Figure S1

a hMSC

EP / LP

SA-β-gal-positive cells (fold), EP vs LP, ***

b hMSC

EP / LP

Clonal expansion ability (fold), EP vs LP, ***

c hMSC

$LMNA^{+/+}$ / $LMNA^{G608G/+}$ / $LMNA^{G608G/G608G}$

SA-β-gal-positive cells (fold), $LMNA^{+/+}$, $LMNA^{G608G/+}$, $LMNA^{G608G/G608G}$, **, ***

d hMSC

$LMNA^{+/+}$ / $LMNA^{G608G/+}$ / $LMNA^{G608G/G608G}$

Clonal expansion ability (fold), $LMNA^{+/+}$, $LMNA^{G608G/+}$, $LMNA^{G608G/G608G}$, ***

e Primary hMSC

Young #1 #2 #3 #4
Old #1 #2 #3 #4

Clonal expansion ability (fold), Young vs Old, *

f Primary hMSC

	Young				Old				kDa
	#1	#2	#3	#4	#1	#2	#3	#4	
CLOCK									100
Fold	0.92	0.83	1.18	1.07	0.86	0.65	0.57	0.42	
β-actin									37

g hMSC

$LMNA^{+/+}$ / $LMNA^{G608G/+}$ / $LMNA^{G608G/G608G}$

CLOCK — 100 kDa
Lamin A/C — 75 kDa *
β-actin — 37 kDa

h

HR donor vetor

5' Homology arm 3' Homology arm

$CLOCK^{+/+}$
Exons 1 2 3 4 5 6 7 8 24

CRISPR/Cas9 HR

Homozygous mutant ($CLOCK^{neo/neo}$)

FLPo recombination

$CLOCK^{-/-}$
1 2 3 4 6 7 8 24

i hESC

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

CLOCK — 100 kDa
β-actin — 37 kDa

j $CLOCK^{+/+}$ / $CLOCK^{-/-}$

k

meF → OCT4
← meR

○ Unmethylated CpG
● Methylated CpG

$CLOCK^{+/+}$ $CLOCK^{-/-}$
n=7 n=7

l $CLOCK^{+/+}$ / $CLOCK^{-/-}$

Clonal expansion ability (fold), $CLOCK^{+/+}$ vs $CLOCK^{-/-}$, ns

m

Cell cycle phase (%), $CLOCK^{+/+}$, $CLOCK^{-/-}$, ns
S / G0/G1 / G2/M

n NANOG / OCT4 / SOX2 / DNA / Merge

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

o Ki67 / DNA

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

Ki67-positive cells (%), $CLOCK^{+/+}$ vs $CLOCK^{-/-}$, ns

p FOXA2 / DNA SMA / DNA Tuj1 / DNA

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

Endoderm / Mesoderm / Ectoderm

Teratoma

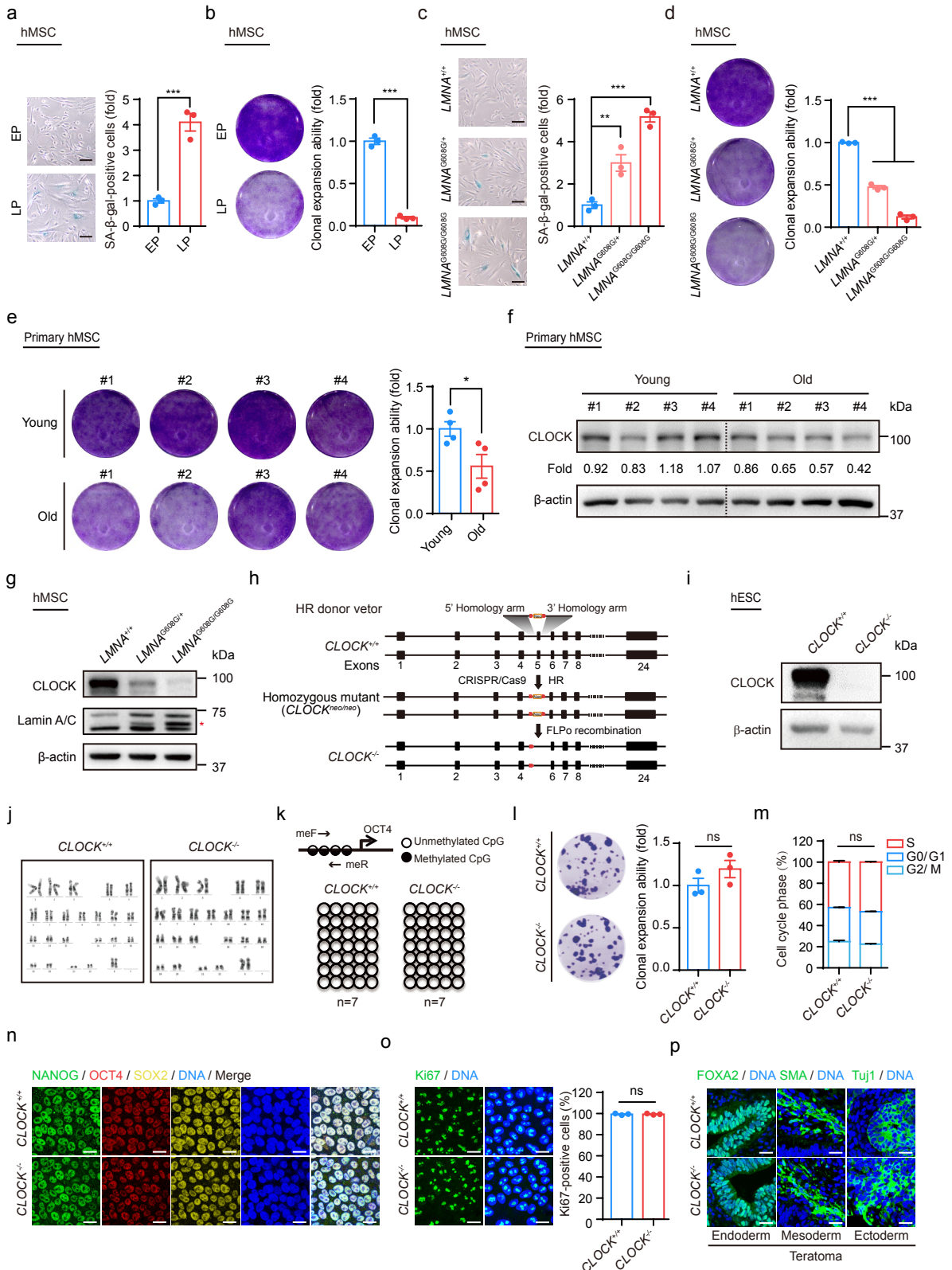**Supplementary information, Fig. S1 Characterization of senescent hMSCs and generation of CLOCK-deficient hESCs. a** SA-β-gal staining of EP (P4) and LP (P13) *CLOCK*$^{+/+}$ hMSCs. Data are presented as means ± SEM. *n* = 3 biological replicates. ***p* < 0.001 (Two-tailed unpaired Student's *t*-test). Scale bars, 100 μm. **b** Clonal expansion assay of EP (P4) and LP (P12) *CLOCK*$^{+/+}$ hMSCs. Data are presented as means ± SEM. *n* = 3 biological replicates. ***p* < 0.001 (Two-tailed unpaired Student's *t*-test). **c** SA-β-gal staining of HGPS-specific hMSCs. Data are presented as means ± SEM. *n* = 3 biological replicates. **p < 0.01; ***p* < 0.001 (Two-tailed unpaired Student's *t*-test). Scale bars, 100 μm. **d** Clonal expansion assay of HGPS-specific hMSCs. Data are presented as means ± SEM. *n* = 3 biological replicates. ***p* < 0.001 (Two-tailed unpaired Student's *t*-test). **e** Clonal expansion assay of primary hMSCs derived from young and old individuals. Data are presented as means ± SEM. *n* = 4 biological replicates. *p < 0.05 (Two-tailed unpaired Student's *t*-test). **f** Representative image of western blot analysis of CLOCK in young and old human primary MSCs. β-actin was used as the loading control. *n* = 4 biological replicates. **g** Representative image of western blot analysis of CLOCK in HGPS-specific (*LMNA*$^{G608G/+}$ and *LMNA*$^{G608G/G608G}$) hMSCs. β-actin was used as the loading control. * represents the band of progerin. **h** Schematic showing the gene editing strategy for *CLOCK* (exon 5) using CRISPR/Cas9-mediated HR in hESCs. **i** Western blot analysis of CLOCK in both *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hESCs. β-actin was used as the loading control. Data are representative of three independent experiments. **j** G-banding karyotyping of *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hESCs. **k** DNA methylation analysis at the *OCT4* promoter in *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hESCs. **l** Clonal expansion assay of *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hESCs. Data are presented as means ± SEM. *n* = 3 biological replicates. ns, nonsignificant (Two-tailed unpaired Student's *t*-test). **m** Cell cycle analysis of *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hESCs. Data are presented as means ± SEM. *n* = 3 biological replicates. ns, nonsignificant (Two-tailed unpaired Student's *t*-test). **n** Immunofluorescence analysis of the pluripotency markers NANOG, OCT4, and SOX2 in *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hESCs. Scale bars, 25 μm. **o** Immunofluorescence analysis of Ki67 in *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hESCs. Data are presented as means ± SEM. *n* = 3 biological replicates. ns, nonsignificant (Two-tailed unpaired Student's *t*-test). Scale bars, 25 μm. **p** Immunofluorescence analysis of representative markers of the three germ layers in teratomas derived from *CLOCK*$^{+/+}$ and *CLOCK*$^{-/-}$ hESCs. Scale bars, 25 μm.