Figure S4

a

hMSC

Genome methylation by Dam → Genome isolation and DpnI digestion → PCR amplification of methylated fragments → Library construction → Next-generation sequencing

Dam

Dam-EMD

DamID signal

b

Dam   Dam-EMD

☐ ○ *CLOCK*^{+/+}
☐ ○ *CLOCK*^{-/-}

PC3 (9.28% explained var.)
PC1 (42.5% explained var.)
PC2 (14.54% explained var.)

c

Genomic coverage (%)

CLOCK^{+/+}   *CLOCK*^{-/-}

d

Relative DamID signal
(*CLOCK*^{-/-} vs. *CLOCK*^{+/+})

-1.35   0   1.15

Chr. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X

e

H3K9me3
ChIP-seq

Euclidean distance

250
200
150
100
50
0

CLOCK^{+/+} - - - + + +
CLOCK^{-/-} + + + - - -

f

H3K9me3 ChIP-seq

CLOCK^{+/+}   *CLOCK*^{-/-}

H3K9me3 signal (CPM)

5,000
4,000
3,000
2,000
1,000

996 mountains   848 mountains

Mountains   Mountains
ranked by H3K9me3 signal   ranked by H3K9me3 signal

g

***

H3K9me3 signal (log$_2$(CPM+1))

12
10
8
6
4
2
0

CLOCK^{+/+}   *CLOCK*^{-/-}

h

Overlapping LADs

1.00
0.75
0.50
0.25
0.00

iLAD
LAD

CLOCK^{+/+}   *CLOCK*^{-/-}

i

ATAC-seq

Euclidean distance

200
150
100
50
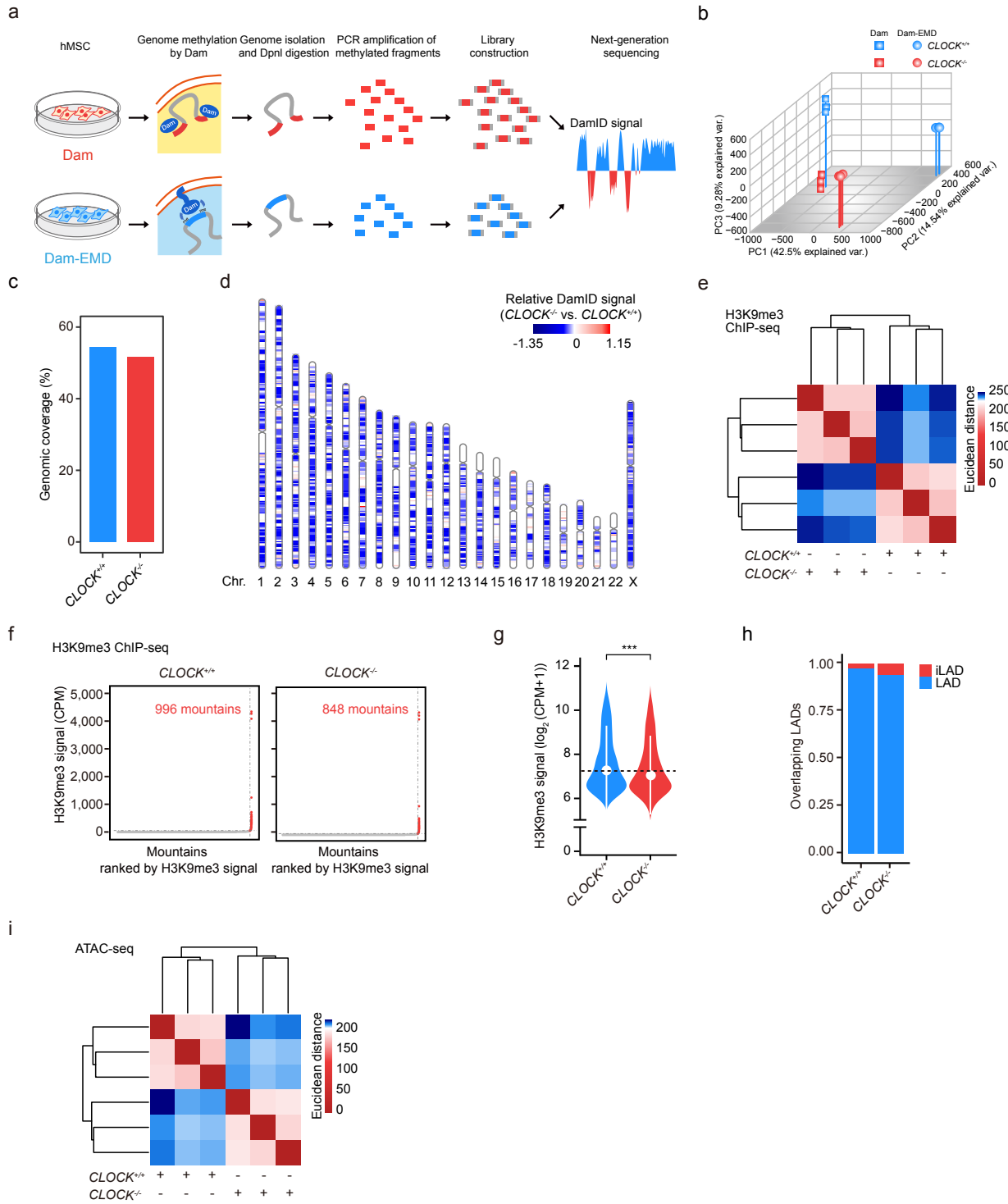0

CLOCK^{+/+} + + + - - -
CLOCK^{-/-} - - - + + +

Supplementary information, Fig. S4 Epigenomic analyses of *CLOCK*^{-/-} hMSCs. a The flow chart of the DamID-seq strategy. **b** Principal component analysis to assess the reproducibility of DamID-seq data in late-passage *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hMSCs (P9). **c** Bar chart showing the genomic coverage of LADs in late-passage *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hMSCs (P9). **d** Chromosome ideogram showing the relative DamID signals at LAD regions of *CLOCK*$^{-/-}$ hMSCs compared to those of late-passage *CLOCK*$^{+/+}$ hMSCs (P9) across the 23 pairs of chromosomes. The color key from blue to red indicates low to high of the relative DamID signals at each LAD region, respectively. **e** Heatmap showing the Euclidean distance analysis to assess the reproducibility of H3K9me3 ChIP-seq data in late-passage *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hMSCs (P9). The color key of the Euclidean distance from red to blue indicates strong to weak correlation, respectively. **f** Distribution of the H3K9me3 ChIP-seq signal at H3K9me3 peaks revealed two classes of H3K9me3 peaks in late-passage *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hMSCs (P9). H3K9me3 peaks are plotted in increasing order based on the H3K9me3 signal. H3K9me3 mountains are defined as H3K9me3 peaks above the inflection points of the curves. **g** Violin plot showing the H3K9me3 signals at H3K9me3 mountains in late-passage *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hMSCs (P9). The white circles represent the median values, and the white lines represent the values within the IQR from smallest to largest. ***$p < 0.001$ (Two-sided Wilcoxon rank-sum test). **h** Bar plot showing the percentages of H3K9me3 mountains located in LAD regions in late-passage *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hMSCs (P9). **i** Heatmap showing the Euclidean distance analysis to determine the reproducibility of ATAC-seq data in late-passage *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hMSCs (P9). The color key of the Euclidean distance from red to blue indicates strong to weak correlation, respectively.