 iPSC-derived HPCs and K562 cell lines (Figure 6C–D; Supplemental Figure 8C). Using a trypan blue exclusion assay, we also assessed cell viability after JQ1 treatment. We found that HPCs derived from MDS-

25-G1 iPSC showed a more significant decrease in viability than HPCs derived from the control and N-25-A1 lines (Figure 6E–F; Supplemental Figure 8D). This cytotoxic effect was also observed in HPCs derived from other MDS-iPSC clones and CD34⁺ MDS cells from the patient after JQ1 treatment (Supplemental Figure 9A–B). Furthermore, we confirmed the effect of JQ1 in the EVI1-GFP t(3;8) K562 cell line model reported previously. (12) JQ1 administration resulted in a modest reduction in the degree of GFP expression within t(3;8) K562 cells (Figure 6G). Additionally, H3K27ac ChIP sequencing revealed a reduction in the level of H3K27ac modifications in BENC after JQ1 treatment (Figure 6H; Supplemental Figure 9C). These results suggest that JQ1 inhibition of the BENC super-enhancer impairs the growth of t(3;8) MDS cells by suppressing aberrantly activated *MECOM* (Figure 7).

Discussion

Myeloid neoplasms with $t(3;8)(q26.2;q24)$, which often leads to *MECOM* and *MYC* rearrangement, have an extremely poor prognosis with a median survival of 6 months. (13) Myeloid neoplasms with atypical 3q26 rearrangements, including $t(3;8)(q26.2;q24)$, share some similarities with myeloid neoplasms associated with $inv(3)/t(3;3)$. (11, 12) The WHO 2022 classification recognises these myeloid neoplasms with 3q26 rearrangements as AML with defining genetic abnormalities. (15)

The expression of both *EVII* and *MDSI-EVII* was aberrantly upregulated in our patient with MDS $t(3;8)(q26.2;q24)$. *MECOM* translocation reportedly leads to *EVII* overexpression without upregulating *MDSI-EVII* in most cases of atypical 3q26-rearranged AML. (11) The chromosomal breakpoints identified in this study occur commonly between *MDSI* and *EVII* or downstream of *EVII*. (5,11) Lugthart et al. (7) reported positive *MDSI-EVII* expression in 50% of $inv(3)/t(3;3)$ cases and 57% of the $t(3q26)$ group. Hence, *MDSI-EVII* expression appears to be influenced by the breakpoint location. In our case, the breakpoint $t(3;8)(q26.2;q24)$ was located immediately upstream of the *MDSI* promoter, resulting in high *MDSI-EVII* expression.

To date, patient-derived iPSC models have helped elucidate the pathogenic process of many human diseases, including myeloid neoplasms, and serve as a valuable drug discovery platform. (28-31) Our iPSC model of atypical 3q26-rearranged MDS reproduced the *MECOM* expression changes and H3K27ac patterns in the primary patient. A previous study has reported that leukemic DNA methylation and gene expression profiles were reset after reprogramming, but the leukemic properties were reactivated upon haematopoietic differentiation. (22) The current study demonstrates that *MECOM* expression and H3K27ac activation of the *MECOM* promoter and the *MYC* distal enhancer increased after haematopoietic differentiation. However, MDS-iPSCs with $t(3;8)(q26.2;q24)$ exhibited H3K27ac activation at the *MECOM* promoter, even in the pluripotent

state, with higher *MECOM* expression than the control iPSCs. One possibility is that these abnormal histone marks are maintained during MDS cell reprogramming to iPSCs. Alternatively, histone acetylation at the *MECOM* promoter of MDS cells could be reset following reprogramming and acquire aberrant histone activation soon after reprogramming owing to the influence of the translocation.

The BET inhibitor JQ1 has demonstrated activity against MDS/AML cells with inv(3)/t(3;3) through epigenetic silencing of the translocated *GATA2* enhancer. (5) We speculated that repressing super-enhancer-dependent *MECOM* expression could also induce cytotoxicity to MDS/AML clones with atypical 3q26 rearrangement. The previous study reported that the deletion of the *MYC* distal enhancer resulted in the loss of *MECOM* expression. Our data, along with the recent report, support the hypothesis that *MECOM* overexpression in myeloid neoplasms with t(3;8) is driven by the translocation of the *MYC* distal enhancer. (12) This study provides the first proof-of-concept that aberrant gene expression in MDS/AML cells with t(3;8)(q26.2;q24) can be suppressed by BET inhibitors, indicating that iPSCs are valuable tools for uncovering the mechanisms and treatment of enhancer-hijacking.

Despite the promising results, our iPSC model has certain limitations. First, the patient with MDS carried a chromosome 9 deletion along with the translocation of t(3;8)(q26.2;q24) and some recurrent gene mutations related to MDS/AML. These abnormalities could also affect gene expression and disease phenotypes, implying that we did not completely assess the function of t(3;8)(q26.2;q24). Second, the HPCs differentiated from iPSCs do not fully resemble bona fide HSCs with long-term repopulation. Despite these limitations, we modelled enhancer hijacking events in cancers using iPSCs for the first time. This patient-specific iPSC platform may be helpful in precise epigenetic analysis and functional studies of cancers associated with enhancer hijacking, such as myeloid neoplasms with t(3;8)(q26.2;q24).

In conclusion, this study provided novel biological and therapeutic insights into myeloid neoplasms with t(3;8)(q26.2;q24). Using patient-derived iPSCs, we demonstrated that *MYC* distal enhancer translocation and the consequent increase in *MECOM* promoter activity contribute to the abnormal maturation of HPCs and oncogenic processes. Furthermore, we revealed the apoptotic effects of the BET inhibitor JQ1 on MDS cells harbouring t(3;8)(q26.2;q24) by suppressing aberrantly activated *MECOM*. Our results demonstrate the efficacy of iPSC models in elucidating the mechanism of enhancer-hijacking resulting from alterations in chromosomal structure and in identifying prospective therapeutic agents for cancer treatment.

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Author Contributions

M.Nakamura., K.C., and Y.Y. conceived and designed the research; M.Nakamura. performed most of the experiments, analysed the data, and wrote the manuscript; K.C. established MDS-iPSC lines and performed some experiments, analysed the data, and wrote the manuscript; M.Narita, Y.M., and M.Nishikawa provided essential experimental support; Y.O. assisted with the ChIP-seq; M.H. collected patient blood samples and advised on the experimental design; Y.N. performed targeted capture sequencing and whole genome sequencing; D.I., R.D., S.O., and A.T.-K. advised on the experimental design and wrote the manuscript; Y.Y. supervised the overall research and wrote the manuscript. All authors interpreted the data and reviewed and approved the final manuscript.

Disclosure of conflicts of interest

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Table

Table 1. Clinical and hematological characteristics.

Figure legends

Figure 1.

Translocation of 8q24/*MYC* to 3q26/*MECOM* causes aberrant *MECOM* expression in a patient with MDS carrying t(3;8)(q26.2;q24).

(A) Morphology of bone marrow cells. May Grünwald-Giemsa staining revealed blast cells with large nuclei, scant cytoplasm, and no cytoplasmic granules. Magnification of the objective lens was 40×. Scale bar, 50 µm. (B, C) Immunostaining of bone marrow clots of *MECOM* (B) and *MYC* (C). Magnification of the objective lens was 40×. Scale bar, 50 µm. (D) Karyotype analysis of bone marrow cells. (E) Schematic diagram of whole-genome sequencing results for the MDS clones. This figure was created using BioRender.com. (F) Schematic representation of iPSC generation from blood cells of a patient with t(3;8)(q26.2;q24) and genetic information of the iPSC lines. The top panels show all recurrent gene mutations and chromosomal abnormalities detected in the starting cells used for reprogramming and their frequency. The bottom panels present the individual iPSC lines corresponding to their genetic profile. The MDS-iPSC lines were denoted as MDS-25-A3, MDS-25-B8, MDS-25-G1, MDS-25-J1, and MDS-25-J4 iPSCs, and the isogenic normal iPSC lines as N-25-A1 and N-25-B1 iPSCs. VAF, variant allele frequency. The figure was created using BioRender.com.

Figure 2.

MDS-iPSCs exhibit reduced haematopoietic potential and decreased clonogenic capacity.

(A) Schema of a protocol for the haematopoietic differentiation from iPSCs. The images were created using BioRender.com. (B) Representative flow cytometry data in the N-25-A1, MDS-25-G1, and MDS-25-J4 iPSC lines on day 17. Percentages of CD34⁺ HPCs in CD45⁺ cells with the N-25-A1, MDS-25-G1, and MDS-25-J4 are shown in the right-hand graph. The results of three independent experiments are shown. (C) Representative flow cytometry data of CD235a⁺ cells in the N-25-A1, MDS-25-G1, and MDS-25-J4 iPSC lines on day 17. (D) May Grünwald-Giemsa

staining of CD45⁺ (left) and CD45⁺CD34⁺ (right) haematopoietic cells derived from the MDS-25-G1 iPSC lines. Dysplastic changes (arrow point to pseudo Pelger-Huet cells) and leukemic blast morphology (arrowhead) are observed. Magnification of the objective lens was 100×. Scale bar, 20 µm. (E) Haematopoietic colony formation assay of HPCs derived from one control iPSC, two isogenic normal iPSC (N-25-A1, N-25-B1), and three MDS-iPSC (MDS-25-G1, MDS-25-J1, and MDS-25-J4) lines. Each line was tested in three independent experiments. (F) Representative images of GM (top left) and mixed (top right) colonies derived from the N-25-A1 line and GM (bottom) colonies derived from the MDS-25-G1 line. Magnification of the objective lens: 10×. Scale bar, 100 µm. *P* values were calculated using a one-way analysis of variance with Dunnett's correction. ****P* < 0.001. Data are presented as means ± SD.

Figure 3.

MDS-iPSC-derived HPCs exhibit an aberrant gene expression pattern similar to that of primary myeloid neoplasm cells.

(A) The protocol scheme for the haematopoietic differentiation from iPSCs and an RNA-seq sample collection time point. The image was created using BioRender.com. (B) Principal component analysis of HPCs derived from the N-25-A1 and the MDS-25-G1 lines with RNA-seq. (C) Volcano plot presenting the differential gene expression between HPCs derived from the N-25-A1 and MDS-25-G1 lines. Each dot indicates one gene; red dots represent log₂ fold change (log₂FC) of >1 or -1.0 and adjusted *P* value (padj) < 0.05. (D, E) Gene set enrichment analysis of the VALK_AML_CLUSTER_2 (D) and the VALK_AML_CLUSTER_6 (E) data sets showed enrichment of MDS-25-G1 iPSC-derived HPCs.

Figure 4.

HPCs derived from MDS-iPSCs show higher *MECOM* expression levels, comparable to those in MDS cells from the patient.

(A, B) Expression levels of *MDS1-EVII* (A) and *MECOM* (B) in iPSCs and HPCs derived from one control iPSC line, two isogenic normal iPSC lines, and one MDS-iPSC line. Each line was tested in three independent experiments. Expression levels were normalised to the level of *GAPDH*. (C–E) Expression levels of *MDS1-EVII*, *MECOM*, and *MYC* in HPCs derived from control iPSC, N-25-A1, N-25-B1, and MDS-25-G1 lines, CD34⁺ HPCs from the patient, and K562. The results of three independent experiments are shown. Expression levels were normalised to the level of *GAPDH*. *P* values were calculated using a one-way analysis of variance with Dunnett's correction. ***P* < 0.01; ns, not significant. Data are presented as means ± SD.

Figure 5.

***MECOM* promoter hyperactivation upon interaction with *MYC* super-enhancer in t(3;8) MDS.**

(A) Upper part: presenting chr8q24, H3K27ac ChIP-seq data for HPCs derived from control iPSC (grey) and MDS-iPSC (red, MDS-25-G1) lines, CD34⁺ HPCs (orange) from the patient, and K562 (green). A chromosome 8 breakpoint in the patient with t(3;8)(q26.2;q24). Lower part: H3K27ac ChIP-seq data as in the upper part but presenting a close-up of the +1.7 Mb *MYC* super-enhancer (BENC) with previously characterised individual enhancer modules A-I. (25) (B) Upper part: similar to (A) but close-up of the *MECOM* gene. A chromosome 3 breakpoint in the patient with t(3;8)(q26.2;q24). Lower left part: H3K27ac ChIP-seq data for control iPSCs (light grey), MDS-25-G1 iPSCs (light red), MDS-25-J1 iPSCs (pink), HPCs derived from control iPSC (grey), MDS-25-G1 iPSC (red) lines, CD34⁺HPCs (orange) from the patient, and K562 (green) at the *EVII* locus. The peak located at the *EVII* transcriptional start site marks the promoter region. Lower right part: Similar to the left part but comparing *MDS1* promoter activation. These schemas were created using BioRender.com.

Figure 6.

JQ1 suppresses aberrant *MECOM* expression and induces apoptosis in t(3;8) MDS cells.

(A, B) Expression levels of *MDS1-EVII* (A) and *MECOM* (B) in HPCs derived from MDS-iPSCs (MDS-25-G1) after JQ1 treatment. The results of three or more independent experiments are shown. Expression levels were normalised to the level of *GAPDH*. (C) Assessment of apoptosis in HPCs derived from N-25-A1 iPSC (top) and MDS-25-G1 (bottom) lines after JQ1 treatment. Representative flow cytometry plots for Annexin V and 7-AAD staining with percentages for each gate are shown. (D) Relative number of Annexin V-positive cells in HPCs derived from control iPSC (grey), N-25-A1 (blue), K562 (green), and MDS-25-G1 (red) lines after JQ1 treatment. The cell number of Annexin V-positive cells in each DMSO-treated cell line was set to 1. (E, F) Cell viability in HPCs derived from N-25-A1 (E) and MDS-25-G1 (F) lines after JQ1 treatment, assessed by trypan blue exclusion assay. (G) Flow cytometry data of GFP expression in the EVI1-GFP t(3;8) K562 model 3 days after 500 nM JQ1 treatment. (H) H3K27ac-ChIP sequencing data post-JQ1 treatment in t(3;8) K562 cells on the +1.7 Mb *MYC* super-enhancer (BENC). *P* values were calculated using a one-way analysis of variance with Dunnett's correction and a 2-way analysis of variance with Tukey's correction. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001; ns, not significant. DMSO, dimethyl sulfoxide. Data are presented as means ± SD.

Figure 7.

Schematic representation of the results.

MECOM overexpression is driven by enhancer hijacking caused by the translocation t(3;8)(q26.2;q24), involving a super-enhancer (BENC). Administration of the BET inhibitor JQ1 suppressed super-enhancer activity and abrogated the aberrant expression of *MECOM*. The figure was created using BioRender.com.

Table 1 Clinical and hematological characteristics

Age	Sex	Peripheral blood findings				Bone marrow findings		Treatment and follow-up		
		WBC, ×10 ⁹ /L	Hb, g/dL	Plt, ×10 ⁹ /L	Blast, %	Blast, %	Dysplasia	Treatment	Survival, months	Outcome
72	M	9.63	5.1	322	10	19.6	Yes (MKs)	5-Azacitidine	5	Dead

WBC, white blood cell; Hb, hemoglobin; RBC, red blood cell; Plt, platlet; MKs, megakaryocytes.

Figure 1

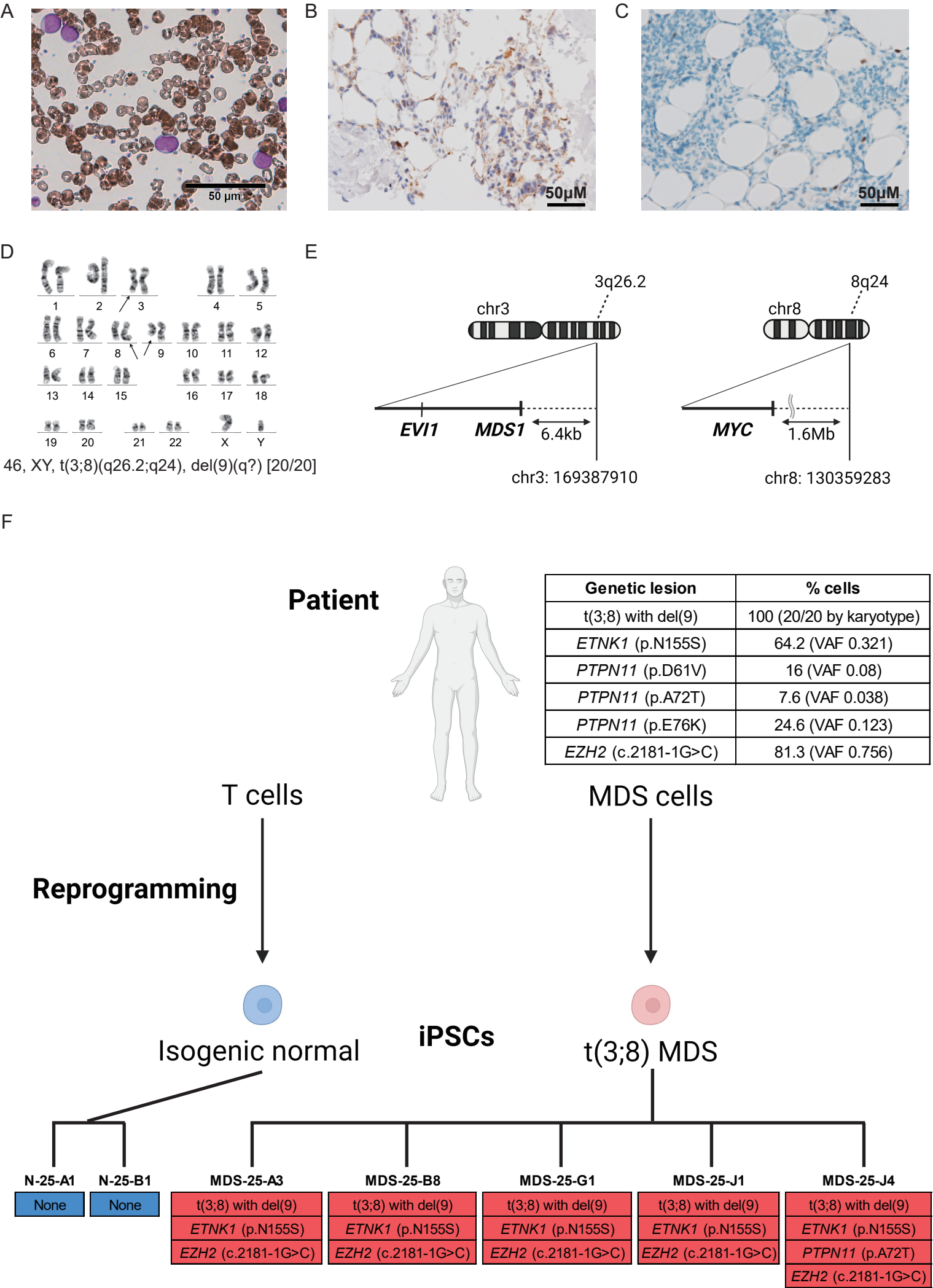


Figure 2

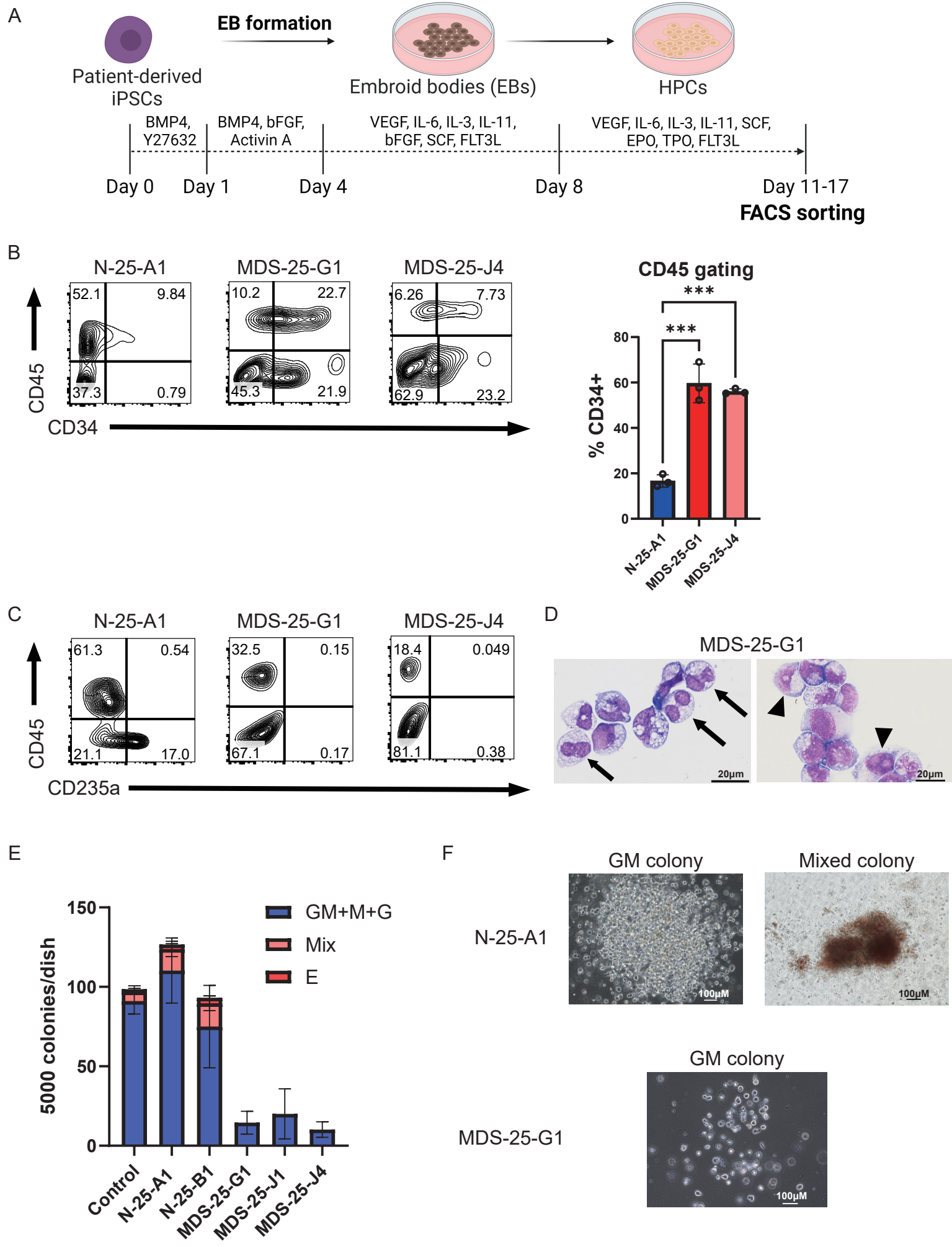
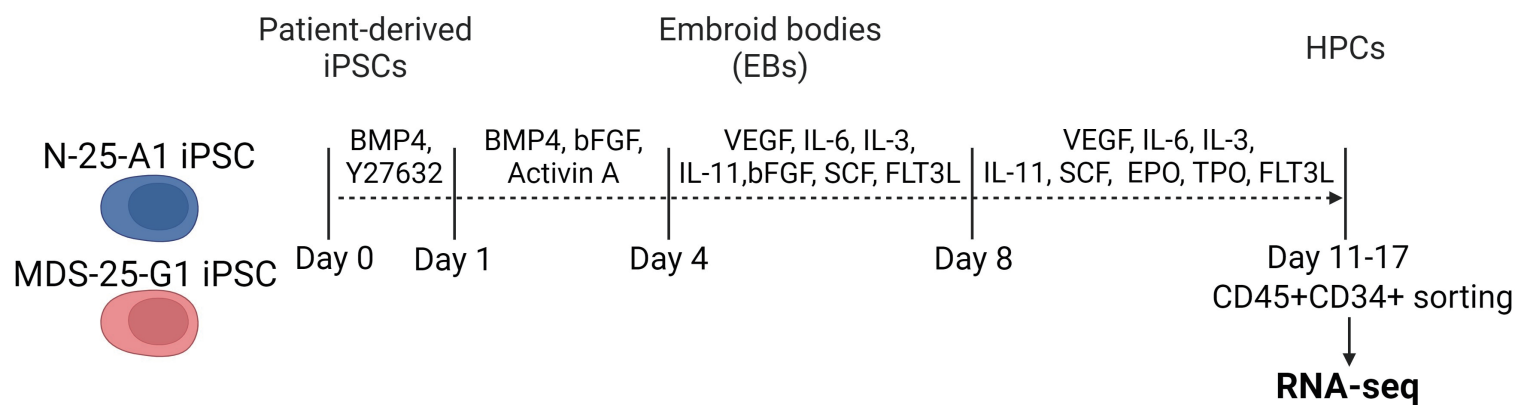
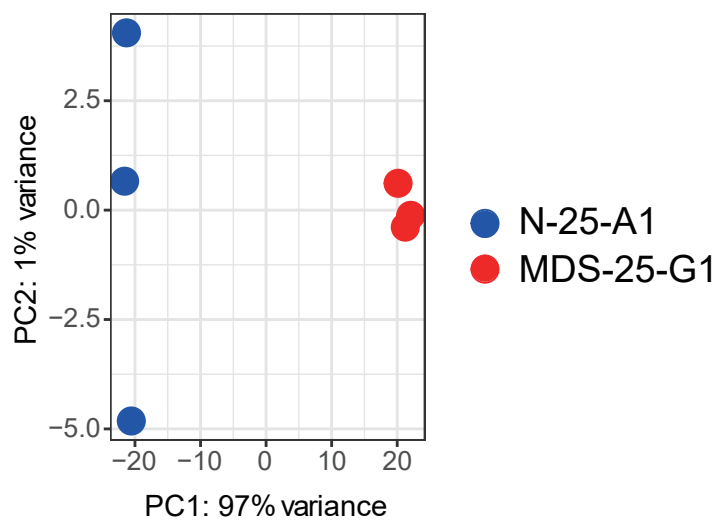


Figure 3

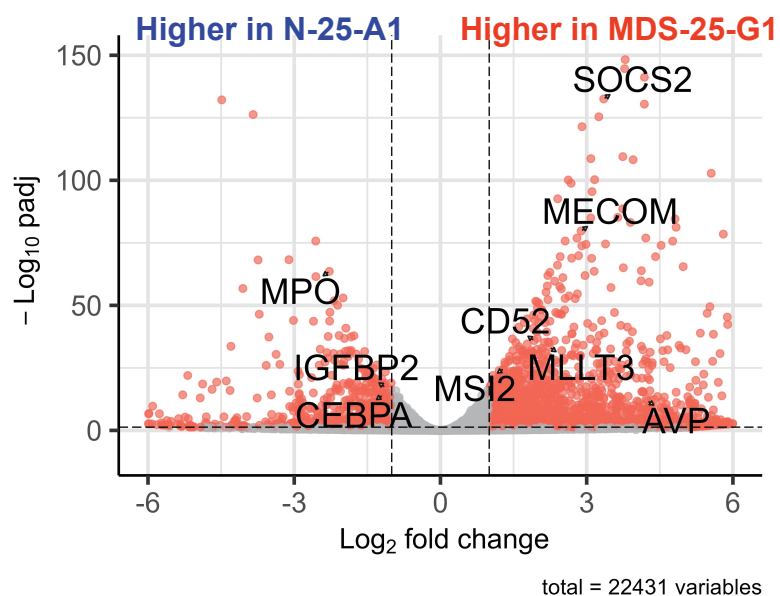
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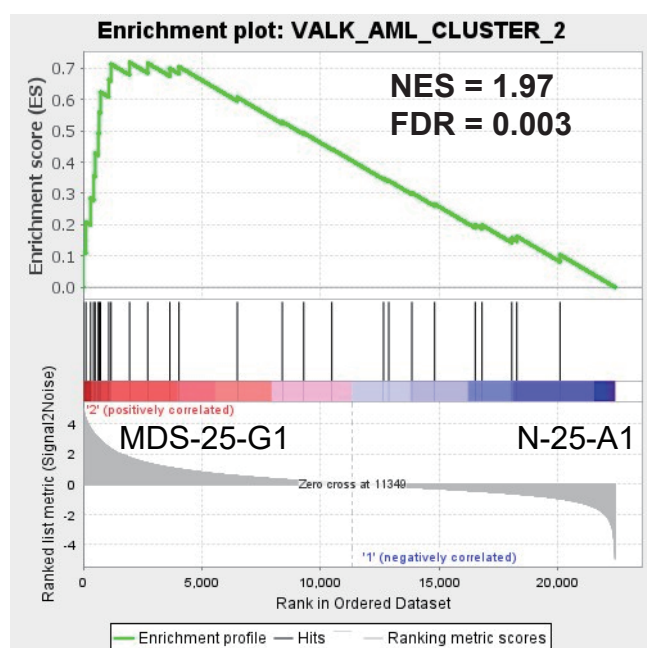
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C



D



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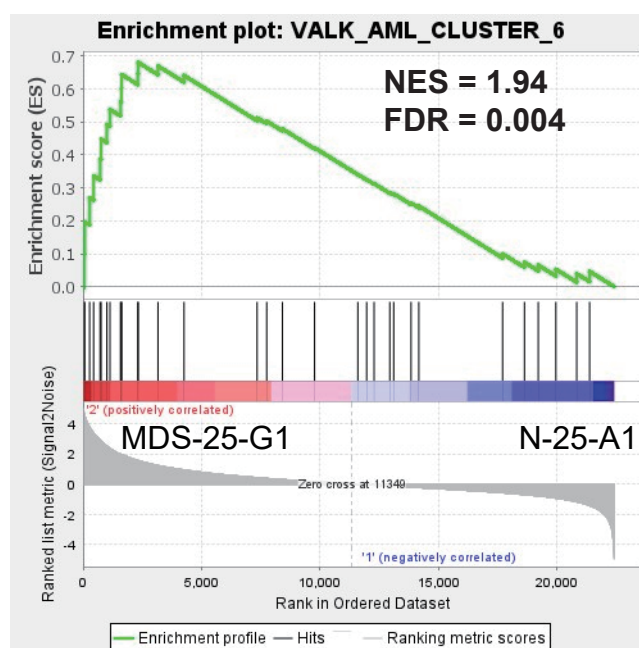


Figure 4

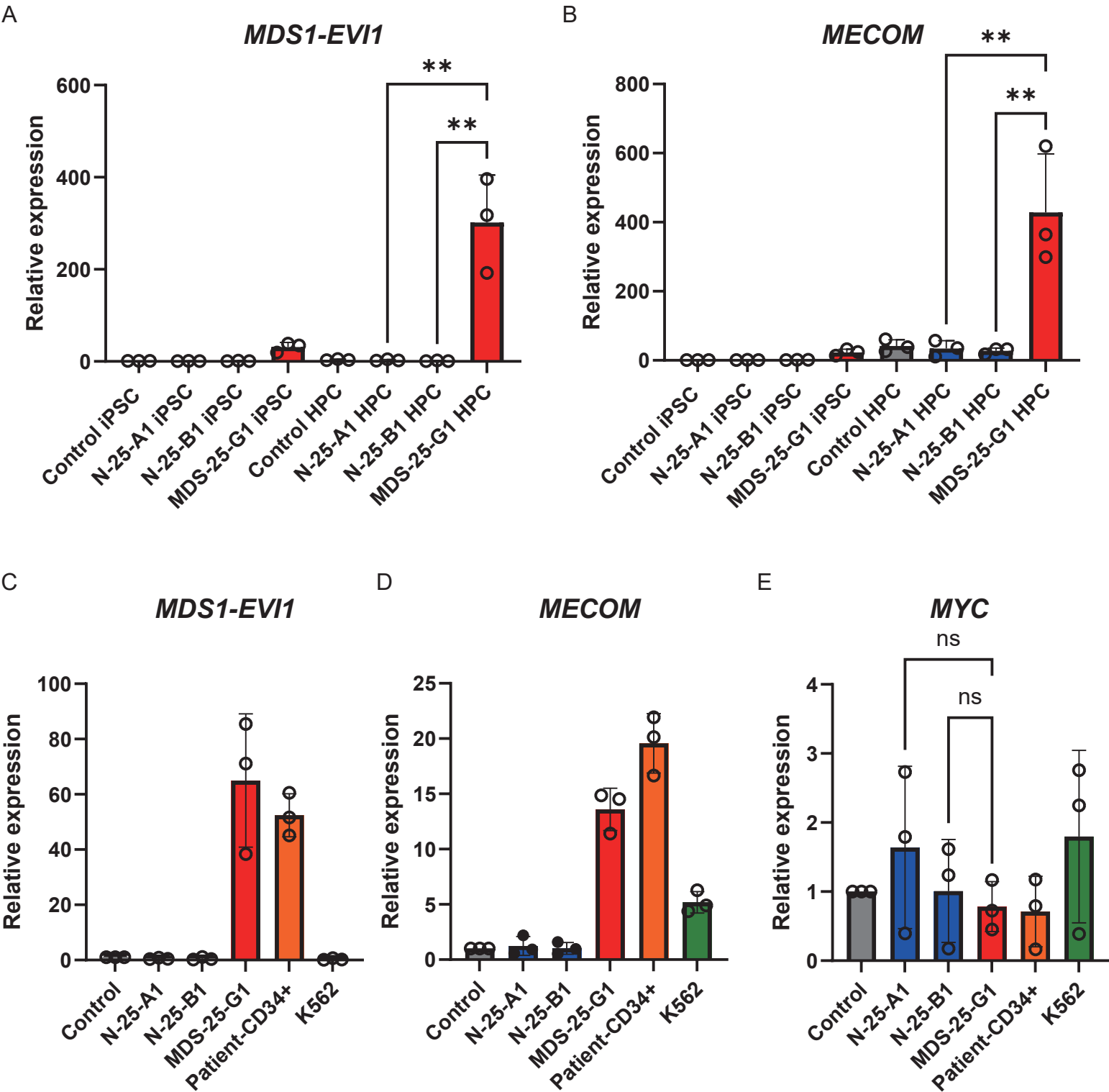
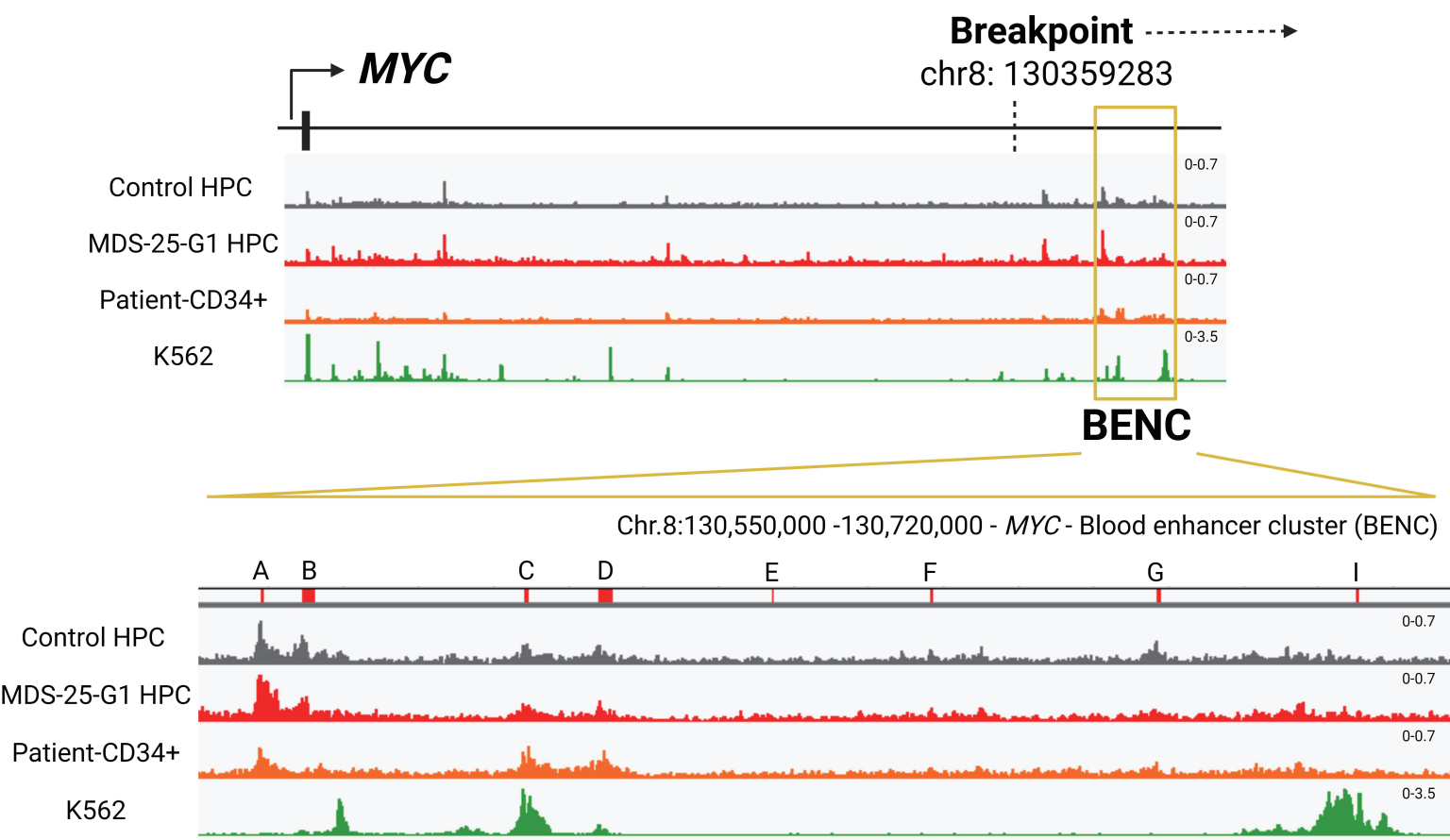


Figure 5

A



B

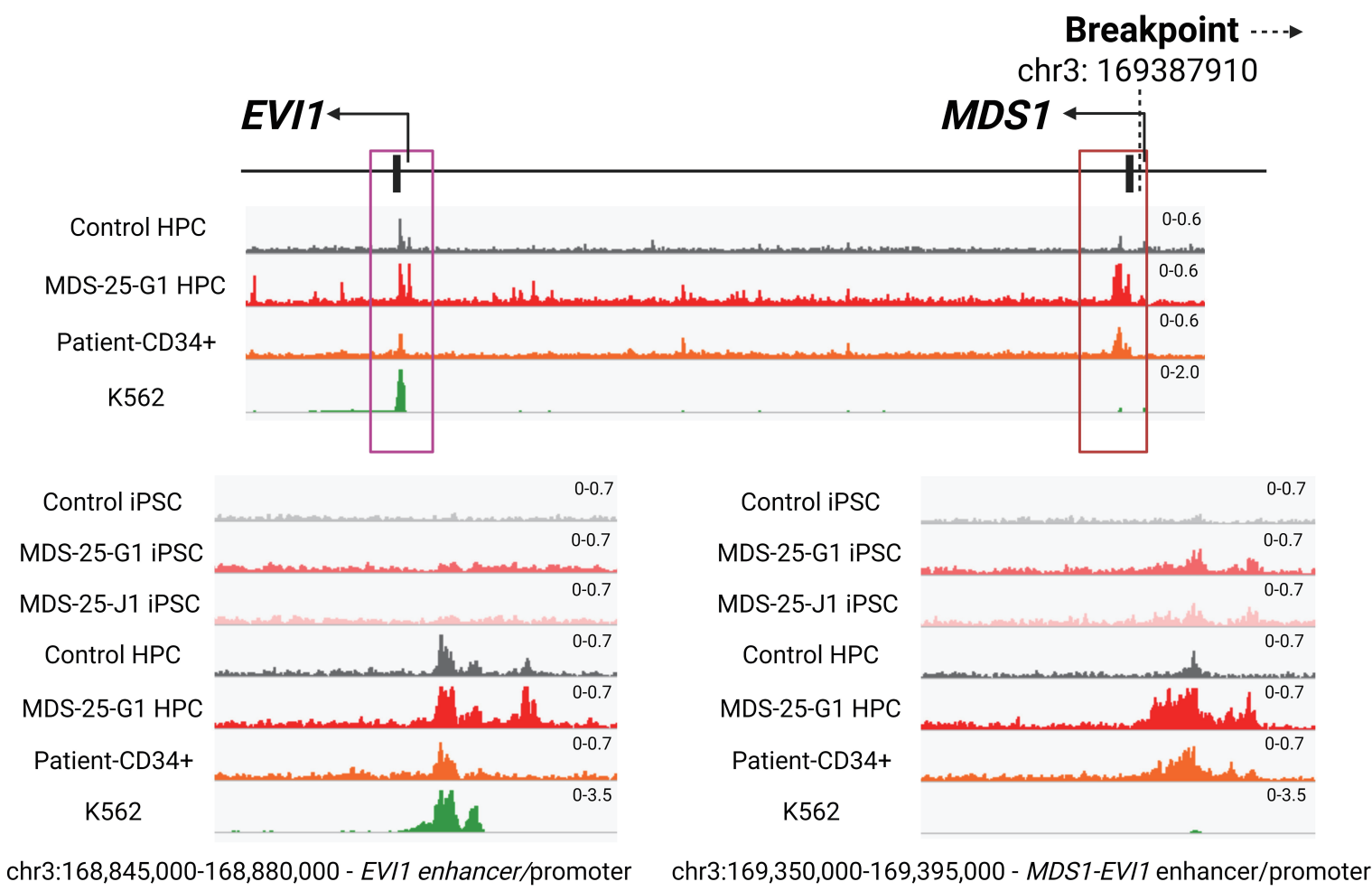
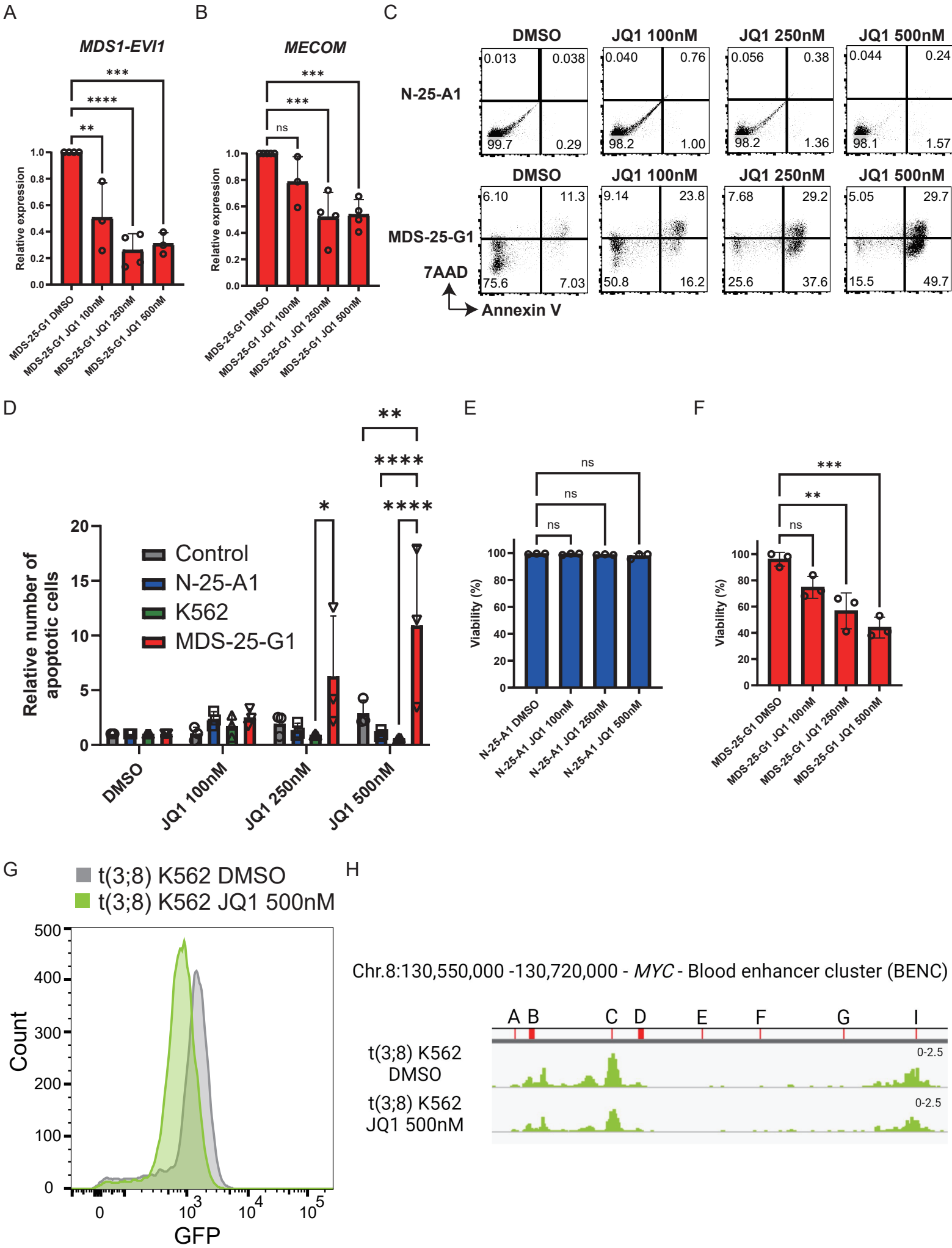
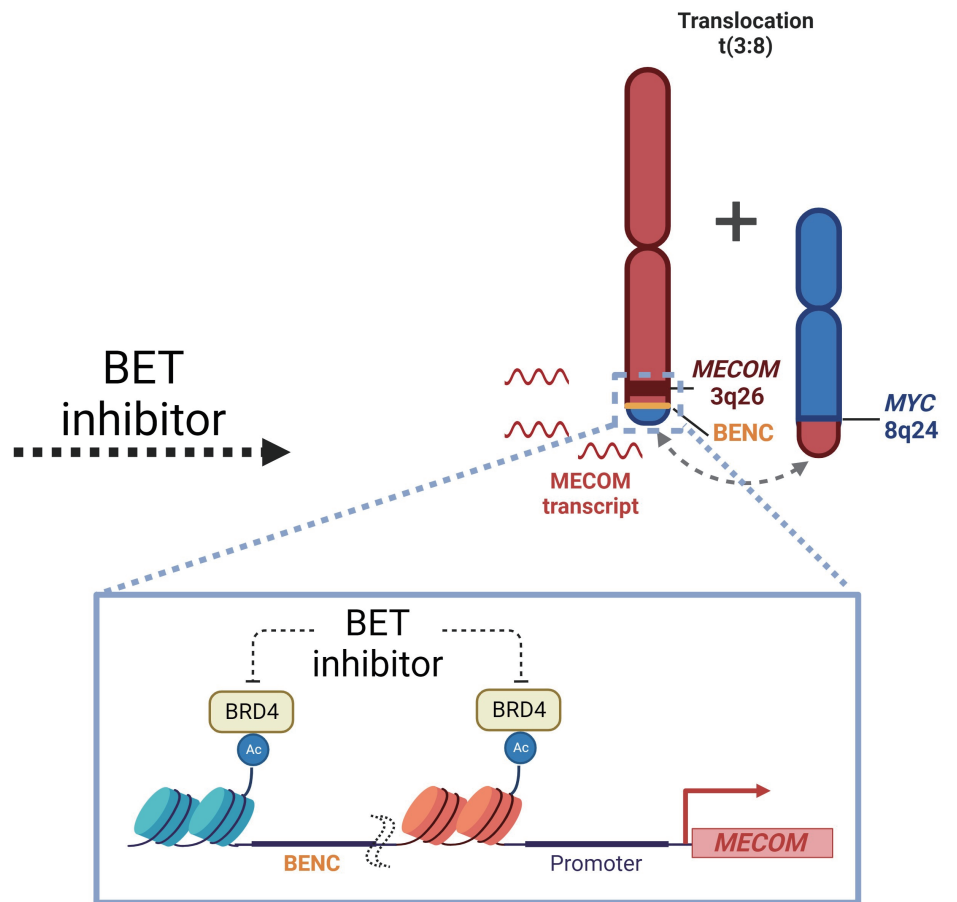


Figure 6





Supplemental methods

Maintenance of iPSC lines

On-feeder iPSC lines were maintained on STO feeder cells in primate ES cell medium (ReproCell) supplemented with 4 ng/mL recombinant bFGF (Wako), as previously described. (1) Feeder-free iPSC lines were cultured in StemFit AK02N medium (Takara Bio) as previously reported. (2)

Haematopoietic differentiation of iPSCs

Haematopoietic differentiation of iPSCs was performed as previously described with minor modifications. (3) Briefly, cells were dissociated into single cells using Accumax (Innovative Cell Technologies) and seeded on a low-binding plate to form embryoid bodies, which were subsequently differentiated in suspension.

Cell lines and culture

K562 and t(3;8) K562 cell lines were cultured in RPMI 1640 + L-glutamine (FUJIFILM Wako Pure Chemical Corporation), 10% foetal bovine serum (Gibco), 50 U/mL penicillin, and 50 µg/mL streptomycin (Gibco). Cells were incubated at 37 °C and 5% CO₂ and passaged every 2 to 3 days.

RNA extraction and quantitative RT-PCR analysis

RNA was isolated using the miRNeasy Micro/Mini Kit (Qiagen), following the manufacturer's protocol. cDNA was synthesised using ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO). Quantitative real-time RT-PCR was performed using the StepOne Real-Time PCR

System (Thermo Fisher Scientific) with TB Green Premix Ex Taq II (Takara Bio). Data were normalised to the expression level of *GAPDH*. The primers used for this analysis are listed in Supplemental Table 4.

Flow cytometric analysis and cell sorting

Flow cytometry analysis and cell sorting were performed using a fluorescence-activated cell sorting (FACS) Aria2 flow cytometer (BD Biosciences). The embryoid bodies were dissociated using 0.25% trypsin, followed by pipetting 10–20 times. Dead cells were removed using 7-AAD (Thermo Fisher Scientific). Data were analysed using FACS Diva v9.0 and FlowJo v10.

Antibodies

Antibodies against CD45-BV421 (BL304032; BioLegend), CD45-BB515 (564585; BD Biosciences), CD34-PE (348057; BD Biosciences), CD235a-APC (551336; BD Biosciences), CD33-BB515 (564588, BD Biosciences), CD11b-BB515 (564517, BD Biosciences), and CD41a-APC (559777, BD Biosciences) were used for FACS analysis and cell sorting. The CD34 MicroBead Kit (Miltenyi Biotec, 130-046-702) and CD45 MicroBead Kit (Miltenyi Biotec, 130-045-801) were used for magnetic-activated cell sorting (MACS).

May Grünwald–Giemsa staining

May Grünwald–Giemsa staining was performed using May Grünwald stain solution (Wako) and Giemsa solution (Wako) following the manufacturer's instructions.

Immunostaining

Immunostaining of TRA-1-60 (BD Biosciences, 560173), SSEA-4 (BD Biosciences, 560308), and Hoechst 33342 (Thermo Fisher Scientific, H3570) was performed using standard immunostaining protocols.

PCR and Sanger sequencing

Genomic DNA was extracted using a DNeasy Blood and Tissue Kit (QIAGEN). For all PCRs, the KOD One PCR Master Mix (TOYOBO) was used following the manufacturer's protocol. The primers used for this analysis are listed in Supplemental Table 3. PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen) and subjected to Sanger sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific).

RNA-seq

RNA was extracted from CD45⁺CD34⁺ HPCs derived from isogenic normal iPSCs and MDS-iPSCs. According to the manufacturer's instructions, we constructed a library using Illumina Stranded Total RNA Prep Ligation with Ribo-Zero Plus. The adapter sequences were subjected to trimming using cutadapt-1.14. (4) The reads mapped to ribosomal RNA sequences were removed using Bowtie2 and Samtools. (5,6) The processed reads were subsequently aligned to the human genome (GRCh38 sourced from the UCSC Genome Browser) through STAR (version 2.5.3a), and data quality was evaluated using RSeQC (version 2.6.4). (7,8) HTSeq (version 0.6.1) was used to count the reads with the GENCODE annotation file (version 27). (9,10) The count was normalised using DESeq2 (version 1.40.2) in R (version 4.3.1). (11) Principal component analysis and the Wald test were performed using the DESeq2 package. We created a volcano plot using the EnhancedVolcano package. (12) Gene set enrichment analysis (13,14) was performed on gene sets from the Molecular Signatures Database (<https://www.broadinstitute.org/msigdb>). (15) Gene sets with an FDR q-value < 0.05 were considered significantly enriched.

Whole-genome sequencing

DNA (50 ng) was subjected to library preparation using the KAPA Hyper Prep Kit, followed by sequencing using NovaSeq 6000 with a target depth of 45× for tumours and 30× for normal

control samples in 150 bp paired-end mode. Mutation calling was performed using the Genomon2 pipeline (v.2.6), as previously described. (16)

Targeted capture sequencing

Genomic DNA was extracted from patient peripheral blood or bone marrow using a QIAamp DNA Mini Kit (Qiagen). Target regions were enriched via liquid-phase hybridisation using a SureSelect custom kit (Agilent Technologies) following the manufacturer's protocol. Subsequently, the purified library was subjected to high-throughput sequencing analysis using an Illumina NovaSeq 6000 system in the 150 bp paired-end mode. Sequencing reads were aligned to the reference human genome (hg19) using the Burrows–Wheeler Aligner, and mutation calls were conducted using genomon-pipeline 2.6.2, as reported previously. (17)

ChIP-seq

ChIP-seq experiments were conducted using feeder-free iPSCs, CD45⁺CD34⁺ HPCs, and K562 cells. A Dead Cell Removal Kit (Miltenyi Biotec, 130-090-101) was used to eliminate dead cells from feeder-free iPSCs and HPCs. Cells were fixed in PBS with 1% formaldehyde (Thermo Fisher Scientific) and gentle mixing for 10 min at room temperature. The reaction was stopped by adding a glycine solution (10×; Cell Signaling Technology) and incubating for 5 min at room temperature, followed by two rounds of cold PBS washing. The cells were processed using the SimpleChIP Plus Sonication Chromatin IP Kit (Cell Signaling Technology) according to the manufacturer's protocol. The chromatin was sonicated with Covaris E220 (Covaris) using a microTUBE AFA Fiber Pre-Slit Crimp-Cap (130 µL), as detailed in Supplemental Table 7. Immunoprecipitation of the cross-linked chromatin was performed using antibodies directed against histone H3 lysine 27 acetylation (H3K27ac) (Cell Signaling Technology) and normal rabbit IgG (Cell Signaling Technology). After purification of the ChIPed DNA, ChIP-seq libraries were constructed using a ThruPLEX DNA-Seq Kit (Takara) according to the manufacturer's

protocol and sequenced using NextSeq 500 or NovaSeq 6000 (Illumina). Sequencing reads were aligned to the hg19 reference genome using bowtie2 (5) following adapter trimming using Cutadapt. (4) Duplicates and reads falling within blacklisted regions (ENCODE) were removed using Picards and bedtools, respectively. For visualisation, bigwig files were created using the bamCoverage tool from deepTools, (18) with the options of normalisation using Bins Per Million mapped reads (BPM), smoothLength 60, and binSize 20. Peak calling was performed using Homer. (19) The coverage was calculated using bedtools. (20) All ChIP-seq data were visualized using IGV, where the y-axis represents normalised BPM. (21) The ChIP-seq experiments were performed in two biological replicates with input controls. We also performed ChIP quantitative PCR (ChIP-qPCR) using H3K27ac, normal rabbit IgG, and input control samples with positive and negative control primers (Supplemental Table 8). The ChIP-qPCR results were analysed using the Percent Input method. The Percent Input was calculated using the following formula: $2\% \times 2^{(Ct \text{ 2\%Input Sample} - Ct \text{ IP Sample})}$ where Ct (threshold cycle) represents the average threshold cycle of the PCR reaction.

Treatment with JQ1

FACS-sorted iPSC-derived CD45⁺CD34⁺ HPCs, MACS-sorted CD34⁺ primary MDS cells, and K562 treated with 100, 250, or 500 nM JQ1 (Sigma-Aldrich, SML1524) in Iscove's Modified Dulbecco's Medium (Gibco) with 20% foetal bovine serum (Gibco) medium. The cytokines used to treat the HPCs from iPSCs were human interleukin-6 (IL-6; 50 ng/mL, R&D Systems), human interleukin-3 (IL-3; 10 ng/mL, R&D Systems), human stem cell factor (SCF; 100 ng/mL, R&D Systems), human Fms-like tyrosine kinase 3 ligand (FLT3L; 100 ng/mL, R&D Systems), and human thrombopoietin (TPO; 50 ng/mL, R&D Systems). Samples were collected 24 h after treatment for qPCR analysis and 72 h after treatment for the cytotoxicity assay. Cytotoxicity was assessed using Annexin V-APC (BD Biosciences, 550474) and 7-AAD (Thermo Fisher Scientific) staining solutions according to the manufacturer's instructions. The stained cells were analysed

using a FACS Aria2 flow cytometer. The trypan blue exclusion assay was conducted using 0.5%-Trypan Blue Stain Solution (Nacalai Tesque) and measured with a TC20 Automated Cell Counter (Bio-Rad). The change in GFP expression in t(3;8) K562 cells was analysed using a FACS Aria2 flow cytometer after 72 h of JQ1 treatment. ChIP sequencing of the t(3;8) K562 cells was performed 1 h after JQ1 treatment.

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Supplemental Tables

Supplemental Table 1. Targeted sequencing data of patient bone marrow cells.

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Supplemental Figure Legends

Supplemental Figure 1.

FISH analysis for *MECOM* and *MYC* used to detect t(3;8).

(A) Schematic representation of the *MECOM* FISH probes used to detect t(3;8). The figure was created using BioRender.com. (B) FISH analysis for *MECOM* with probe (Poseidon™ Repeat Free™ EVI t(3;3), inv(3) triple-colour, break probe). (C) FISH analysis for *c-MYC* with probe (LSI MYC. Dual-colour, break-apart rearrangement probes). FISH, fluorescence *in situ* hybridisation.

Supplemental Figure 2.

Whole-genome sequencing revealed the breakpoints located at 3q26.2 upstream of the *MDS1-EVII* promoter and 8q24 downstream of *MYC*.

(A) Copy number profiles assessed using the sequence-based CNACS algorithm (17) in the patient with MDS carrying t(3;8)(q26.2;q24). Blue dots indicate total copy numbers, whereas red and green dots indicate allele-specific copy numbers of heterozygous single-nucleotide polymorphisms (SNPs). (B) Visualisation of whole genome sequencing data containing breakpoints on chromosomes 3 and 8 using Integrative Genomics Viewer. (C) Sequence data near breakpoints. The upper part shows the control sequence data, and the lower part shows the sequence data of a patient with MDS with t(3;8)(q26.2;q24). One chromosome site showed a CA insertion compared to the control. (D) Schematic representation of 3q26.2 and 8q24. The figure was created using [BioRender.com](https://www.biorender.com).

Supplemental Figure 3.

Characterization of iPSC lines derived from the patient with MDS carrying t(3;8)(q26.2;q24)

(A) Morphology of MDS-iPSCs. Magnification of the objective lens was 10×. Scale bar, 200 μm.

(B–F) Karyotypes of three MDS-iPSC lines (B–D) and two isogenic normal iPSC lines (E, F). (G) Sanger sequencing confirmed that the breakpoints in MDS-iPSCs were the same as those in MDS cells. (H) Expression levels of *NANOG*, *OCT3/4*, and *SOX2* in PBMCs, one control iPSC line derived from a healthy donor, two isogenic normal iPSC lines, and five MDS-iPSC lines relative to those in PBMCs collected from a healthy donor. Each iPSC line was tested in three independent experiments. (I) Representative immunofluorescent staining for TRA-1-60 (top left) and SSEA-4 (bottom left) in MDS-25-G1 iPSC. The nuclei of all cells were stained with Hoechst 33342 (right panel). Magnification of the objective lens: 10×. Scale bar, 200 μm.

Supplemental Figure 4.

Characterization of the immunophenotype of HPCs from N-25-A1 and MDS-25-G1 iPSC lines.

(A) Representative flow cytometry data of CD11b, CD41a, and CD33 cells in the N-25-A1 and MDS-25-G1 iPSC lines on day 17.

Supplemental Figure 5.

Sustained *MECOM* overexpression in t(3;8) MDS cells after reprogramming.

(A, B) Expression levels of *MDS1-EVII* (A) and *MECOM* (B) in a control iPSC line, two isogenic normal iPSC lines, and one MDS-iPSC line (MDS-25-G1). Each iPSC line was tested in three independent experiments. The expression was normalised to that of *GAPDH*. *P* values were calculated using a one-way analysis of variance with Dunnett's correction. ***P* < 0.01. Data are presented as means ± SD.

Supplemental Figure 6.

H3K27ac activity at the *MDS-EVII* and *EVII* in iPSC, iPSC-derived HPCs, primary CD34⁺ MDS cells, and K562.

(A, B) Bar plots showing the accessibility and activity of *MDSI-EVII* (A) and *MECOM* (B) as measured by H3K27ac ChIP-seq (N = 2) in HPCs derived from control iPSC, MDS-25-G1 lines, CD34⁺ HPCs from the patient, and K562. (C, D) Bar charts illustrating the accessibility and activity of *MDSI-EVII* (C) and *MECOM* (D) as assessed by H3K27ac ChIP-seq (N = 2) in control iPSC, MDS-25-G1 lines, and MDS-25-J1 lines. The Y axis indicates the counts per million (CPM) for the *MDSI-EVII* (A, C) and *MECOM* (B, D) locus.

Supplemental Figure 7.

H3K27ac status of *NANOG*, *OCT3/4*, *SOX2*, and *GATA2* enhancer in iPSCs and iPSC-derived HPCs.

(A–C) H3K27ac ChIP-seq data for control iPSCs (light grey), MDS-25-G1 iPSCs (light red), MDS-25-J1 iPSCs (pink), HPCs derived from control iPSCs (grey), and MDS-iPSCs (red, MDS-25-G1) at the *NANOG* (A), *POU5F1* (*OCT3/4*) (B), and *SOX2* loci (C). (D) H3K27ac ChIP-seq data for control iPSCs (light grey), MDS-25-G1 iPSCs (light red), MDS-25-J1 iPSCs (pink), HPCs derived from control iPSCs (grey), MDS-iPSCs (red, MDS-25-G1), CD34⁺ HPCs (orange) from the patient, and K562 (green) at the *GATA2* locus.

Supplemental Figure 8.

Effects of JQ1 in control iPSC-derived HPCs and K562.

(A, B) Expression of *MDSI-EVII* and *MECOM* in HPCs derived from the control iPSC line after JQ1 treatment. Four independent experiments were conducted for each sample. The expression was normalised to that of *GAPDH*. (C) Assessment of apoptosis in HPCs derived from control iPSC (top) and K562 (bottom) after JQ1 treatment. Representative flow cytometry plots of Annexin V and 7-AAD staining are shown with percentages for each gate. (D) Cell viability in HPCs derived from control iPSC after JQ1 treatment assessed by trypan blue exclusion assay. *P* values were calculated using a one-way analysis of variance with Dunnett's correction. ***P* <

0.01; ns, not significant. DMSO, dimethyl sulfoxide. Data are presented as means $\pm$ SD.

Supplemental Figure 9.

Effects of JQ1 in MDS-iPSC-derived HPCs, CD34⁺ primary MDS, and t(3;8) K562 cells.

(A, B) Assessment of apoptosis in HPCs derived from MDS-25-A3, MDS-25-J4 (A), and CD34⁺ primary MDS cells from the patient (B) after JQ1 treatment. Representative flow cytometry plots of Annexin V and 7-AAD staining are shown with percentages for each gate. (C) Bar plots depicting the accessibility and activity of the *MYC* super-enhancer module C as measured by H3K27ac ChIP-seq (N = 2) in t(3;8) K562 cells after DMSO and JQ1 treatment. The Y axis indicates the counts per million (CPM) for the C module.

Supplemental Table 1 Targeted sequencing data of patient bone marrow cells

Gene	Genome position	Ref	Alt	VAF
<i>ETNK1</i>	chr12: 22811995	A	G	0.321
<i>PTPN11</i>	chr12: 112888166	A	T	0.08
<i>PTPN11</i>	chr12: 112888198	G	A	0.038
<i>PTPN11</i>	chr12: 112888210	G	A	0.123
<i>EZH2</i>	chr7: 148504799	C	G	0.756

Supplemental Table 2

Human iPSC lines used in differentiation experiments

ID	Name of iPSC lines	Source		Karyotype
		Origin	T / Non T cell	
CiRA01184A	N-25-A1	PBMC	T	46, XY
CiRA01184B	N-25-B1	PBMC	T	46, XY
CiRA01184C	MDS-25-G1	PBMC	Non T cell	46, XY, t(3;8)(q26.2;q24), del(9)(q?)
CiRA01184D	MDS-25-J1	PBMC	Non T cell	46, XY, t(3;8)(q26.2;q24), del(9)(q?)
CiRA01184E	MDS-25-A3	PBMC	Non T cell	46, XY, t(3;8)(q26.2;q24), del(9)(q?)
CiRA01184F	MDS-25-B8	PBMC	Non T cell	46, XY, t(3;8)(q26.2;q24), del(9)(q?)
CiRA01184G	MDS-25-J4	PBMC	Non T cell	46, XY, t(3;8)(q26.2;q24), del(9)(q?)

Supplemental Table 3

List of primers used for PCR

Target		Sequence (5'-3')
Breakpoint_1	Forward	TGCTTTCTGCCGTGGTCAC
	Reverse	GCCTGGTTTCTTGTAGTCTTAATGAACA
Breakpoint_2	Forward	CCACTGCAGAAACCCTAGACTAACA
	Reverse	GAAGTTGGACATATCCTAGCCCTGTG
<i>ETNK1</i>	Forward	CATCAAAGTAAGGGGTTTCTGG
	Reverse	TCTGCCCCTTGCTGTTGTAG
<i>PTPN11</i>	Forward	TGTAACCTTTTATTTGTCCCCTTG
	Reverse	AGCAGCAGACTTTGTGGTCA
<i>EZH2</i>	Forward	ACACTTTGCAGCTGGTGAGA
	Reverse	GTCAGGCTCTGGGAATGCTT

Supplemental Table 4

List of primers used for RT-PCR

Genes		Sequence (5'-3')
<i>NANOG</i>	Forward	TGGCTGCCGTCTCTGGCTATAGAT
	Reverse	AAGCCTCCCAATCCCAAACAATAC
<i>OCT3/4</i>	Forward	CCCCAGGGCCCCATTTTGGTACC
	Reverse	ACCTCAGTTTGAATGCATGGGAGAGC
<i>SOX2</i>	Forward	TTCACATGTCCCAGCACTACCAGA
	Reverse	TCACATGTGTGAGAGGGGCAGTGTGC
<i>GAPDH</i>	Forward	TGCACCACCAACTGCTTAGC
	Reverse	GGCATGGACTGTGGTCATGAG
<i>MDS1-EVII</i>	Forward	GAAAGACCCCAGTTATGGATGG
	Reverse	GTACTTGAGCCAGCTTCCAACA
<i>MECOM</i>	Forward	AAACTCGAAAGCGAGAATGATCT
	Reverse	TGGTGGCGAATTAAATTGGACTT
<i>MYC</i>	Forward	ATACATCCTGTCCGTCCAAGCAGA
	Reverse	ACGCACAAGAGTTCCGTAGCTG

Supplemental Table 5 **Sanger sequencing results of multiple iPSC lines**

iPSC	<i>ETNK1</i> p.N155S	<i>PTPN11</i> p.D61V	<i>PTPN11</i> p.A72T	<i>PTPN11</i> p.E76K	<i>EZH2</i> c.2181-1G>C	Karyotype
N-25-A1	Negative	Negative	Negative	Negative	Negative	46, XY
N-25-B1	Negative	Negative	Negative	Negative	Negative	46, XY
MDS-25-A3	Positive	Negative	Negative	Negative	Positive	46, XY, t(3;8)(q26.2;q24), del(9)(q?)
MDS-25-B8	Positive	Negative	Negative	Negative	Positive	46, XY, t(3;8)(q26.2;q24), del(9)(q?)
MDS-25-G1	Positive	Negative	Negative	Negative	Positive	46, XY, t(3;8)(q26.2;q24), del(9)(q?)
MDS-25-J1	Positive	Negative	Negative	Negative	Positive	46, XY, t(3;8)(q26.2;q24), del(9)(q?)
MDS-25-J4	Positive	Negative	Positive	Negative	Positive	46, XY, t(3;8)(q26.2;q24), del(9)(q?)

Supplemental Table 6

Gene set enrichment analysis results (FDR < 0.05)

NAME	SIZE	NES	NOM p-val	FDR q-val	FWER p-val
WINZEN DEGRADED VIA KHSRP	87	2.07923	0	0.004719644	0.005
RICKMAN_HEAD_AND_NECK_CANCER_B	34	2.0529277	0	0.00473104	0.01
SANA_TNF_SIGNALING_UP	73	2.051454	0	0.003154027	0.01
HOLLERN_EMT_BREAST_TUMOR_DN	99	2.04E+00	0	0.002603064	0.011
SEAVEY_EPITHELIOID_HEMANGIOENDOTHELIOMA	75	2.04E+00	0	0.002082451	0.011
WP_OVERVIEW_OF_PROINFLAMMATORY_AND_PROFIBROTIC_MEDIATORS	62	2.02E+00	0	0.003160447	0.02
BLANCO_MELO_RESPIRATORY_SYNCYTIAL_VIRUS_INFECTION_A594_CELLS_UP	250	2.0143187	0	0.003114498	0.023
WUNDER_INFLAMMATORY_RESPONSE_AND_CHOLESTEROL_UP	50	2.0045197	0	0.003676466	0.031
KEGG_ALLOGRAFT_REJECTION	28	2.0007658	0	0.003372364	0.032
ONDER_CDH1_TARGETS_2_DN	376	1.9949726	0	0.003982032	0.041
KEGG_GRAFT_VERSUS_HOST_DISEASE	28	1.9885048	0	0.003961891	0.045
RICKMAN_HEAD_AND_NECK_CANCER_C	51	1.9879639	0	0.003709987	0.046
ZHAN_MULTIPLE_MYELOMA_LB_UP	45	1.9855521	0	0.003643625	0.049
LINDSTEDT_DENDRITIC_CELL_MATURATION_B	47	1.9768256	0	0.003993251	0.056
BLANCO_MELO_HUMAN_PARAINFLUENZA_VIRUS_3_INFECTION_A594_CELLS_UP	180	1.9750341	0	0.003789622	0.057
SANA_RESPONSE_TO_IFNG_UP	65	1.9743809	0	0.003730269	0.06
BENPORATH_PRC2_TARGETS	364	1.9722495	0	0.003789254	0.065
CROMER_TUMORIGENESIS_DN	31	1.9711263	0	0.00357874	0.065
VALK_AML_CLUSTER_2	27	1.9694333	0	0.003440515	0.066
RICKMAN_HEAD_AND_NECK_CANCER_A	74	1.9666762	0	0.003363101	0.068
KERLEY_RESPONSE_TO_CISPLATIN_UP	42	1.958366	0	0.004060775	0.087
GHANDHI_BYSTANDER_IRRADIATION_UP	70	1.9570278	0	0.003919712	0.088
NOUSHMEHR_GBM_SILENCED_BY_METHYLATION	40	1.9565164	0	0.00374929	0.088
SEKI_INFLAMMATORY_RESPONSE_LPS_UP	65	1.9553875	0	0.003632842	0.089
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	56	1.9543788	0	0.003563106	0.091
MISIAK_ANAPLASTIC_THYROID_CARCINOMA_DN	19	1.9542354	0	0.003426063	0.091
SATO_SILENCED_BY_DEACETYLTATION_IN_PANCREATIC_CANCER	36	1.9445565	0	0.003861044	0.107
BLANCO_MELO_COVID19_SARS_COV_2_POS_PATIENT_LUNG_TISSUE_UP	134	1.9389113	0	0.00409521	0.116
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_10D_UP	169	1.9383385	0	0.003986317	0.117
REACTOME_ANTIGEN_PRESENTATION_FOLDING_ASSEMBLY_AND_PEPTIDE_LOADING_OF_CLASS_I_MHC	29	1.9383035	0	0.00385344	0.117
VALK_AML_CLUSTER_6	32	1.9376972	0	0.003790071	0.119
MIKKELSEN_NPC_HCP_WITH_H3K27ME3	188	1.9357132	0	0.003819898	0.124
KEGG_AUTOIMMUNE_THYROID_DISEASE	28	1.932658	0	0.004019742	0.133
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_16D_DN	112	1.928436	0	0.004290665	0.145
MIKKELSEN_NPC_HCP_WITH_H3K4ME3_AND_H3K27ME3	155	1.9282414	0	0.004168074	0.145
KEGG_TYPE_1_DIABETES_MELLITUS	34	1.9171942	0	0.004945522	0.173
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_3D_UP	164	1.917145	0	0.004811859	0.173
MEISSNER_NPC_HCP_WITH_H3K4ME2_AND_H3K27ME3	206	1.9160961	0	0.004809979	0.178
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_8D_UP	142	1.9125277	0	0.00497855	0.189

SCHLESINGER_METHYLATED_DE_NOVO_IN_CANCER	69	1.9096096	0	0.00506713	0.198
GAVIN_FOXP3_TARGETS_CLUSTER_P2	72	1.9094685	0	0.004943542	0.198
BEIER_GLIOMA_STEM_CELL_DN	66	1.9082853	0	0.004960982	0.204
MEISSNER_NPC_HCP_WITH_H3_UNMETHYLATED	404	1.9027056	0	0.005571759	0.231
ABE_INNER_EAR	34	1.8968718	0.00126582	0.006325041	0.259
ANDERSEN_CHOLANGIOCARCINOMA_CLASS2	126	1.893977	0	0.006500901	0.268
BRACHAT_RESPONSE_TO_CISPLATIN	21	1.8921057	0	0.006647369	0.274
FOROUTAN_INTEGRATED_TGFB_EMT_DN	63	1.8903779	0	0.006788659	0.285
COWLING_MYCN_TARGETS	33	1.8891361	0	0.00676492	0.289
SEITZ_NEOPLASTIC_TRANSFORMATION_BY_8P_DELETION_UP	60	1.8887823	0	0.00664634	0.289
MIKKELSEN_MEF_HCP_WITH_H3K27ME3	333	1.8882978	0	0.00658941	0.291
SMID_BREAST_CANCER_RELAPSE_IN_BONE_UP	65	1.888053	0	0.006478703	0.292
REACTOME_FORMATION_OF_THE_CORNIFIED_ENVELOPE	34	1.8855442	0	0.006608099	0.304
BIOCARTA_INFLAM_PATHWAY	18	1.8830593	0	0.006785707	0.316
FOROUTAN_TGFB_EMT_DN	95	1.8809049	0	0.006975044	0.33
BLANCO_MELO_BETA_INTERFERON_TREATED_BRONCHIAL_EPITHELIAL_CELLS_UP	319	1.8807613	0	0.00688275	0.331
WP_PROTEASOME_DEGRADATION	58	1.8795135	0	0.006878309	0.336
REACTOME_TNFS_BIND_THEIR_PHYSIOLOGICAL_RECEPTORS	22	1.8757654	0	0.007221696	0.358
LANDEMAINE_LUNG_METASTASIS	17	1.8752332	0	0.007162246	0.359
NOURUZI_NEPC_ASCL1_TARGETS	95	1.8751017	0	0.007088746	0.361
XIE_ST_HSC_SIPR3_OE_DN	21	1.87355	0	0.007206869	0.372
SABATES_COLORECTAL_ADENOMA_DN	184	1.871941	0	0.007213067	0.377
WP_ALLOGRAFT_REJECTION	68	1.8712268	0	0.007234259	0.381
BLANCO_MELO_COVID19_SARS_COV_2_INFECTION_CALU3_CELLS_UP	276	1.8698033	0	0.007269464	0.386
HAHTOLA_MYCOSIS_FUNGOIDES_CD4_UP	58	1.8648239	0	0.007717717	0.406
WP_OVARIAN_INFERTILITY	32	1.8643731	0	0.007730296	0.41
BLANCO_MELO_COVID19_BRONCHIAL_EPITHELIAL_CELLS_SARS_COV_2_INFECTION_UP	112	1.863234	0	0.007814504	0.418
REACTOME KERATINIZATION	36	1.8622493	0	0.007839475	0.425
BOQUEST_STEM_CELL_DN	201	1.8617457	0	0.00778013	0.428
REACTOME_ENDOGENOUS_STEROLS	20	1.8616774	0.00137741	0.007667375	0.428
BOQUEST_STEM_CELL_CULTURED_VS_FRESH_DN	23	1.8610392	0.00136799	0.007611856	0.432
PEDERSEN_METASTASIS_BY_ERBB2_ISOFORM_3	16	1.8600817	0	0.00767835	0.438
MIKKELSEN_IPS_WITH_HCP_H3K27ME3	53	1.8563071	0	0.008202805	0.463
CHARAFE_BREAST_CANCER_BASAL_VS_MESENCHYMAL_UP	93	1.8468698	0	0.009463707	0.511
BOSCO_EPITHELIAL_DIFFERENTIATION_MODULE	35	1.8461603	0.0012837	0.009437914	0.516
MIKKELSEN_MCV6_LCP_WITH_H3K4ME3	129	1.8458024	0	0.009362761	0.518
ZHAN_MULTIPLE_MYELOMA_PR_DN	47	1.8447919	0	0.009339144	0.522
FRASOR_RESPONSE_TO ESTRADIOL_DN	66	1.8427185	0	0.009500643	0.539
CERVERA_SDHB_TARGETS_I_UP	99	1.8413377	0	0.009609415	0.547
HELLEBREKERS_SILENCED_DURING_TUMOR_ANGIOGENESIS	72	1.8363042	0	0.010337834	0.578
MOSERLE_IFNA_RESPONSE	29	1.8354775	0.00130378	0.010397686	0.585
MEISSNER_BRAIN_HCP_WITH_H3K27ME3	143	1.8347045	0	0.01044529	0.592
MA_RAT_AGING_UP	94	1.8337802	0	0.010514153	0.596

PARK_TRETINOIN_RESPONSE_AND_PML_RARA_FUSION	30	1.8333244	0	0.010513025	0.598
FOSTER_KDM1A_TARGETS_UP	177	1.83307	0	0.010444075	0.6
REACTOME_METABOLIC_DISORDERS_OF_BIOLOGICAL_OXIDATION_ENZYMES	24	1.8315417	0.00135135	0.010543798	0.607
PLASARI_TGFB1_SIGNALING_VIA_NFIC_10HR_UP	46	1.8290999	0	0.010794699	0.617
JEON_SMAD6_TARGETS_UP	22	1.8289222	0	0.010692404	0.617
BREDEMEYER_RAG_SIGNALING_VIA_ATM_NOT_VIA_NFKB_DN	39	1.8275182	0	0.010861349	0.626
YAO_HOXA10_TARGETS_VIA_PROGESTERONE_DN	18	1.8249156	0	0.011281559	0.65
BROWNE_HCMV_INFECTION_2HR_UP	31	1.8218155	0.00132979	0.011893787	0.672
KIM_PTEN_TARGETS_UP	16	1.8216405	0	0.011794197	0.672
WIERENGA_STAT5A_TARGETS_UP	191	1.8213328	0	0.01171721	0.674
MIKKELSEN_ES_ICP_WITH_H3K4ME3_AND_H3K27ME3	91	1.8193688	0	0.011906866	0.685
NABA_SECRETED_FACTORS	185	1.8192137	0	0.011810444	0.685
PID_IL8_CXCRI_PATHWAY	28	1.8189083	0	0.011775865	0.688
MARKEY_RB1_ACUTE_LOF_DN	208	1.8186163	0	0.011672836	0.69
MARTENS_TRETINOIN_RESPONSE_UP	470	1.8184674	0	0.011601375	0.691
CHUANG_OXIDATIVE_STRESS_RESPONSE_UP	25	1.8163519	0.00271739	0.012024192	0.707
AIGNER_ZEB1_TARGETS	29	1.8157862	0	0.011979366	0.71
VERHAAK_AML_WITH_NPM1_MUTATED_UP	170	1.81513	0	0.011992213	0.718
SENGUPTA_NASOPHARYNGEAL_CARCINOMA_WITH_LMPI_DN	109	1.8133448	0	0.012200569	0.728
HAN_JNK_SINGALING_UP	32	1.8133298	0	0.012090293	0.728
GAURNIER_PSMID4_TARGETS	58	1.8123802	0	0.012110756	0.732
HARRIS_BRAIN_CANCER_PROGENITORS	28	1.8115319	0.001287	0.012112443	0.736
BOQUEST_STEM_CELL_UP	206	1.8106656	0	0.012114235	0.738
MEISSNER_NPC_HCP_WITH_H3K27ME3	37	1.8088114	0	0.012428099	0.743
KIM_LRRC3B_TARGETS	28	1.8086308	0	0.012338421	0.745
REACTOME_INTERFERON_GAMMA_SIGNALING	82	1.8072487	0	0.012434761	0.751
ROY_WOUND_BLOOD_VESSEL_DN	16	1.8060678	0.00282486	0.012564085	0.759
SCHOEN_NFKB_SIGNALING	31	1.803855	0	0.012880157	0.767
RASHI_RESPONSE_TO_IONIZING_RADIATION_2	117	1.8037766	0	0.01276412	0.767
KEGG_CELL_ADHESION_MOLECULES_CAMS	110	1.8023902	0	0.012971643	0.782
SATO_SILENCED_BY_METHYLATION_IN_PANCREATIC_CANCER_1	330	1.8017267	0	0.013032599	0.787
BASSO_CD40_SIGNALING_UP	99	1.8003843	0	0.013167463	0.791
ICHIBA_GRAFT_VERSUS_HOST_DISEASE_35D_DN	42	1.7997581	0	0.013143884	0.793
PHONG_TNF_TARGETS_UP	61	1.7996306	0	0.013030575	0.793
SIMBULAN_PARP1_TARGETS_UP	26	1.7986363	0	0.013226982	0.801
CERVERA_SDHB_TARGETS_2	99	1.7966883	0	0.013491781	0.805
MADAN_DPPA4_TARGETS	36	1.795627	0	0.013569457	0.81
REACTOME_PPTIDE_LIGAND_BINDING_RECEPTORS	111	1.7926984	0	0.014119231	0.828
COLDREN_GEFITINIB_RESISTANCE_DN	184	1.7923988	0	0.01408085	0.828
RICKMAN_HEAD_AND_NECK_CANCER_E	40	1.7908255	0.00123457	0.014338057	0.833
SENESE_HDAC2_TARGETS_DN	110	1.7898791	0	0.014498256	0.838
ZHENG_IL22_SIGNALING_UP	49	1.788244	0	0.014793367	0.85
CHARAFE_BREAST_CANCER_LUMINAL_VS_MESENCHYMAL_UP	387	1.7872702	0	0.014826588	0.853

SMID_BREAST_CANCER_LUMINAL_A_UP	62	1.7870263	0.00114943	0.014739108	0.855
CHIANG_LIVER_CANCER_SUBCLASS_CTNNB1_DN	145	1.7869729	0	0.014630483	0.855
ZHAN_MULTIPLE_MYELOMA_CD1_DN	45	1.7865765	0	0.014656467	0.857
GAVIN_FOXP3_TARGETS_CLUSTER_P4	86	1.7849263	0	0.015056522	0.868
ZHANG_RESPONSE_TO_IKK_INHIBITOR_AND_TNF_UP	199	1.7832086	0	0.015377907	0.875
SAUL_SEN_MAYO	102	1.7824824	0	0.015441264	0.878
ACOSTA_PROLIFERATION_INDEPENDENT_MYC_TARGETS_DN	110	1.7816715	0	0.015582647	0.883
CONCANNON_APOPTOSIS_BY_EPOXOMICIN_UP	224	1.7803224	0	0.01587823	0.89
KIM_GLI2_TARGETS_UP	77	1.7803149	0	0.015766751	0.89
WP_IMMUNE_INFILTRATION_IN_PANCREATIC_CANCER	29	1.7795143	0	0.015783114	0.891
HECKER_IPNB1_TARGETS	82	1.7791711	0	0.015757674	0.892
FUJII_YBX1_TARGETS_UP	36	1.7779074	0.00128866	0.015981944	0.897
MISSIAGLIA_REGULATED_BY_METHYLATION_UP	112	1.7774765	0	0.015934883	0.899
BROWNE_INTERFERON_RESPONSE_GENES	62	1.7773377	0	0.015840705	0.899
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	27	1.777248	0.0013089	0.015761308	0.9
PLASARI_TGFB1_TARGETS_10HR_DN	229	1.7767303	0	0.015777143	0.902
MEISSNER_NPC_HCP_WITH_H3K4ME2	395	1.7756293	0	0.016006177	0.906
VECCHI_GASTRIC_CANCER_EARLY_DN	268	1.7747085	0	0.01613917	0.908
PID_ERB_GENOMIC_PATHWAY	15	1.7746575	0	0.016046856	0.908
BOSCO_INTERFERON_INDUCED_ANTIVIRAL_MODULE	71	1.7728887	0	0.016353738	0.913
BLANCO_MELO_COVID19_SARS_COV_2_INFECTION_A594_ACE2_EXPRESSION_CELLS_RUXOLITINIB_DN	59	1.7719152	0	0.016494567	0.914
WP_OLIGODENDROCYTE_SPECIFICATION_AND_DIFFERENTIATION_LEADING_TO_MYELIN_COMPONENTS_FOR_CNS	23	1.7714671	0	0.016498128	0.914
NABA_MATRISOME_ASSOCIATED	443	1.7703749	0	0.016687797	0.919
JAEGER_METASTASIS_DN	172	1.7697593	0	0.016797913	0.921
BILD_HRAS_ONCOGENIC_SIGNATURE	225	1.7685747	0	0.016982144	0.925
SERVITJA_ISLET_HNF1A_TARGETS_DN	71	1.7669531	0	0.017351862	0.932
NELSON_RESPONSE_TO_ANDROGEN_DN	16	1.7661037	0.00292398	0.017499324	0.935
NAKAYAMA_SOFT_TISSUE_TUMORS_PCAL_UP	67	1.7640383	0	0.017916435	0.942
DUNNE_TARGETS_OF_AML1_MTG8_FUSION_UP	52	1.763751	0	0.017861558	0.944
WP_CYTOKINES_AND_INFLAMMATORY_RESPONSE	17	1.7636082	0.00138696	0.017776752	0.946
KORKOLA_EMBRYONIC_CARCINOMA_VS_SEMINOMA_DN	20	1.7610223	0.00277778	0.018348241	0.951
SATO_SILENCED_BY_METHYLATION_IN_PANCREATIC_CANCER_2	37	1.7595794	0	0.018641438	0.952
KEGG_VIRAL_MYOCARDITIS	59	1.7587157	0	0.018853158	0.956
SERVITJA_LIVER_HNF1A_TARGETS_DN	101	1.7586416	0	0.018740565	0.956
BOSCO_ALLERGEN_INDUCED_TH2_ASSOCIATED_MODULE	136	1.7550194	0	0.019729992	0.966
REACTOME_GROWTH_HORMONE_RECEPTOR_SIGNALING	20	1.7540497	0.00139276	0.01990119	0.966
WANG_THOC1_TARGETS_DN	17	1.7537235	0.00142045	0.019877741	0.967
WP_MAMMARY_GLAND_DEVELOPMENT_PATHWAY_PREGNANCY_AND_LACTATION_STAGE_3_OF_4	26	1.7514113	0.00133333	0.020527368	0.971
GEISS_RESPONSE_TO_DSRNA_UP	38	1.7503902	0.00258732	0.020684617	0.972
ROSS_AML_WITH_MLL_FUSIONS	74	1.7495865	0	0.020817379	0.972
ZHAN_MULTIPLE_MYELOMA_MS_UP	46	1.7485596	0	0.021017075	0.973
MIKKELSEN_IPS_ICP_WITH_H3K27ME3	27	1.7469517	0.0026738	0.02140696	0.974
VALK_AML_CLUSTER_7	27	1.7468697	0.00132275	0.021307696	0.974

WP_FGF23_SIGNALING_IN_HYPOPHOSPHATEMIC_RICKETS_AND_RELATED_DISORDERS	CORRE_MULTIPLE_MYELOMA_UP	58	1.7468504	0.00116959	0.021187112	0.974
		16	1.7462641	0	0.02122379	0.975
YAMASHITA_LIVER_CANCER_STEM_CELL_DN		47	1.7462008	0.0012987	0.021132922	0.975
	LIU_VAV3_PROSTATE_CARCINOGENESIS_UP	73	1.7458649	0	0.021098051	0.975
LIM_MAMMARY_STEM_CELL_DN		377	1.7407655	0	0.022616668	0.983
	WANG_MLL_TARGETS	237	1.7406175	0	0.022546554	0.983
BAKKER_FOXO3_TARGETS_UP		52	1.7399551	0.00118765	0.022607256	0.984
	ICHIBA_GRAFT_VERSUS_HOST_DISEASE_D7_UP	101	1.7391825	0	0.02276395	0.985
YAN_ESCAPE_FROM_ANOIKIS		21	1.739135	0.00541272	0.022656687	0.985
	HOWLIN_CITED1_TARGETS_2_UP	15	1.7386831	0.001443	0.022667669	0.986
REACTOME_SUMOYLATION_OF_INTRACELLULAR_RECEPTORS		28	1.7380985	0.00255428	0.02277344	0.986
	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	179	1.7370008	0	0.022920797	0.987
AMIT_EGF_RESPONSE_240_MCF10A		19	1.7358389	0.00141243	0.023175897	0.987
	SABATES_COLORECTAL_ADENOMA_UP	85	1.733128	0	0.023833768	0.989
ONDER_CDHI_TARGETS_3_DN		31	1.7321304	0.00380711	0.023992088	0.989
	NIKOLSKY_BREAST_CANCER_7Q21_Q22_AMPLICON	61	1.7320497	0	0.023871977	0.989
OHM_METHYLATED_IN_ADULT_CANCERS		23	1.7310151	0.00277393	0.024019096	0.989
	MEBARKI_HCC_PROGENITOR_FZD8CRD_DN	314	1.7305857	0	0.024083326	0.989
REACTOME_NUCLEAR_RECEPTOR_TRANSCRIPTION_PATHWAY		45	1.7242801	0.00243605	0.02627735	0.993
	PELLICCIOтта_HDAC_IN_ANTIGEN_PRESENTATION_DN	49	1.7224665	0.00121951	0.02681196	0.994
GHANDHI_DIRECT_IRRADIATION_UP		86	1.7216215	0	0.026960773	0.994
	ADDYA_ERYTHROID_DIFFERENTIATION_BY_HEMIN	72	1.7214376	0	0.026878452	0.994
DELACROIX_RAR_TARGETS_UP		36	1.7204964	0.00255102	0.027064638	0.994
	KIM_RESPONSE_TO_TSA_AND_DECITABINE_UP	95	1.7201644	0	0.027022095	0.994
PLASARI_TGFB1_TARGETS_10HR_UP		169	1.7185004	0	0.027460353	0.994
	LJ_PROSTATE_CANCER_EPIGENETIC	28	1.7174478	0.00263158	0.027786968	0.996
KEGG_ASTHMA		23	1.7171448	0.00278164	0.027741423	0.996
	WIERENGA_STATS_A_TARGETS_GROUP2	51	1.7156388	0.00123001	0.028184738	0.996
NAKAJIMA_EOSINOPHIL		24	1.7151251	0.00415512	0.028252741	0.996
	AMUNDSON_DNA_DAMAGE_RESPONSE_TP53	16	1.7146497	0.00286944	0.028372744	0.996
XIE_ST_HSC_SIPR3_OE_UP		165	1.7144297	0	0.02833001	0.996
	GRAHAM_CML_DIVIDING_VS_NORMAL_QUIESCENT_DN	83	1.7141099	0	0.028335117	0.996
REACTOME_CLASS_A_1_RHODOPSIN_LIKE_RECEPTORS		185	1.7131965	0	0.028566202	0.996
	LEE_NEURAL_CREST_STEM_CELL_DN	99	1.7130272	0	0.028476283	0.996
PEDERSEN_TARGETS_OF_611CTF_ISOFORM_OF_ERBB2		67	1.7130022	0	0.028354598	0.996
	SCHUETZ_BREAST_CANCER_DUCTAL_INVASIVE_DN	71	1.7113327	0.00114025	0.028962612	0.997
FOROUTAN_PRODRAK_TGFB_EMT_DN		78	1.7110821	0	0.029001145	0.997
	BAUS_TTF2_TARGETS_UP	20	1.7104912	0.00272851	0.029048884	0.998
REACTOME_TNFR2_NON_CANONICAL_NF_KB_PATHWAY		92	1.7102996	0	0.028967833	0.998
	CHUNG_BLISSER_CYTOTOXICITY_DN	47	1.7098027	0.00120627	0.029033631	0.999
DALESSIO_TSA_RESPONSE		18	1.7096145	0.00571429	0.028985107	0.999
	ODONNELL_TARGETS_OF_MYC_AND_TFRC_UP	73	1.7095848	0.00236967	0.028851612	0.999
REACTOME_COLLAGEN_DEGRADATION		50	1.7087663	0	0.02903777	0.999

ALTEMEIER_RESPONSE_TO_LPS_WITH_MECHANICAL_VENTILATION	117	1.7080883	0	0.029146234	0.999
SENGUPTA_NASOPHARYNGEAL_CARINOMA_DN	242	1.7071528	0	0.029422587	1
MIKKELSEN_MEF_ICP_WITH_H3K27ME3	116	1.7058419	0	0.0298022	1
WP_EPO_RECEPTOR_SIGNALING	25	1.7044935	0.00527009	0.030187838	1
WU_SILENCED_BY_METHYLATION_IN_BLADDER_CANCER	42	1.7042793	0.00126422	0.030148733	1
IZADPANAH_STEM_CELL_ADIPOSE_VS_BONE_DN	92	1.7025181	0	0.03073857	1
RHEIN_ALL_GLUCCORTICOID_THERAPY_UP	71	1.7024783	0.00116009	0.03061494	1
MIKKELSEN_MEF_HCP_WITH_H3_UNMETHYLATED	131	1.7022887	0	0.030570518	1
REACTOME_SENESCENCE_ASSOCIATED_SECRETORY_PHENOTYPE_SASP	52	1.7016952	0.00235849	0.030728394	1
TAVOR_CEBPA_TARGETS_UP	50	1.700443	0.00118483	0.031034455	1
SMIRNOV_RESPONSE_TO_IR_2HR_UP	50	1.6996197	0	0.03129538	1
CHEMNITZ_RESPONSE_TO_PROSTAGLANDIN_E2_DN	342	1.6985686	0	0.031537194	1
HORIUCHI_WTAP_TARGETS_UP	286	1.6978207	0	0.03173074	1
JOHNSTONE_PARVB_TARGETS_2_UP	139	1.6959944	0	0.032329503	1
CHIANG_LIVER_CANCER_SUBCLASS_UNANNOTATED_UP	73	1.6959	0.00230415	0.032207314	1
SCHRAETS_MLL_TARGETS_UP	28	1.6957921	0.00399467	0.032119602	1
HOQUE_METHYLATED_IN_CANCER	47	1.6957105	0.00120773	0.032020308	1
WP_CONGENITAL_GENERALIZED_LIPODYSTROPHY	17	1.6924496	0.00412655	0.03342246	1
BIOCARTA_NUCLEARRS_PATHWAY	22	1.6922342	0.00132979	0.03334702	1
NABA_CORE_MATRISOME	192	1.6918464	0	0.033362396	1
RIGGI_EWING_SARCOMA_PROGENITOR_UP	355	1.6910281	0	0.033553053	1
FLORIO_NEOCORTEX_BASAL_RADIAL_GLIA_UP	235	1.6905613	0	0.033592068	1
KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION	31	1.6902864	0.00520156	0.033589996	1
NEMETH_INFLAMMATORY_RESPONSE_LPS_UP	82	1.6902361	0	0.033467114	1
LU_IL4_SIGNALING	87	1.68909	0	0.03386232	1
TONKS_TARGETS_OF_RUNX1_RUNXIT1_FUSION_HSC_DN	188	1.6882917	0	0.034030877	1
REACTOME_CYTOCHROME_P450_ARRANGED_BY_SUBSTRATE_TYPE	33	1.6881839	0.00503145	0.033939537	1
KIM_MYCN_AMPLIFICATION_TARGETS_DN	100	1.6864309	0	0.03467687	1
REACTOME_PROTEIN_PROTEIN_INTERACTIONS_AT_SYNAPSES	71	1.6851805	0.00114286	0.03505656	1
REACTOME_COLLAGEN_BIOSYNTHESIS_AND_MODIFYING_ENZYMES	59	1.6848623	0.00121507	0.035040565	1
J1_METASTASIS_REPRESSED_BY_STK11	22	1.684316	0.00693481	0.03515022	1
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS	28	1.6840096	0.0040107	0.035134077	1
PAL_PRMT5_TARGETS_DN	20	1.6807674	0.01578947	0.03639434	1
TSENG_ADIPOGENIC_POTENTIAL_UP	26	1.6804953	0.00406504	0.036357675	1
WIEDERSCHAIN_TARGETS_OF_BMI1_AND_PCGF2	50	1.6795388	0.00241546	0.03666676	1
WAMUNYOKOLI_OVARIAN_CANCER_LMP_UP	246	1.6793629	0	0.036599502	1
KEGG_TYPE_II_DIABETES_MELLITUS	39	1.678691	0.00379267	0.036780246	1
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS_VIA_7ALPHA_HYDROXYCHOLESTEROL	18	1.6766497	0.00556328	0.037719548	1
DER_IFN_ALPHA_RESPONSE_UP	72	1.6764716	0	0.037659664	1
HUPER_BREAST_BASAL_VS_LUMINAL_UP	33	1.6755897	0.0089172	0.037928328	1
LIANG_SILENCED_BY_METHYLATION_2	44	1.6755172	0.00124069	0.03778535	1
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_16D_UP	159	1.6748055	0	0.03801371	1
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	141	1.6741847	0	0.03815133	1

WP_PHOSPHODIESTERASES_IN_NEURONAL_FUNCTION	41	1.6728753	0.00126263	0.038651083	1
SANSOM_APC_TARGETS_DN	296	1.6727536	0	0.03857048	1
NAKAMURA_CANCER_MICROENVIRONMENT_UP	19	1.671117	0.00140056	0.039354995	1
PID_IL12_2PATHWAY	52	1.6708246	0.00122399	0.039349336	1
KIM_ALL_DISORDERS_CALB1_CORR_DN	27	1.6705225	0.00413223	0.039369203	1
OHGUCHI_LIVER_HNF4A_TARGETS_DN	109	1.6686295	0	0.040207885	1
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_6HR_UP	74	1.6681318	0.00113379	0.040318638	1
DAZARD_UV_RESPONSE_CLUSTER_G2	23	1.667883	0.00406504	0.040333807	1
PEREZ_TP53_AND_TP63_TARGETS	178	1.6670728	0	0.040641844	1
REACTOME_RA_BIOSYNTHESIS_PATHWAY	16	1.6661139	0.00854701	0.04097948	1
LEIN_PONS_MARKERS	76	1.6660851	0	0.04083554	1
PEDERSEN_METASTASIS_BY_ERBB2_ISOFORM_1	41	1.6657115	0.00866337	0.04088866	1
TIAN_TNF_SIGNALING_VIA_NFKB	24	1.6650002	0.00264901	0.04104776	1
GERY_CEBP_TARGETS	114	1.664439	0	0.041166555	1
REACTOME_REGULATION_OF_INSULIN_LIKE_GROWTH_FACTOR_TRANSPORT_AND_UPTAKE_BY_INSULIN_LIKE_GROWTH_FACTOR_BINDING_PROTEINS_IGFBPS	92	1.6638671	0	0.04127415	1
MISHRA_CARCINOMA_ASSOCIATED_FIBROBLAST_UP	22	1.6636509	0.00979021	0.041233532	1
WP_DEVELOPMENT_AND_HETEROGENEITY_OF_THE_ILC_FAMILY	23	1.6619052	0.00544959	0.04203071	1
SATO_SILENCED_EPIGENETICALLY_IN_PANCREATIC_CANCER	31	1.6617954	0.00520156	0.04195611	1
VALK_AML_WITH FLT3_ITD	39	1.6616386	0.00125156	0.04190994	1
SMID_BREAST_CANCER_LUMINAL_B_UP	134	1.6613262	0	0.04192613	1
NABA_ECM_AFFILIATED	106	1.6606057	0	0.042162668	1
PEPPER_CHRONIC_LYMPHOCYTIC_LEUKEMIA_DN	15	1.6601416	0.00438597	0.042277314	1
HELLER_HDAC_TARGETS_SILENCED_BY_METHYLATION_UP	391	1.6596856	0	0.04231264	1
KEGG_ABC_TRANSPORTERS	35	1.6574379	0.00372671	0.043426007	1
WP_GLYCOSPHINGOLIPID_METABOLISM	17	1.657092	0.00689655	0.043477304	1
ONDER_CDH1_SIGNALING_VIA_CTNNB1	75	1.6568792	0.00115075	0.04346744	1
ROSS_AML_OF_FAB_M7_TYPE	68	1.6565377	0	0.04348792	1
REACTOME_ACTIVATED_NOTCH1_TRANSMITS_SIGNAL_TO_THE_NUCLEUS	30	1.6561743	0.01088435	0.043548416	1
PID_P75_NTR_PATHWAY	63	1.655555	0.00472813	0.04373549	1
WANG_ESOPHAGUS_CANCER_VS_NORMAL_UP	112	1.6550013	0.00109649	0.04388506	1
HELLER_SILENCED_BY_METHYLATION_UP	232	1.6548737	0	0.04381739	1
WP_PATHOGENESIS_OF_SARSCOV2_MEDIATED_BY_NSP9NSP10_COMPLEX	18	1.6544154	0.00578871	0.043948844	1
PHONG_TNF_RESPONSE_NOT_VIA_P38	321	1.6542816	0	0.043884594	1
IVANOVA_HEMATOPOIESIS_STEM_CELL_LONG_TERM	178	1.6535703	0	0.04417264	1
MAHADEVAN_IMATINIB_RESISTANCE_DN	15	1.6532625	0.0058309	0.0442424	1
GAUSSMANN_MLL_AF4_FUSION_TARGETS_D_UP	32	1.6527997	0.00533333	0.04438383	1
WP_MARKERS_OF_KIDNEY_CELL_LINEAGE	40	1.651703	0.00630517	0.04479095	1
SUH_COEXPRESSED_WITH_ID1_AND_ID2_UP	17	1.6515445	0.00716332	0.04471206	1
BOQUEST_STEM_CELL_CULTURED_VS_FRESH_UP	364	1.6514826	0	0.044588488	1
REACTOME_NEF_MEDIATES_DOWN_MODULATION_OF_CELL_SURFACE_RECEPTORS_BY_RECRUITING_THEM_TO_CLATHRIN_ADAPTERS	19	1.6513246	0.0097493	0.044494707	1
REACTOME_BILE_ACID_AND_BILE_SALT_METABOLISM	34	1.6501088	0.00909091	0.045020986	1
MUELLER_METHYLATED_IN_GLOBLASTOMA	27	1.6491861	0.00934579	0.045361355	1
LEE_LIVER_CANCER_DENA_DN	51	1.6486278	0.00475059	0.0455753	1

WP_THERMOGENESIS	97	1.6485534	0.00111483	0.045466706	1
MARTIN_NFKB_TARGETS_UP	34	1.6475637	0.00381194	0.04590959	1
REACTOME_COLLAGEN_CHAIN_TRIMERIZATION	36	1.6465836	0.00256082	0.04630537	1
PID_IL23_PATHWAY	28	1.6463773	0.00518135	0.046255343	1
PAPASPYRIDONOS_UNSTABLE_ATEROSCLEROTIC_PLAQUE_UP	51	1.6458997	0.002442	0.046418842	1
PARK_TRETINOLIN_RESPONSE_AND_RARA_PLZF_FUSION	23	1.6457673	0.00664011	0.046334375	1
PID_TAP63_PATHWAY	48	1.6446292	0.00243309	0.0468517	1
PETRETTO_HEART_MASS_QTL_CIS_DN	25	1.6440232	0.00661376	0.047017835	1
YANG_BCL3_TARGETS_UP	300	1.6437106	0	0.047037173	1
LIANG_SILENCED_BY_METHYLATION_UP	23	1.6433989	0.01232877	0.047063004	1
SCHAEFFER_PROSTATE_DEVELOPMENT_48HR_UP	359	1.6425371	0	0.047401868	1
DER_IFN_GAMMA_RESPONSE_UP	69	1.6417525	0.00471143	0.047759674	1
HOEBEKE_LYMPHOID_STEM_CELL_DN	88	1.6414504	0	0.047779325	1
BIOCARTA_EICOSANOID_PATHWAY	21	1.6407943	0.0097629	0.048036523	1
ODONNELL_TFRC_TARGETS_UP	320	1.640255	0	0.048201025	1
TORCHIA_TARGETS_OF_EWSR1_FLI1_FUSION_UP	248	1.6398224	0	0.048288945	1
WP_PRADERWILLI_AND_ANGELMAN_SYNDROME	46	1.6394477	0.00124844	0.048339825	1
COLIN_PILOCYTIC_ASTROCYTOMA_VS_GLIOBLASTOMA_UP	26	1.6391946	0.00953679	0.048342425	1
NOJIMA_SRP2_TARGETS_UP	25	1.639068	0.01246537	0.048273355	1
TURASHVILI_BREAST_LOBULAR_CARCINOMA_VS_DUCTAL_NORMAL_DN	71	1.6377279	0	0.04886716	1
GOLUB_ALL_VS_AML_DN	23	1.637726	0.00813008	0.04871349	1
OLSSON_E2F3_TARGETS_UP	25	1.6374929	0.0096022	0.048717823	1
MASRI_RESISTANCE_TO_TAMOXIFEN_AND_AROMATASE_INHIBITORS_UP	15	1.6367941	0.01900585	0.049018085	1
KANG_IMMORTALIZED_BY_TERT_DN	76	1.6364317	0.00228833	0.04910397	1
SASSON_RESPONSE_TO_FORSKOLIN_UP	84	1.6355493	0.00113895	0.049492624	1
ABBUD_LIF_SIGNALING_1_UP	40	1.6354852	0.00391134	0.049380437	1
MEISSNER_BRAIN_HCP_WITH_H3_UNMETHYLATED	21	1.6351707	0.00539084	0.049417958	1
IKEDA_MIR30_TARGETS_DN	20	1.6351316	0.00703235	0.049271718	1
PODAR_RESPONSE_TO_ADAPHOSTIN_UP	137	1.6350403	0	0.049169775	1
PID_IL8_CXCR2_PATHWAY	34	1.635025	0.00259403	0.049028084	1
WIERENGA_STAT5A_TARGETS_GROUP1	120	1.6338845	0.00108696	0.049594533	1

Supplemental Table 7**Covaris condition**

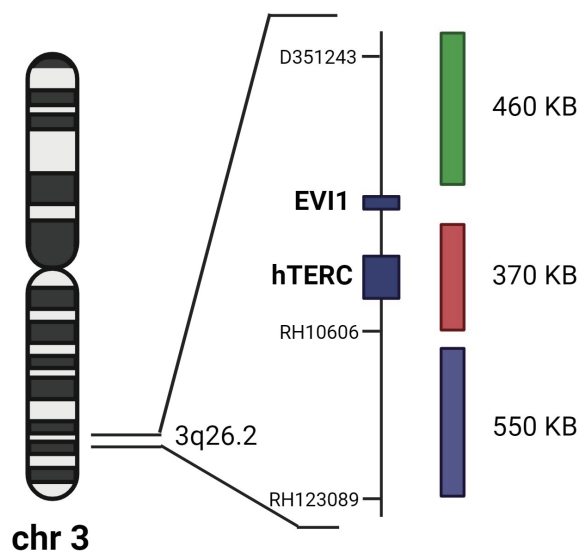
Sample	Peak Incident Power (W)	Duty factor (%)	Cycles/Burst	Duration(min)
iPSC	105	5	200	30
HPCs derived from iPSC	105	5	200	20
Patient's HPCs	175	5	200	30
K562	175	5	200	30

Supplemental Table 8 List of primers used for ChIP-qPCR

Genes		Sequence (5'-3')
<i>RPL30</i>	Forward	CAAGGCAAAGCGAAATTGGT
	Reverse	GCCCGTTCAGTCTCTTCGATT
<i>ZNF180</i>	Forward	TGATGCACAATAAGTCGAGCA
	Reverse	TGCAGTCAATGTGGGAAGTC

Supplemental Figure 1

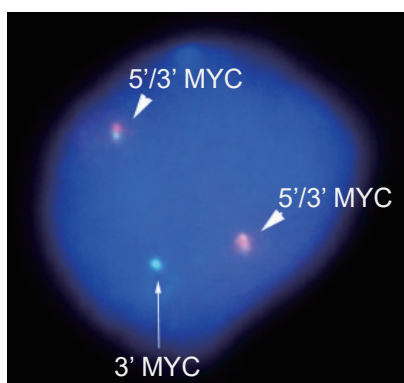
A



B



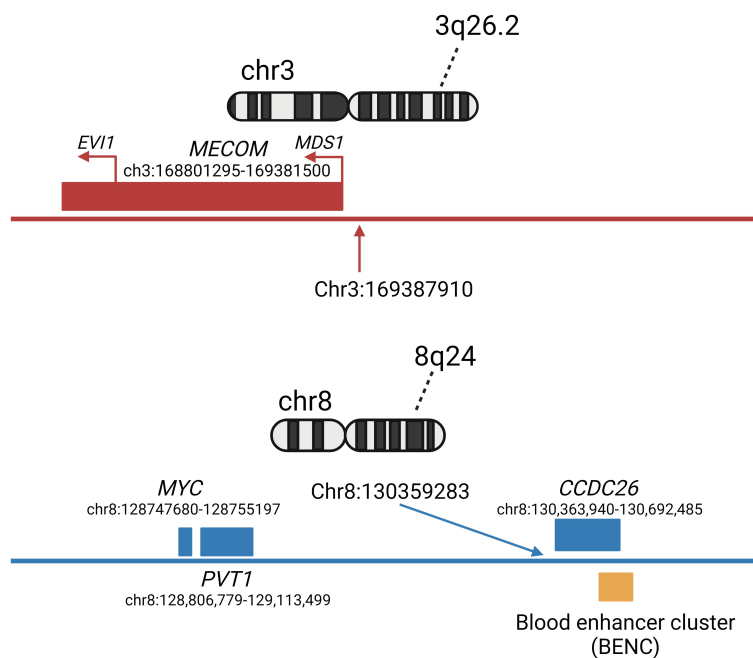
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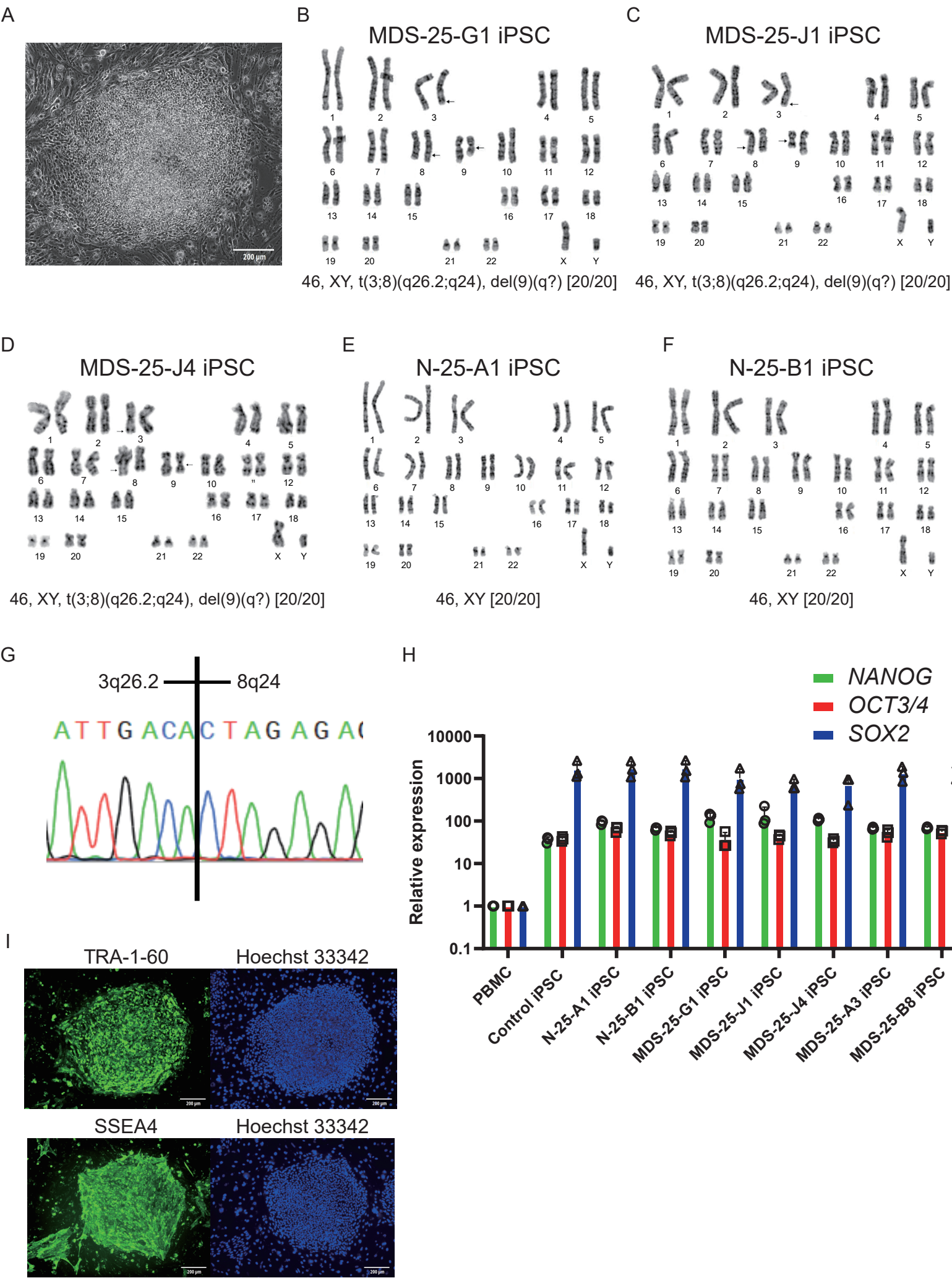
A



D

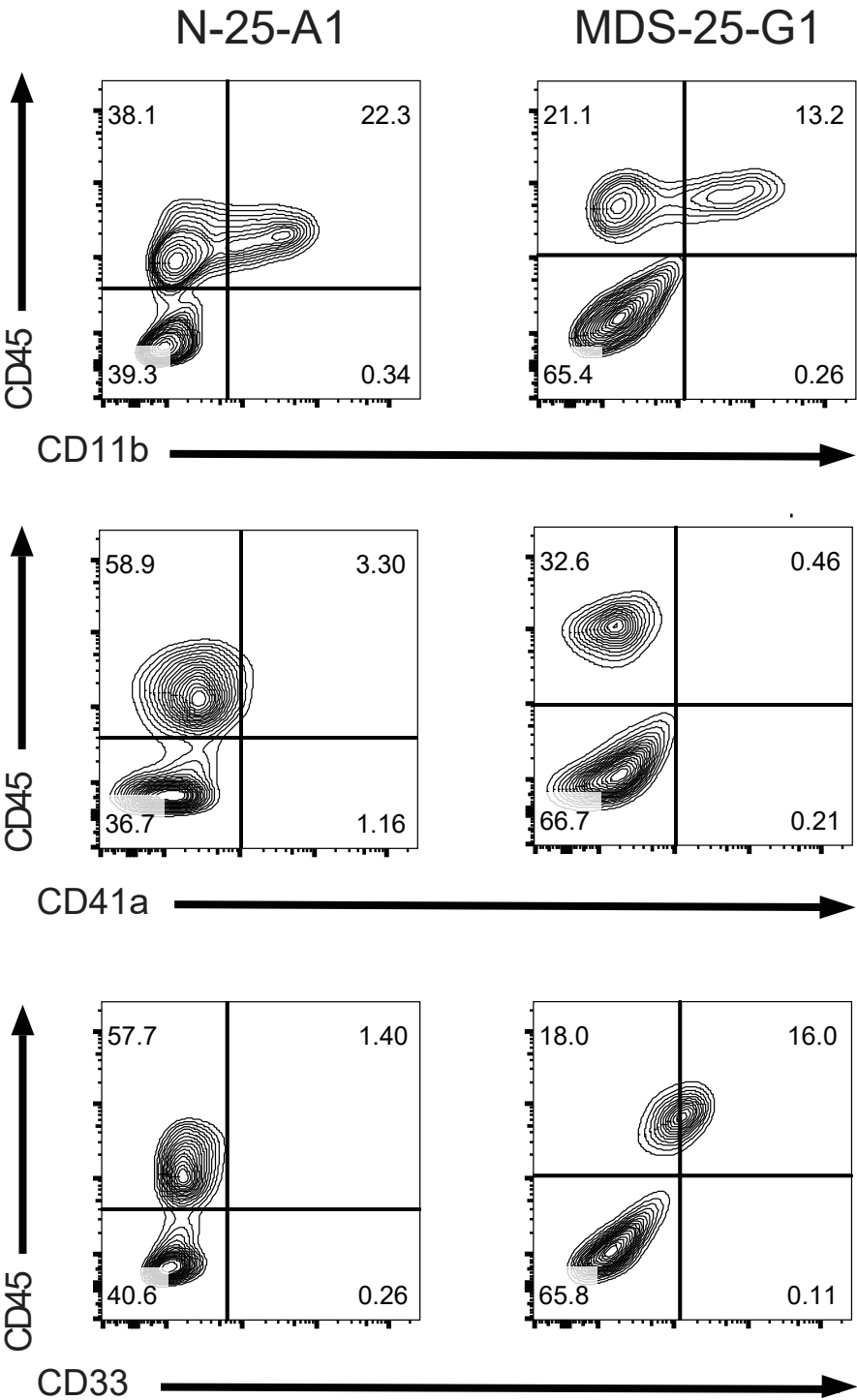


Supplemental Figure 3

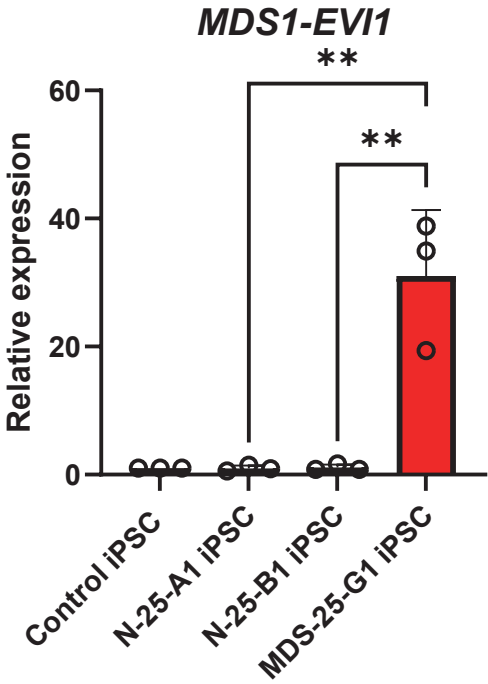


Supplemental Figure 4

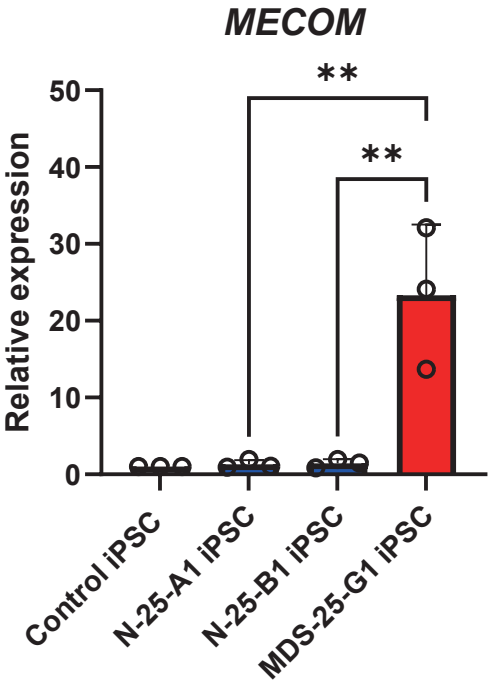
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A

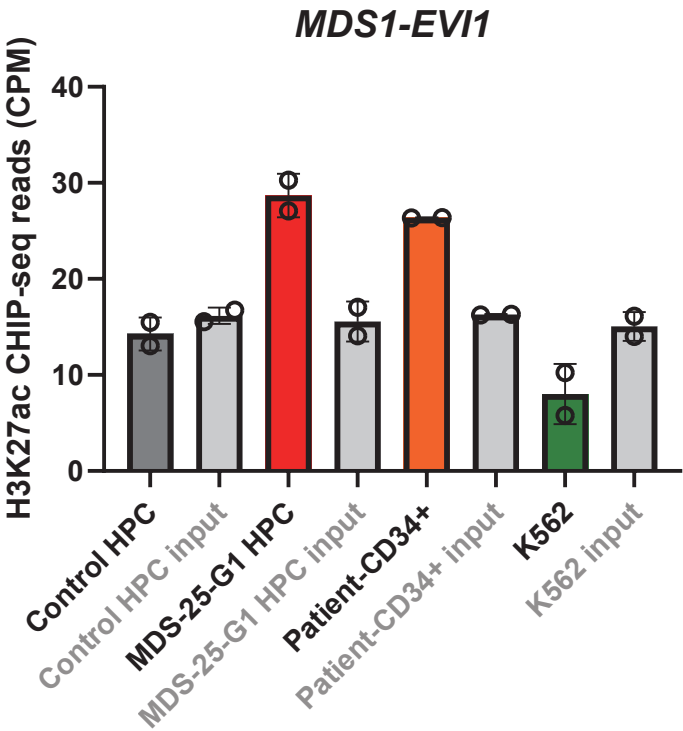


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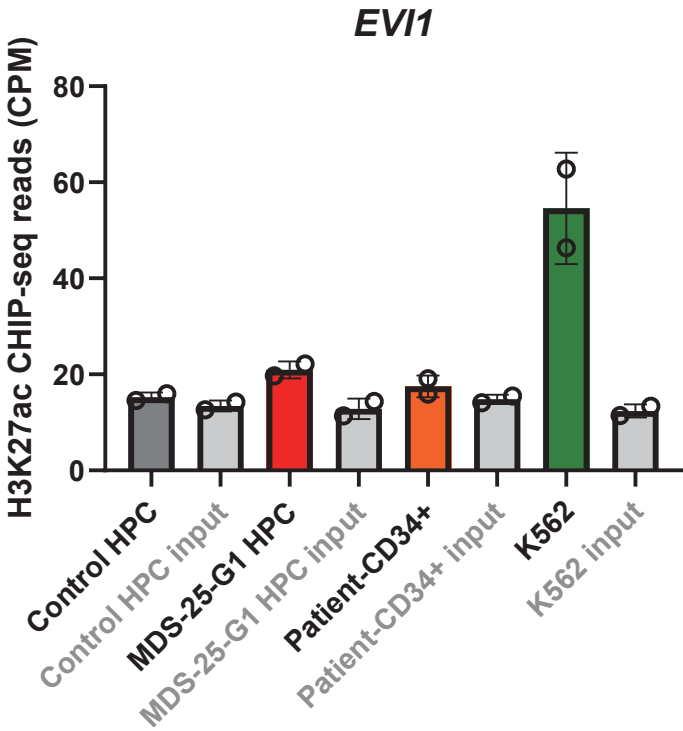


Supplemental Figure 6

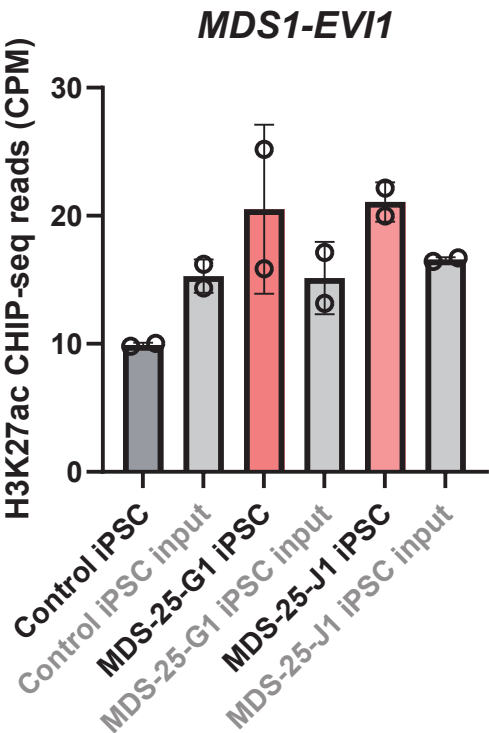
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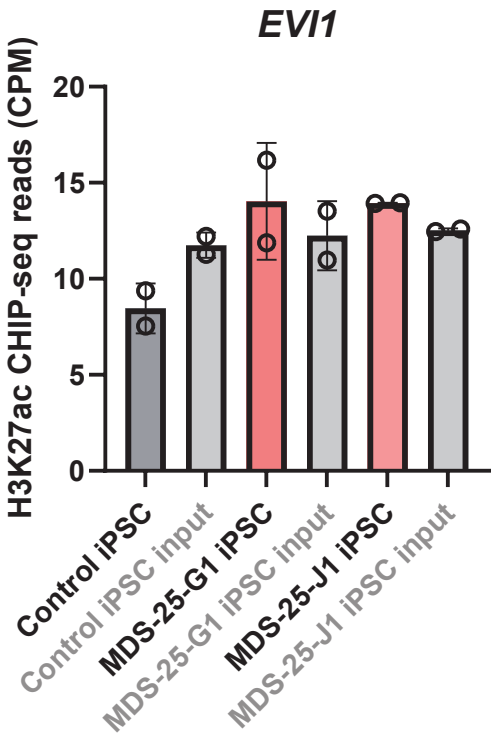
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C

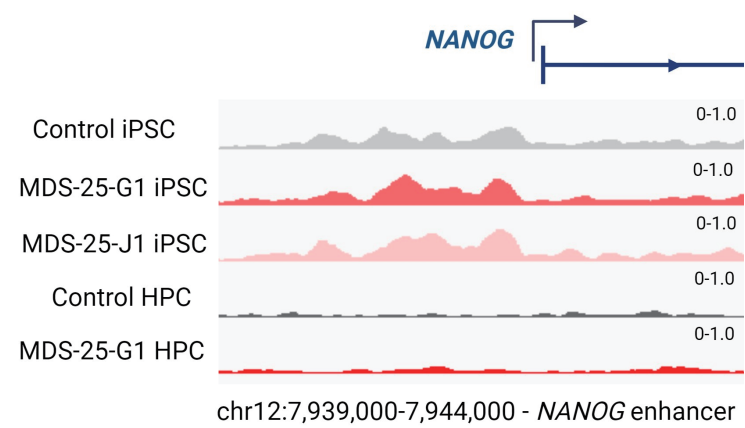


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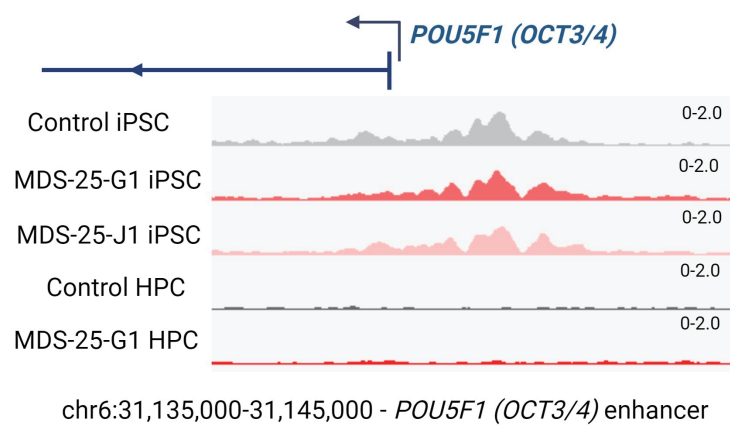


Supplemental Figure 7

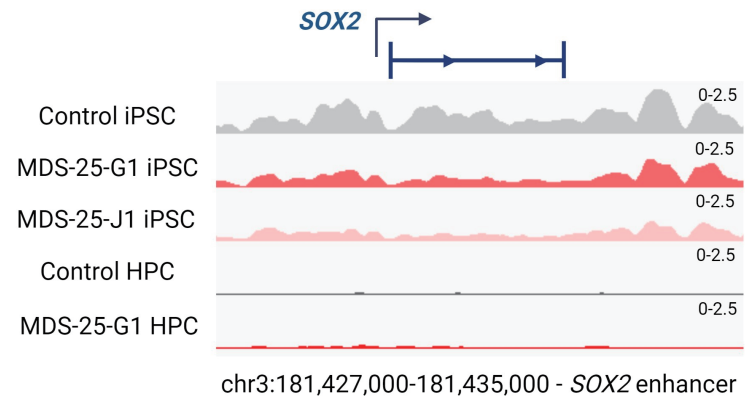
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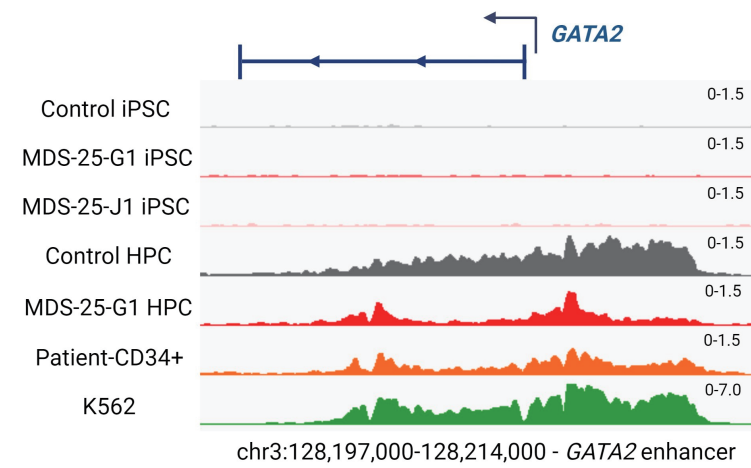
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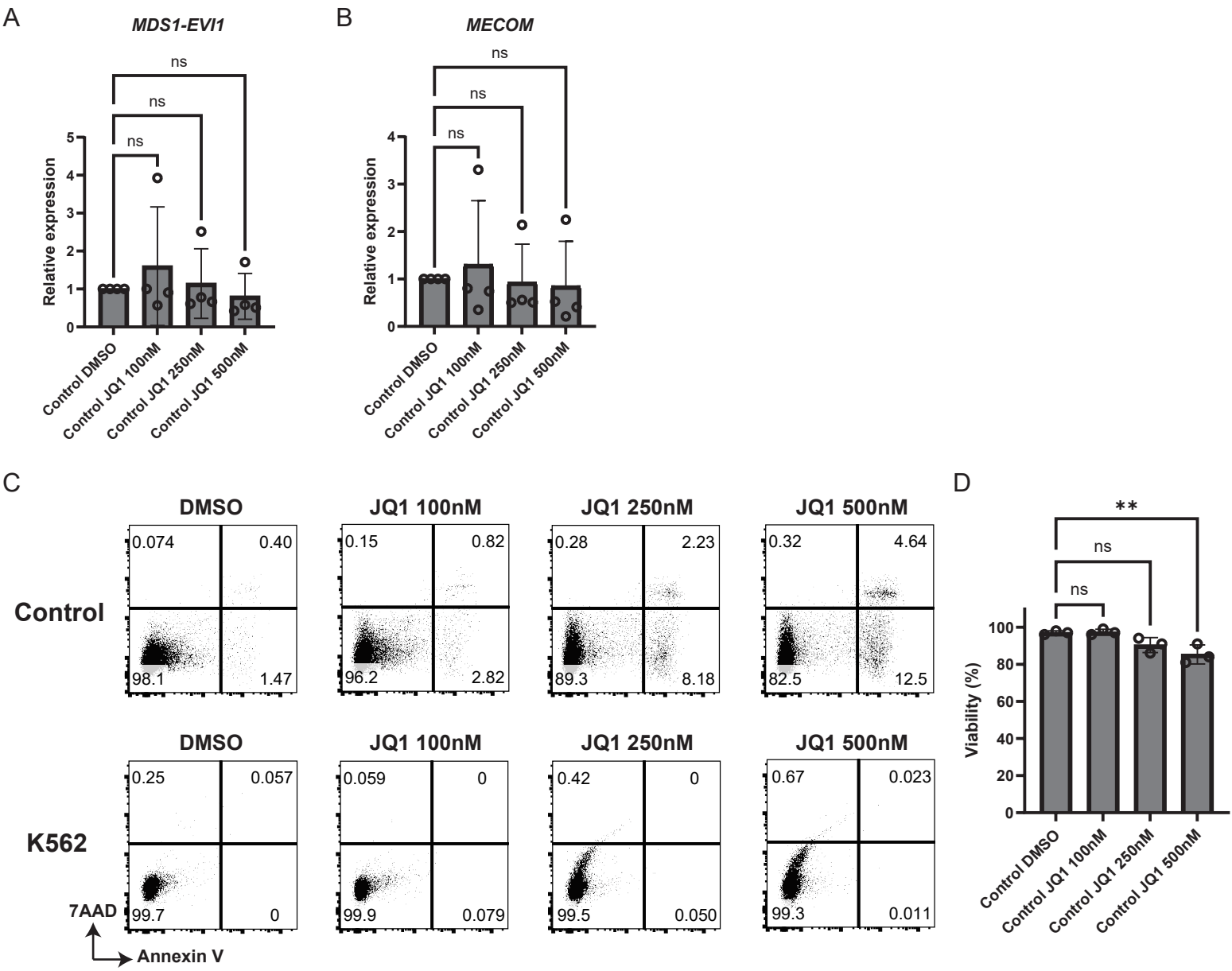
C



D

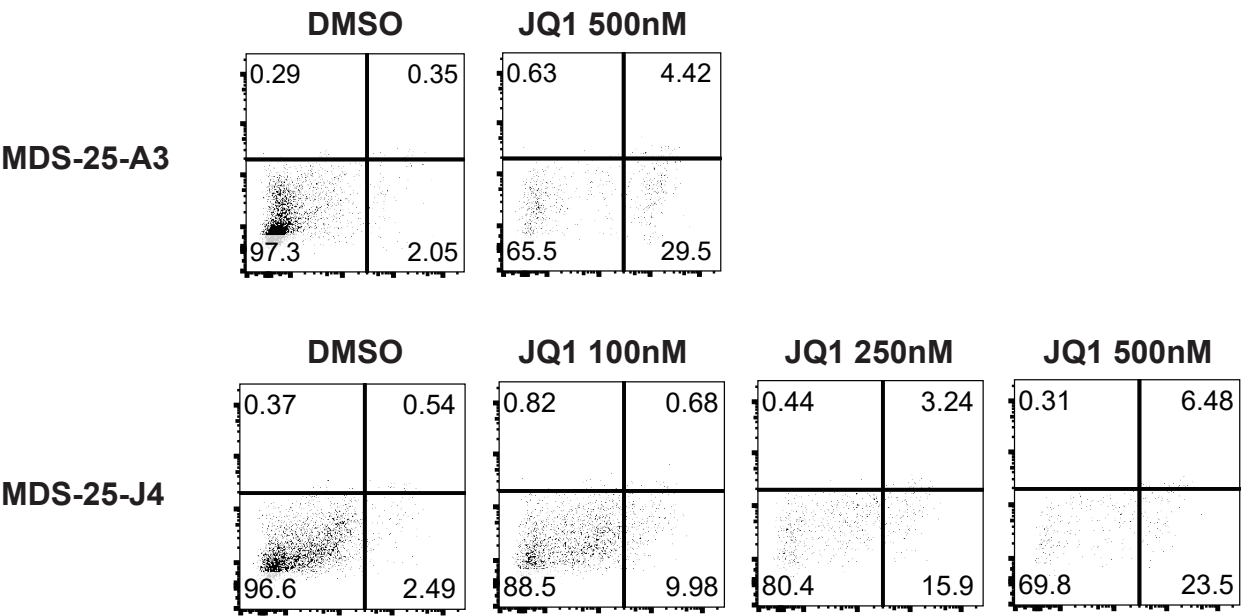


Supplemental Figure 8

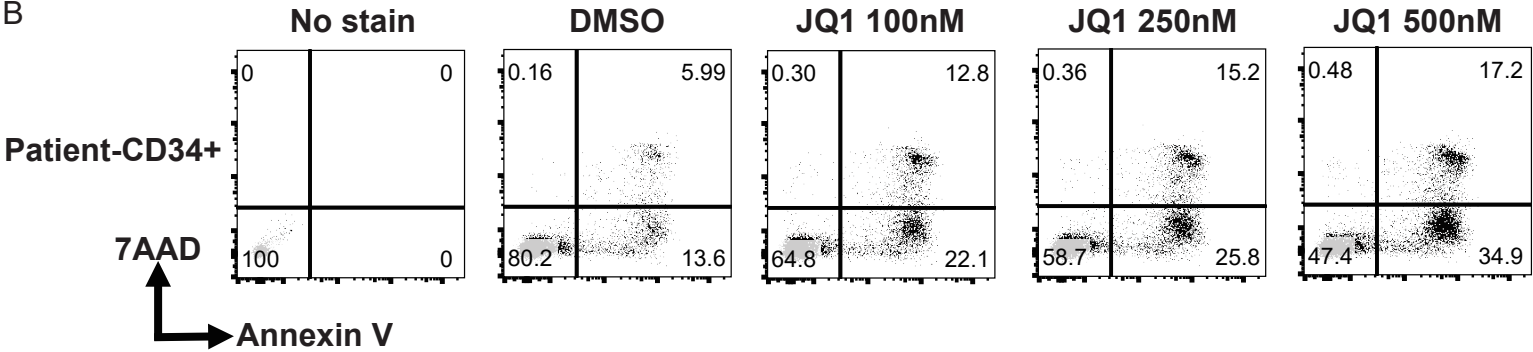


Supplemental Figure 9

A



B



C

