Stem Cell Reports, Volume *9*

Supplemental Information

Inhibition of EZH2 Promotes Human Embryonic Stem Cell Differentiation into Mesoderm by Reducing H3K27me3

Yongxin Yu, Peng Deng, Bo Yu, John M. Szymanski, Tara Aghaloo, Christine Hong, and Cun-Yu Wang

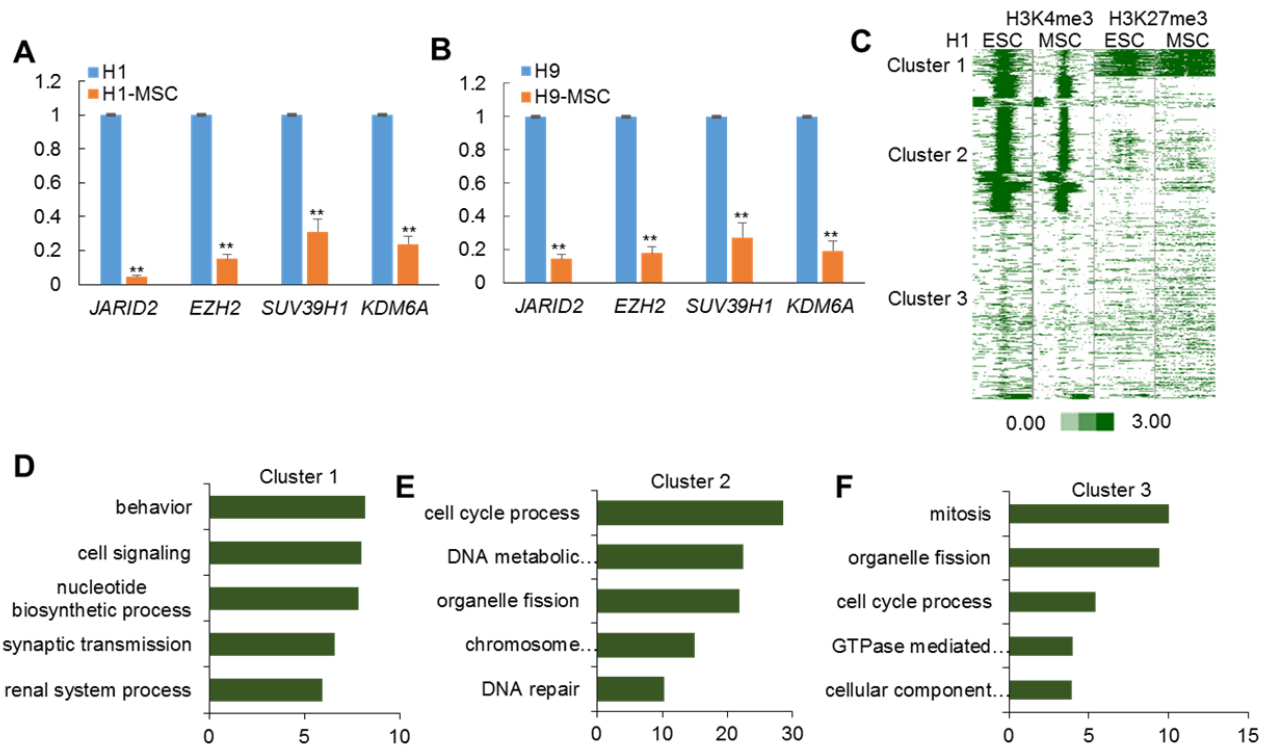

Figure S1, related to Figures 1 and 2, Alterations of bivalent genes in MSCs.
(A) qRT-PCR showed that the expression levels of *JARID2*, *EZH2*, *SUV39H1* and *KDM6A* in H1 hESCs-derived MSCs were downregulated compared with H1 hESCs.
(B) qRT-PCR showed that the expression levels of *JARID2*, *EZH2*, *SUV39H1* and *KDM6A* in H9 hESCs-derived MSCs were downregulated compared with H9 hESCs.
Data are shown as mean ± SD; n = 3 independent experiments. * p< 0.05, ** p<0.01, and *** p<0.001 by Student's t test.
(C) Profiles of H3K4me3 and H3K27me3 around TSS of genes that downregulated in MSCs. Green represents high intensity, and white represents no signal. The profile plot shows the average reads at each relative position to TSS on the x axis with H3K4me3 and H3K27me3 coverage.
(D-F) Gene ontology enrichment analysis of Cluster 1 genes (B), Cluster 2 genes (C) and Cluster 3 genes (D). Bars represent -log10 of P values.

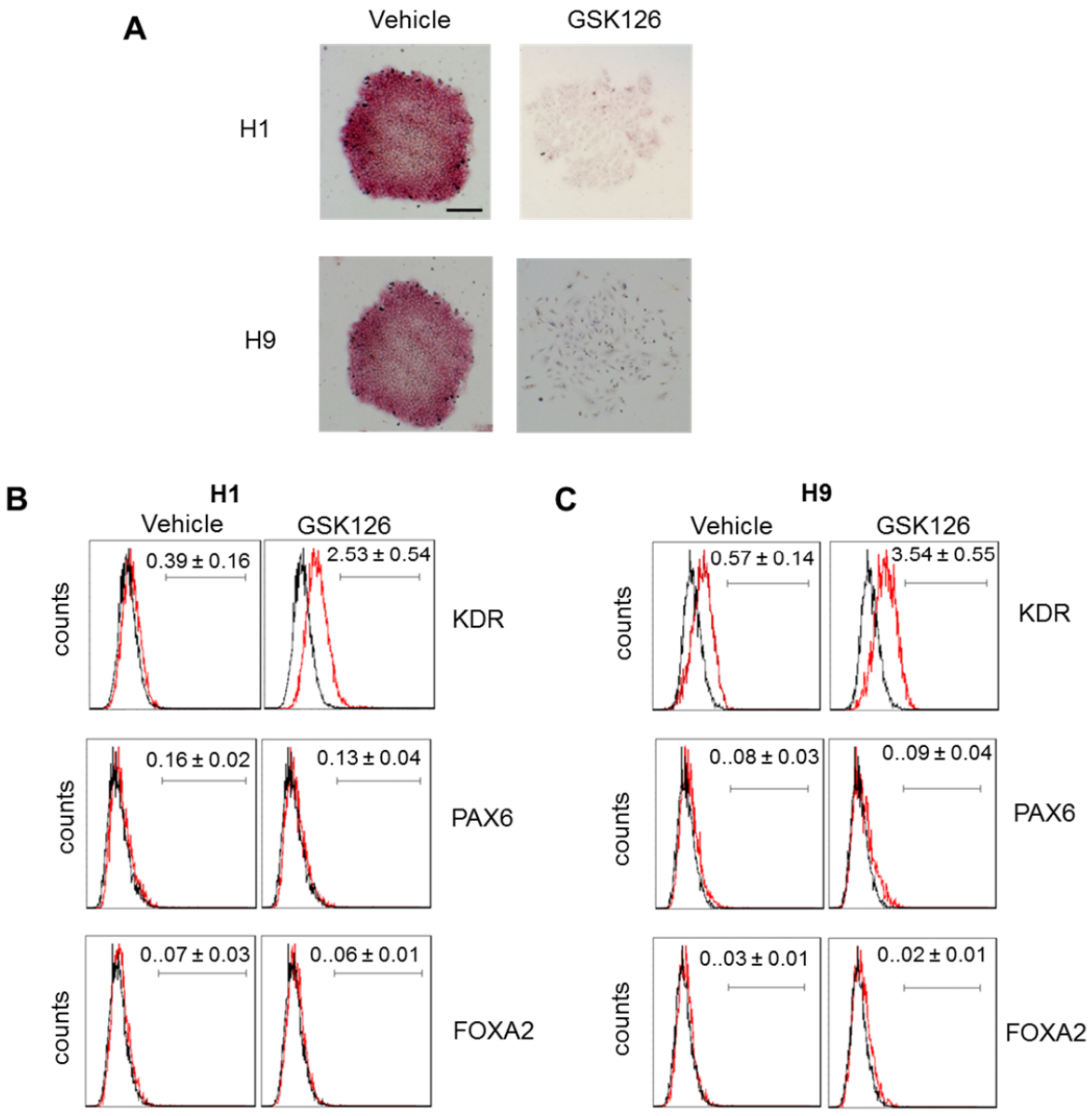

Figure S2, related to Figure 3, Inhibiting EZH2 by GSK126 promotes hESC differentiation.

(A) ALP staining of GSK126-treated hESC. Scale Bar, 200 μm.

(B) FACS staining of KDR-expressing cells.

(C) FACS staining of PAX6- and FOXA2-expressing cells.

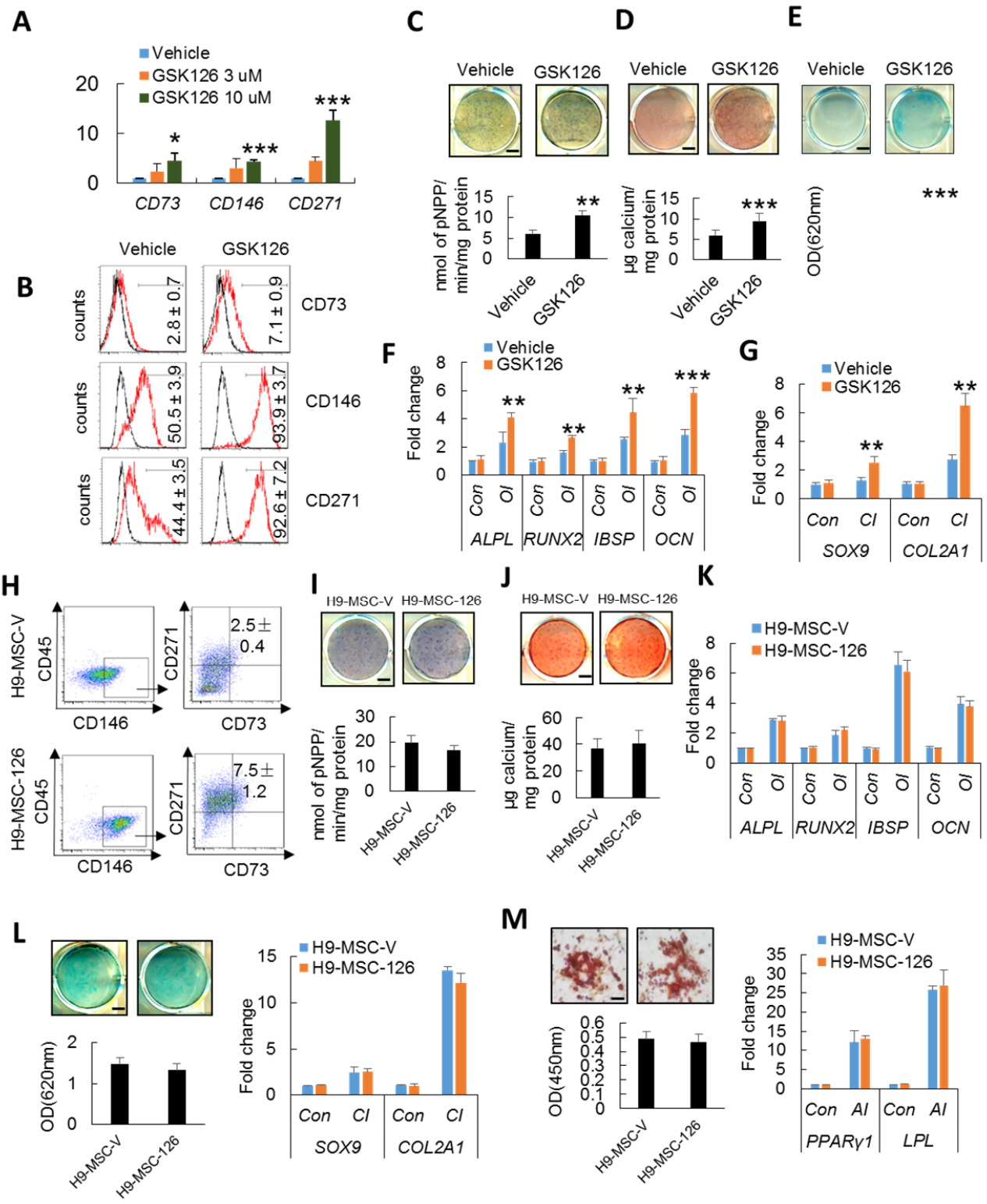
A
Vehicle
GSK126 3 uM
GSK126 10 uM
CD73
CD146
CD271
B
Vehicle
GSK126
counts
2.8 ± 0.7
7.1 ± 0.9
CD73
50.5 ± 3.9
93.9 ± 3.7
CD146
44.4 ± 3.5
92.6 ± 7.2
CD271
C
nmol of pNPP/ min/mg protein
D
µg calcium/ mg protein
E
OD(620nm)
F
Fold change
ALPL
RUNX2
IBSP
OCN
G
SOX9
COL2A1
H
H9-MSC-V
H9-MSC-126
CD45
CD146
CD271
CD73
2.5± 0.4
7.5± 1.2
I
J
K
L
OD(620nm)
M
OD(450nm)
PPARγ1
LPL

Figure S3, related to Figure 4, Effect of GSK126 treatment on mesenchymal lineage commitment of H9 hESCs.

(A) qRT-PCR gene expression analysis of well-known MSC surface markers (*CD73*, *CD146* and *CD271*).

(B) Flow cytometry analysis for CD73, CD146 and CD271 expression of H9 cells treated with DMSO or GSK126.

(C) ALP staining and ALP activity assay after OI for DMSO or GSK126 treated H9 cells. Scale bar, 440 μm.

(D) ARS staining and quantification after 14 days of OI for DMSO or GSK126 treated H9 cells. Scale bar, 440 μm.

(E) Alcian blue staining and quantification after CI for DMSO or GSK126 treated H9 cells. Scale bar, 440 μm.

(F, G) qRT-PCR gene expression analysis of osteogenic markers (*ALPL*, *RUNX2*, *IBSP*, *OCN*) (F) and chondrogenic markers (SOX9 and COL2a1) (G) after lineage specific differentiation in H9 cells treated with or without GSK126.

(H) Proportions of $CD90^{+}CD146^{+}CD271^{+}CD45^{-}$ H9-MSC-V and H9-MSC-126 are compared.

(I) ALP staining and ALP activity assay of H9-MSC-V and H9-MSC-126 after 14 days of OI. Scale bar, 440 μm

(J) ARS staining and quantification of H9-MSC-V and H9-MSC-126 after 14 days of OI.

(K) qRT-PCR gene expression analysis of osteogenic markers (*ALPL*, *RUNX2*, *IBSP*, *OCN*) in H9-MSC-V and H9-MSC-126 after 14 days of OI. Scale bar, 440 μm

(L) Alcian blue staining and quantification (left) and qRT-PCR gene expression analysis of chondrogenic markers (SOX9 and COL2a1) (right) of H9-MSC-V and H9-MSC-126 after 21 days of CI. Scale bar, 440 μm

(M) Oil Red O staining and quantification (left) and qRT-PCR gene expression analysis of adipogenic markers (*PPARG* and *LPL*) (right) of H9-MSC-V and H9-MSC-126 after 21 days of AI. Scale bar, 30 μm

Data are shown as mean ± SD; n = 3 independent experiments. * $p< 0.05$, ** $p<0.01$, and *** $p<0.001$ by Student's t test.

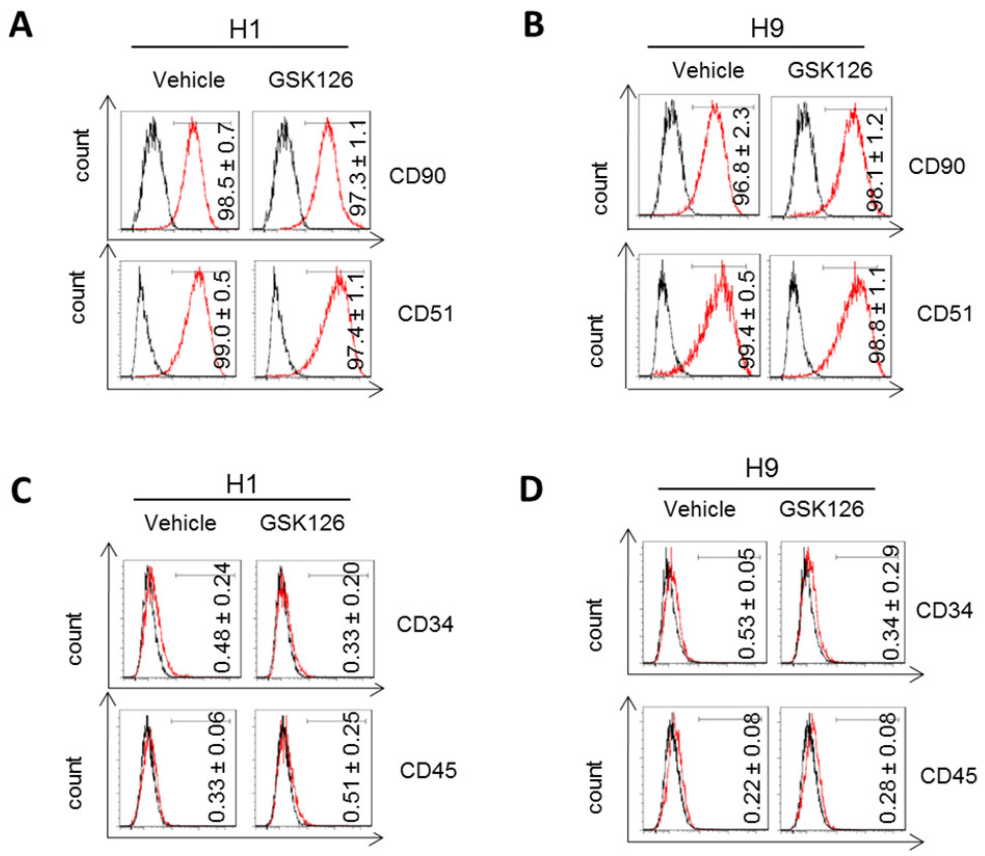

Figure S4, related to Figure 4, Surface markers expression after GSK126 treatment.

(A and B) Flow cytometry analysis for CD90 and CD51 expression of cells treated with DMSO or GSK126 in H1 hESCs (A) and H9 hESCs (B).

(C and D) Flow cytometry analysis for CD34 and CD45 expression of cells treated with DMSO or GSK126 in H1 hESCs (C) and H9 hESCs (D).

Supplemental Experimental Procedures

Raw data for histone H3K4me3 and H3K27me3 ChIP-seq and raw data for RNA-seq of hESCs and hESC-derived lineages were downloaded from NCBI epigenome roadmap (http://www.ncbi.nlm.nih.gov/geo/roadmap/epigenomics/).

All sequencing reads were mapped to NCBI build 37 (hg19) of the human genome using the software Bowtie. The mapped reads were subjected to the algorithm to evaluate the bound regions (peaks) of these reads in the genome. In detail, the genome was divided into 100-bp windows and we calculated the p value for Poisson distribution of ChIP-ed DNA relative to input for each window. Significant peaks were defined as the windows with significant p value less than 10-3. Only reads that aligned to a unique genomic position with no more than two mismatches were retained for the above analysis. When multiple reads mapped to the same position in the genome, only one was counted. Representative ChIP-seq enriched regions were visualized in the Integrated Genome Browser. To assign ChIP-seq enriched regions (peaks) to genes, we employed Cis-regulatory Elements Annotation System (CEAS) to create average profiling of all Refseq genes and overlaps of significant peaks with genomic annotation regions. Genes with significant peaks within 10 kb of their TSSs were considered as bound.

For H1 and the H1 derived cells, the RNA-Seq reads were mapped to human genome (hg19) with TopHat (version 2.0.9). The mapped reads were further analyzed by Cufflinks and the expression levels for each transcript were quantified as Fragments Per Kilobase of transcript per Million mapped reads (FPKM). We used DAVID to analyze functional enrichment in GO terms, KEGG Pathways.