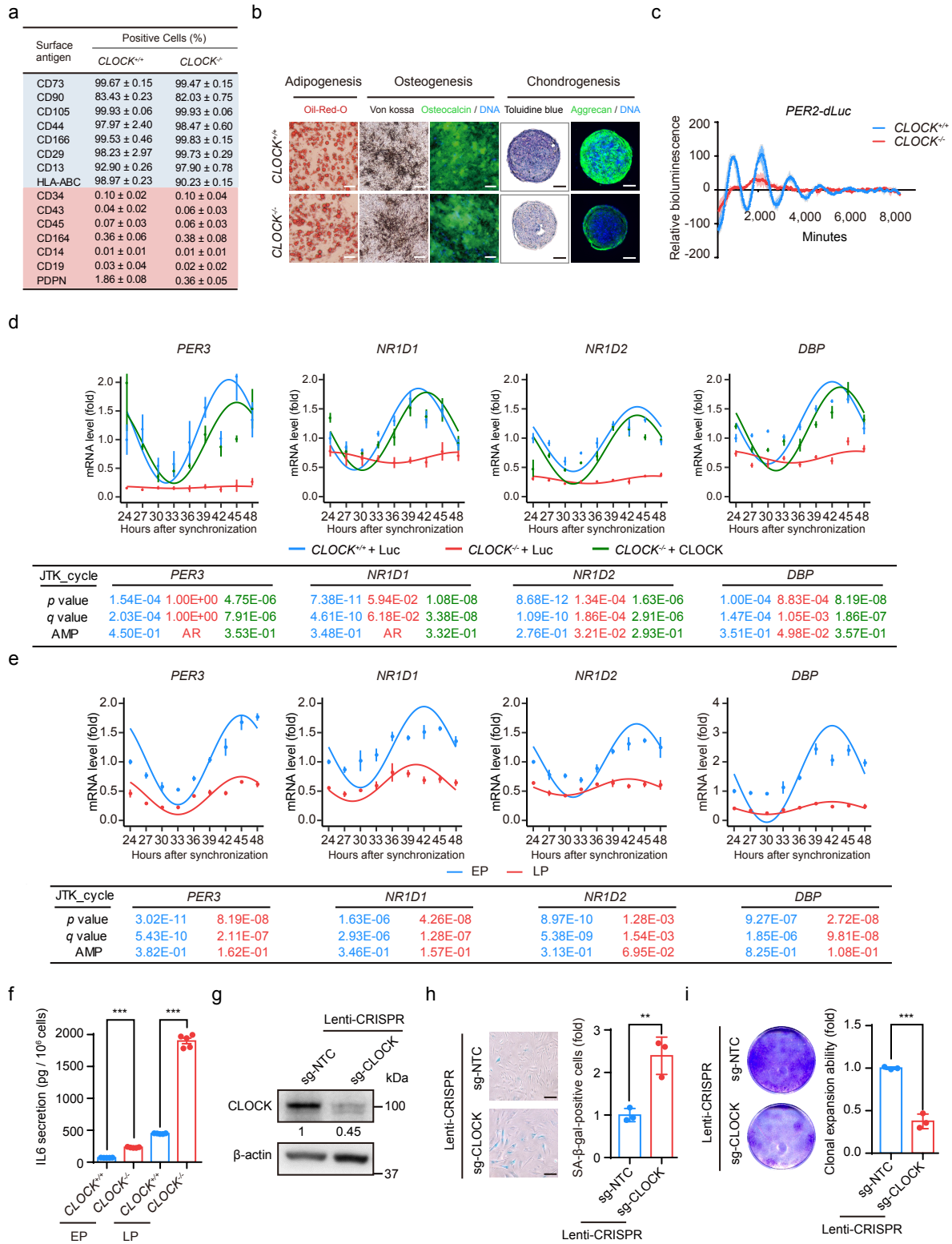


Figure S2



Supplementary information, Fig. S2 Characterization of CLOCK-deficient hMSCs.

a Surface antigen analysis of *CLOCK*^{+/+} and *CLOCK*^{-/-} hMSCs. Data are presented as means \pm SEM. *n* = 3 biological replicates. **b** Characterization of the multilineage differentiation potential of *CLOCK*^{+/+} and *CLOCK*^{-/-} hMSCs. Toluidine blue and Aggrecan staining were used to evaluate chondrogenesis. Von Kossa and Osteocalcin staining were used to evaluate osteogenesis. Oil Red O staining were used to evaluate adipogenesis. Scale bars, 100 μ m. **c** Representative traces of circadian oscillation in *CLOCK*^{+/+} and *CLOCK*^{-/-} hMSCs monitored by a transiently transfected reporter plasmid expressing destabilized Luc driven by the *PER2* gene promoter (*PER2-dLuc*). Continuous monitoring of Luc activity revealed significant amplitude dampening of *PER2::LUC* oscillations in *CLOCK*^{-/-} hMSCs. Data are presented as means \pm SD. *n* = 3 biological replicates. **d** Relative mRNA levels of the indicated genes from forskolin-synchronized *CLOCK*^{+/+} and *CLOCK*^{-/-} hMSCs transduced with lentiviruses expressing Luc or CLOCK measured at the indicated time points. The qPCR data shown above were further analyzed by JTK_Cycle analysis to determine the rhythmicity and amplitude of the peaks (Time course threads of *CLOCK*^{+/+} or *CLOCK*^{-/-} hMSCs with both *p* and *q* values < 0.05 were considered rhythmic). AR, arrhythmic. Data are presented as means \pm SD. *n* = 4. The solid fitted line represents a cosinor-fitted curve that indicates rhythmic expression. Data are representative of two independent experiments. **e** Relative mRNA levels of the indicated genes from forskolin-synchronized EP (P4) and LP (P13) *CLOCK*^{+/+} hMSCs measured at the indicated time points. The RT-qPCR data shown above were further analyzed by JTK_Cycle analysis to determine the rhythmicity and amplitude of the peaks (Time course threads of EP or LP hMSCs with both *p* and *q* values < 0.05 were considered rhythmic). AR, arrhythmic. Data are presented as means \pm SD. *n* = 4. The solid fitted line represents a cosinor-fitted curve that indicates rhythmic expression. Data are representative of two independent experiments. **f** ELISA of IL6 secretion in EP (P4) and LP (P9) *CLOCK*^{+/+} and *CLOCK*^{-/-} hMSCs. Data are presented as means \pm SEM. *n* = 5. ****p* < 0.001 (Two-tailed unpaired Student's *t*-test). **g** Western blot analysis of CLOCK in *CLOCK*^{+/+} hMSCs transduced with lentivirus-CRISPRv2 targeting CLOCK or an NTC sgRNA. β -actin was used as the loading control. Data are representative of three independent experiments. NTC means non-targeting control. **h** SA- β -gal staining in *CLOCK*^{+/+} hMSCs transduced with lentivirus-CRISPRv2 targeting CLOCK or an NTC sgRNA. Data are presented as means \pm SEM. *n* = 3 biological replicates. ***p* < 0.01 (Two-tailed unpaired Student's *t*-test). Scale bars, 100 μ m. NTC means non-targeting control. **i** Clonal expansion assay of *CLOCK*^{+/+} hMSCs transduced with lentivirus-CRISPRv2 targeting CLOCK or an NTC sgRNA. Data are presented as means \pm SEM. *n* = 3 biological replicates. ****p* < 0.001 (Two-tailed unpaired Student's *t*-test). NTC means non-targeting control.