

# **SUPPLEMENTAL MATERIAL**

## **Data S1.**

### **SUPPLEMENTAL METHODS**

#### **Global DNA Methylation**

Global methylation levels of CD34<sup>+</sup> stem cell DNA were measured using the Methylflash Global DNA Methylation ELISA Easy kit (Epigentek, Germany) following the manufacture's recommendations. Briefly, 100 ng of genomic DNA was used for 5-methyl cytosine(5-mC) quantitation. The input DNA was washed and incubated with a capture antibody. The wells were then washed, and detection antibody was applied. Use of enhancer solution and development solution created a color change proportional to the quantity of 5-mC content, and the samples were read colorimetrically on an automated plate reader at 450-nm absorbance. The use of a standard curve enabled the quantification of 5-mC based on absorbance measurements. The data were expressed 5-mC% (5-mC/total DNA (A+G+C+T)).

#### **DNMT Activity Assay**

Nuclear extracts of CD34<sup>+</sup> stem cells were prepared by EpiQuik™ Nuclear Extraction Kit (Epigentek, Germany), and the total DNMT activity of nuclear extracts was detected according to the manufacturer's protocol (EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Kit, Epigentek, Germany), the absorbance of samples taken from each well was measured on a microplate reader (Synergy HT, Bio-Tek) at 450-nm. The DNMT activity was calculated and expressed using the following formula: (Sample OD – Blank OD) / (protein amount (ug) x hour) x1000

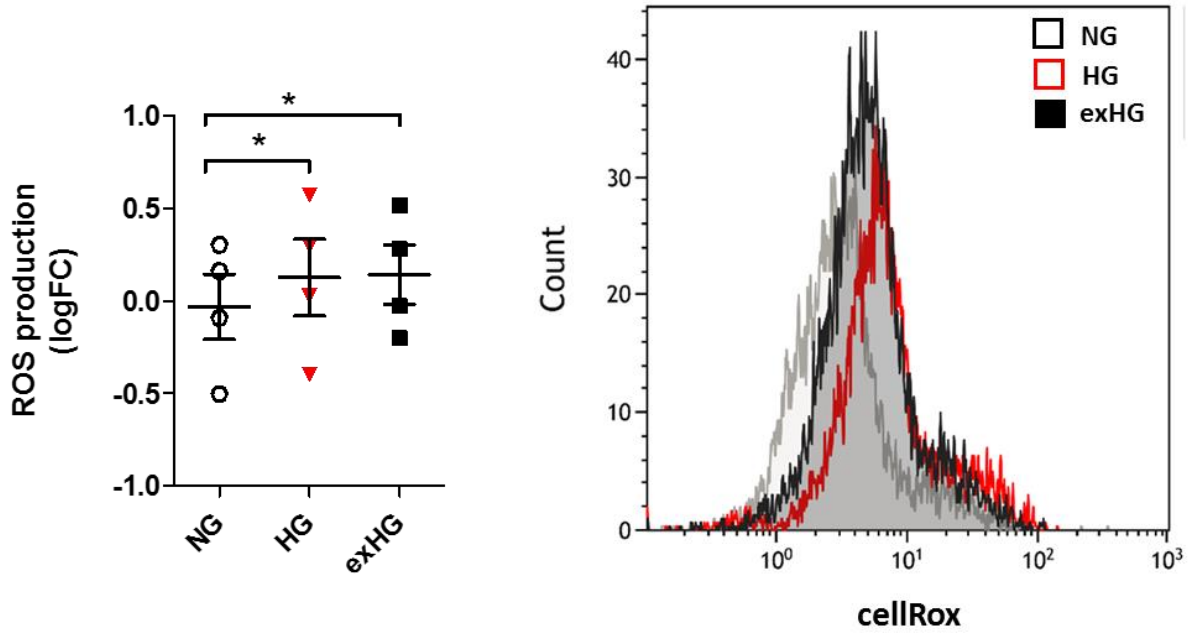
**Table S1. Antibody List.**

| Antibody  | Provider       | Clone or antintibody ID | WB Dilution | ChIP [ ] | MWt. (kDa)  |
|---|----------------|-------------------------|-------------|----------|-------------|
| Histone H4ac (pan-acetyl) antibody (pAb)              | Active Motif   | 39243                   |             | 5 µL     |             |
| Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade | QIAGEN         | GAH-6204                |             | 5 µg     |             |
| Anti-Histone H3K27me antibody - ChIP Grade            | Abcam          | ab6002                  |             | 5 µg     |             |
| Human RNA Polymerase II ChampionChIP™                 | QIAGEN         | GAH-111                 |             | 2 µg/ml  |             |
| MnSOD, Rabbit   | Abcam          | ab13533                 | 1:5000      |          | 25          |
| CAT, Rabbit   | Abcam          | ab52477                 | 1:1000      |          | 60          |
| pSShc, Rabbit   | Abcam          | ab24787                 | 1:500       |          | 66          |
| p-Akt, Rabbit   | Cell Signaling | 9271                    | 1:1000      |          | 60          |
| Akt, Rabbit   | Cell Signaling | 9272                    | 1:1000      |          | 60          |
| DNMT1, Rabbit   | Abcam          | ab19905                 | 1:200       |          | 184-176-144 |
| DNMT3B, Monoclonal Rabbit                             | Cell Signaling | D7070                   | 1:500       |          | 110         |
| GAPDH, Rabbit   | Cell Signaling | 14C10                   | 1:2000      |          | 37          |
| Anti-β-Actin–Peroxidase antibody, Monoclonal Mouse    | Sigma Aldrich  | AC-15                   | 1:10000     |          | 42          |

**Table S2. Primer List.**

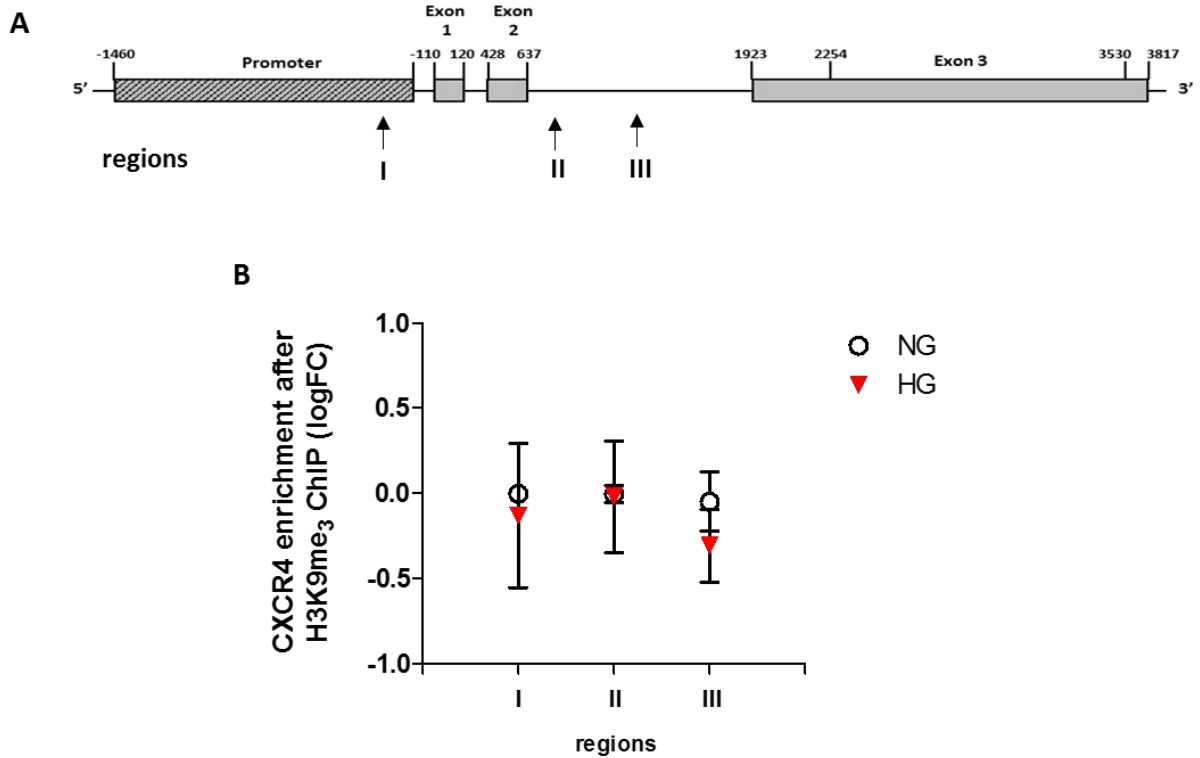
| Primer List |                             |                         |
|-------------|-----------------------------|-------------------------|
| c-DNA       | Name                        | Sequence 5' → 3'        |
|             | <b>MnSOD fw</b>             | CACCACAGCAAGCACCA       |
|             | <b>MnSOD rv</b>             | CTTGTCAAAGGAACCAAAGTCAC |
|             | <b>CAT fw</b>               | CTCGTGGGTTTGCAGTGAA     |
|             | <b>CAT rv</b>               | TGGCTGTGGATAAAAAGATGGA  |
|             | <b>p66<sup>shc</sup> fw</b> | AAGTACAATCCACTCCGGAATGA |
|             | <b>p66<sup>shc</sup> rv</b> | GGGCCCCAGGGATGAAG       |
|             | <b>DNMT1 fw</b>             | GTCAAACCAAAGAACCAACACC  |
|             | <b>DNMT1 rv</b>             | GACTTCTGTGCTTCTTCTCATCT |
|             | <b>DNMT3A fw</b>            | GTAACCTTCCCAGTATGAACAG  |
|             | <b>DNMT3A rv</b>            | CCTGCTTTATGGAGTTTGACCT  |
|             | <b>DNMT3B fw</b>            | AAACCCAACAACACGCAA      |
|             | <b>DNMT3B rv</b>            | TTCTCGGCTCTGATCTTCATC   |
|             | <b>TET2 fw</b>              | AGGAAGAGCAGTAAGGGACT    |
|             | <b>TET2 rv</b>              | GAGGTGATGGTATCAGGAATGG  |
|             | <b>TET3 fw</b>              | GGAACAACCAAAGGAGAAGGA   |
|             | <b>TET3 rv</b>              | CTACCAGGAGCTCACCGA      |
|             | <b>CXCR4 fw</b>             | AGCAGGTAGCAAAGTGACG     |
|             | <b>CXCR4 rv</b>             | CCTCGGTGTAGTTATCTGAAGTG |
| ChIP Assay  |                             |                         |
|             | <b>Region1 CXCR4 fw</b>     | CGCCCAGTTCCTCAACCTAA    |
|             | <b>Region1 CXCR4 rv</b>     | GCAAATAAGCCCGGAGAGAT    |
|             | <b>Region2 CXCR4 fw</b>     | CGGGTAACTGGATCAGTGG     |
|             | <b>Region2 CXCR4 rv</b>     | AAATGAACAAACGGCACCTC    |
|             | <b>Region3 CXCR4 fw</b>     | CACCCTGTGGGACAGAGC      |
|             | <b>Region3 CXCR4 rv</b>     | GCCCCAAGTTTCATTTCTC     |

**Figure S1. Flow cytometric quantification of ROS production in HG and exHG-CD34<sup>+</sup> stem cells.**



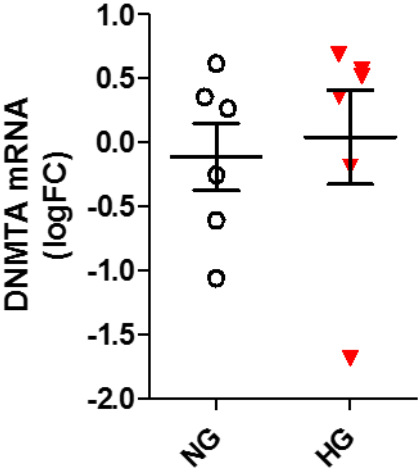
The data are reported as log<sub>2</sub> fold-change (FC) of MFI over control (NG), ( $n=4$ ;  $*p<0.05$  vs NG; 1-way ANOVA followed by Newman-Keuls *post hoc* analysis). exHG indicates ex-high glucose; HG, high glucose; MFI, mean fluorescence intensity; NG, normal glucose; ROS, radical oxygen species.

**Figure S2. A, Schematic representation of the CXCR4 gene with mapped sequences (arrows, region I, II and III). B, ChIP analysis of the CXCR4 promoter for H3K9me3 modification after hyperglycaemia exposure of CD34<sup>+</sup> stem cells.**



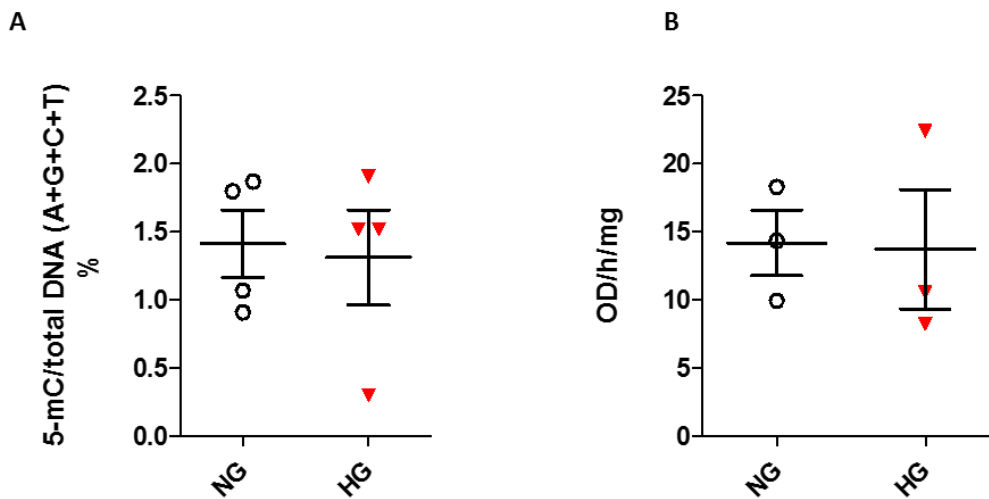
The data, after input normalization are expressed as log<sub>2</sub> fold change over NG ( $n=3$ ; paired  $t$ -test). ChIP indicates chromatin immunoprecipitation; CXCR4, C-X-C chemokine receptor type 4; H3K9me<sub>3</sub>, trimethylation on lysine 9 of histone H3; HG, high glucose; NG, normal glucose.

**Figure S3. Analysis of DNMT3A, expression in CD34<sup>+</sup> stem cells by qPCR.**



The data expressed as log<sub>2</sub> fold change over NG are from at least 6 independent experiments (NG vs HG; paired *t*-test). DNMT3A indicates DNA methyltransferase 3A; HG, high glucose; NG, normal glucose.

**Figure S4. A, Effect of HG exposure on global 5-mC methylation level in CD34<sup>+</sup> stem cells. The data are expressed as 5-mC% (5-mC/total DNA (A+G+C+T)). B, DNMT activity in HG-CD34<sup>+</sup> stem cells after HG exposure.**



The DNMT activity was calculated and expressed using the following formula: (Sample OD – Blank OD) / (protein amount (ug) x hour) x1000. The data are from at least 3 independent experiments (NG vs HG; paired *t*-test). DNMT indicates DNA methyltransferase; HG, high glucose; NG, normal glucose; OD, optical density.