Figure EV1. Quality control measurements of sequencing libraries.

A, B Insert the size distribution of each ATAC-seq library (two libraries per oxygen condition—A) and mapping statistics for each individual replicate (B).

C, D Correlation matrices of H3K27ac ChIP-seq libraries (C) and H3K4me3 ChIP-seq libraries (D).

E Heatmap of H3K4me3 abundance between hypoxia- and normoxia-cultured MSCs.

Data information: n = 2 biologically independent experiments.
Figure EV1.
Figure EV2. Metabolic profiling of hypoxic and normoxic MSCs.
A, B Glucose consumption (A) and lactate production (B) were measured in the media of hypoxia- and normoxia-cultured cells using the Vi-Cell MetaFLEX instrument. n = 3 biologically independent experiments.
C, D Basal (C) and maximal (D) ECAR in hypoxia- and normoxia-cultured MSCs. n = 3 biologically independent experiments.
E qRT-PCR analysis of glycolytic genes in hypoxic and normoxic cells. β-actin was used as an internal control for normalization. n = 3 biologically independent experiments.
F, G Basal (F) and maximal (G) OCR in hypoxia- and normoxia-cultured MSCs. n = 3 biologically independent experiments.
H MFI of hypoxia- and normoxia-cultured cells after staining with the MitoTracker Deep Red FM dye. n = 4 biologically independent experiments.
Data information: Results are shown as mean ± SEM and statistical significance was determined using a two-sided unpaired t-test. Source data are available online for this figure.
Figure EV3: Impaired lipogenesis of normoxia-cultured MSCs is not due to lower CIC levels.

A, B Representative images (A) and quantification of lipid droplets after observing cells under the electron microscope (left) and after staining lipids with Nile Red (right-B). Scale bars, 2 μm for electron microscopy images and 50 μm for confocal images. n = 4 biologically independent experiments and merged results are shown in (B). Results are shown as mean ± SEM and statistical significance was determined using a two-sided unpaired t-test.

C Representative images of hypoxia- and normoxia-cultured cells after immunostaining against SREBP1. Scale bar, 50 μm.

D Representative immunoblots against FASN and ACC1 in hypoxia- and normoxia-cultured cells. β-actin was used as a loading control. n = 3 biologically independent experiments.

Source data are available online for this figure.
Figure EV4. Quality control measurements of commercial MSCs fractionation.

A, B Representative images after immunostaining of normoxic and hypoxic commercially available MSCs against acetyl-lysine and TOMM20 (A) and assessment of cells (%) with mitochondrial acetyl-lysine signal localization (B). Nuclei were stained with DAPI. Quantification of cells (%) with mitochondrial acetyl-lysine signal in (B) from n = 201 hypoxic and n = 205 normoxic individual cells from a representative experiment of two biologically independent experiments is shown in (B). Results are shown as mean ± SEM and statistical significance was determined using a two-sided unpaired t-test. In magnified insets, the intensity of the acetyl-lysine signal was adjusted similarly to all samples for visualization purposes. Scale bar, 25 μm.

C Representative immunoblots against the mitochondrial proteins TOMM20 and MT-CO1 and the cytosolic protein α-tubulin in whole lysates, mitochondrial fractions and cytosolic fractions of normoxia- and hypoxia-cultured MSCs. n = 4 biologically independent experiments.

D Principal component analysis (PCA) plot showing clustering of normoxia- and hypoxia-cultured cells after metabolite extraction from the whole cell, mitochondrial and cytosolic fractions. n = 4 biologically independent experiments.

Source data are available online for this figure.
Figure EV5. Impaired CIC function in normoxic MSCs.

A qRT-PCR analysis of Slc25a1, which encodes citrate carrier. β-actin was used as an internal control for normalization. n = 3 biologically independent experiments. Results are shown as mean ± SEM and statistical significance was determined using a two-sided unpaired t-test.

B Representative images of hypoxia- and normoxia-cultured cells after immunostaining against CIC. Scale bar, 50 μm.

C Representative images after staining hypoxic and normoxic cells using an anti-FLAG antibody. Cells were transfected with either a vector plasmid or a FLAG-CIC-expressing plasmid. TOMM20 was used as a counterstain for mitochondria to confirm proper localization of the exogenously expressed CIC-FLAG protein, as shown in the magnified inset. Transfection and all downstream experiments were done for n = 2 biologically independent experiments. Scale bar; 50 μm.

D–F Representative images after staining cells used in (C) against acetyl-lysine and TOMM20 (D), assessment of the localization, as described above (E), and quantification of nuclear acetyl-lysine signal MFI (F). Nuclei were stained with DAPI. Quantification of nuclear acetyl-lysine MFI from n = 43 hypoxic, n = 63 normoxic and n = 38 normoxic_CIC OE individual cells from a representative experiment of two biologically independent experiments is shown in (F). Results are shown as mean ± SEM and statistical significance was determined with ordinary one-way ANOVA, using the Holm–Sidak’s multiple-comparisons test in Panels (E) and (F). The distribution of data points in (F) is shown as a violin plot, where the mean is indicated by a solid line and the quartiles are indicated with dashed lines. In magnified insets, the intensity of the acetyl-lysine signal was adjusted similarly to all samples, for visualization purposes. Scale bar, 25 μm.

Source data are available online for this figure.