ADDITIONAL FILES

Repressive H3K27me3 drives hyperglycemia-induced oxidative and inflammatory transcriptional programs in human endothelium

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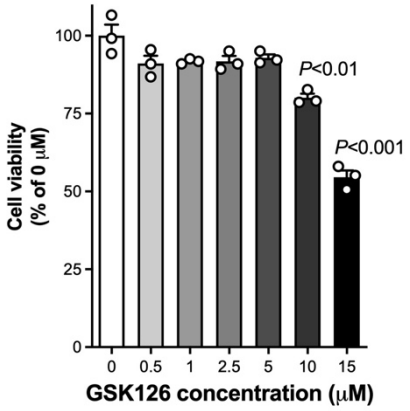

Fig. S1 HAEC viability. Cells were exposed to increasing concentrations of GSK126 (0-15 μmol/l) for 20 h (n=3/group).

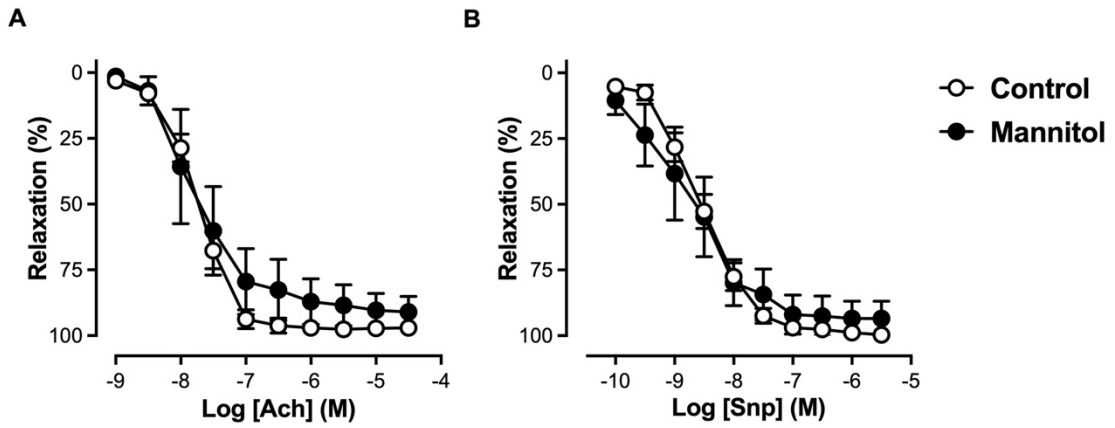

Fig. S2 Mannitol and endothelial function. (A) Endothelium-dependent relaxations to acetylcholine (Ach) and **(B)** endothelium-independent relaxations to sodium nitroprusside (SNP) after 20-hour exposure to normal (5 mmol/l) or high (25 mmol/l) concentrations of mannitol (n=4-6/group).

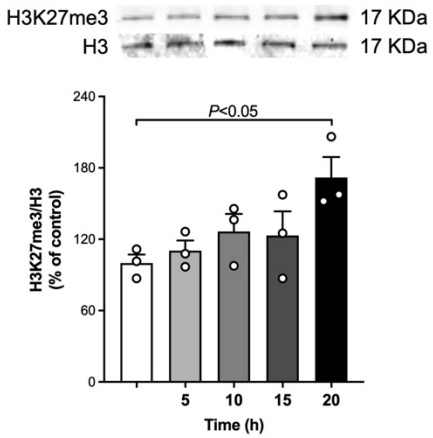

Fig. S3 Time-course of high glucose concentration and H3K27me3 expression. Representative western blot images and relative densitometric quantifications showing H3K27me3 protein in HAEC exposed to high glucose (25 mmol/l). The open bar represents H3K27me3 in cell exposed to normal glucose (5 mmol/l, n=3/group).

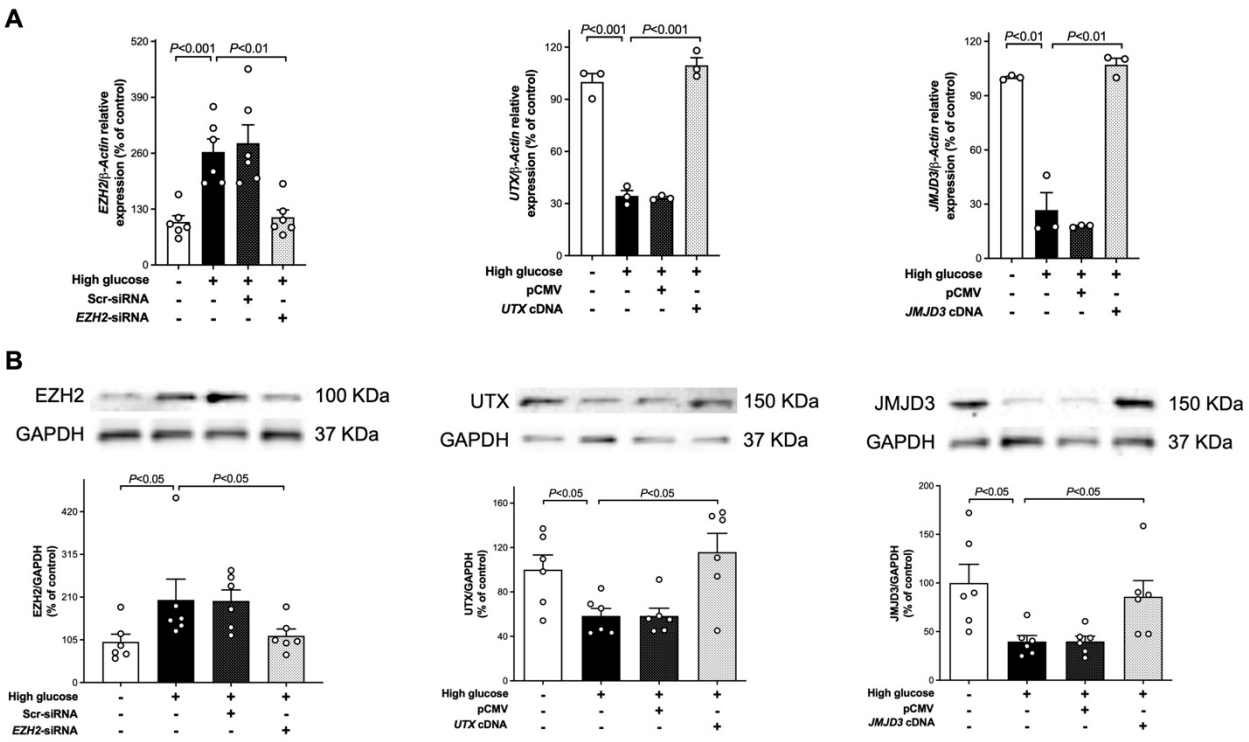

Fig. S4 Reprogramming of chromatin modifying enzymes by EZH2 siRNA, and UTX or JMJD3 overexpressing vectors. (A) mRNA and **(B)** protein expression of EZH2, UTX, and JMJD3 in HAEC exposed to normal (5 mmol/l) and high glucose (25 mmol/l). Scramble-siRNA and pCMV were used as controls for siRNA-mediated knockdown and vector-based overexpression, respectively (n=3-6/group).

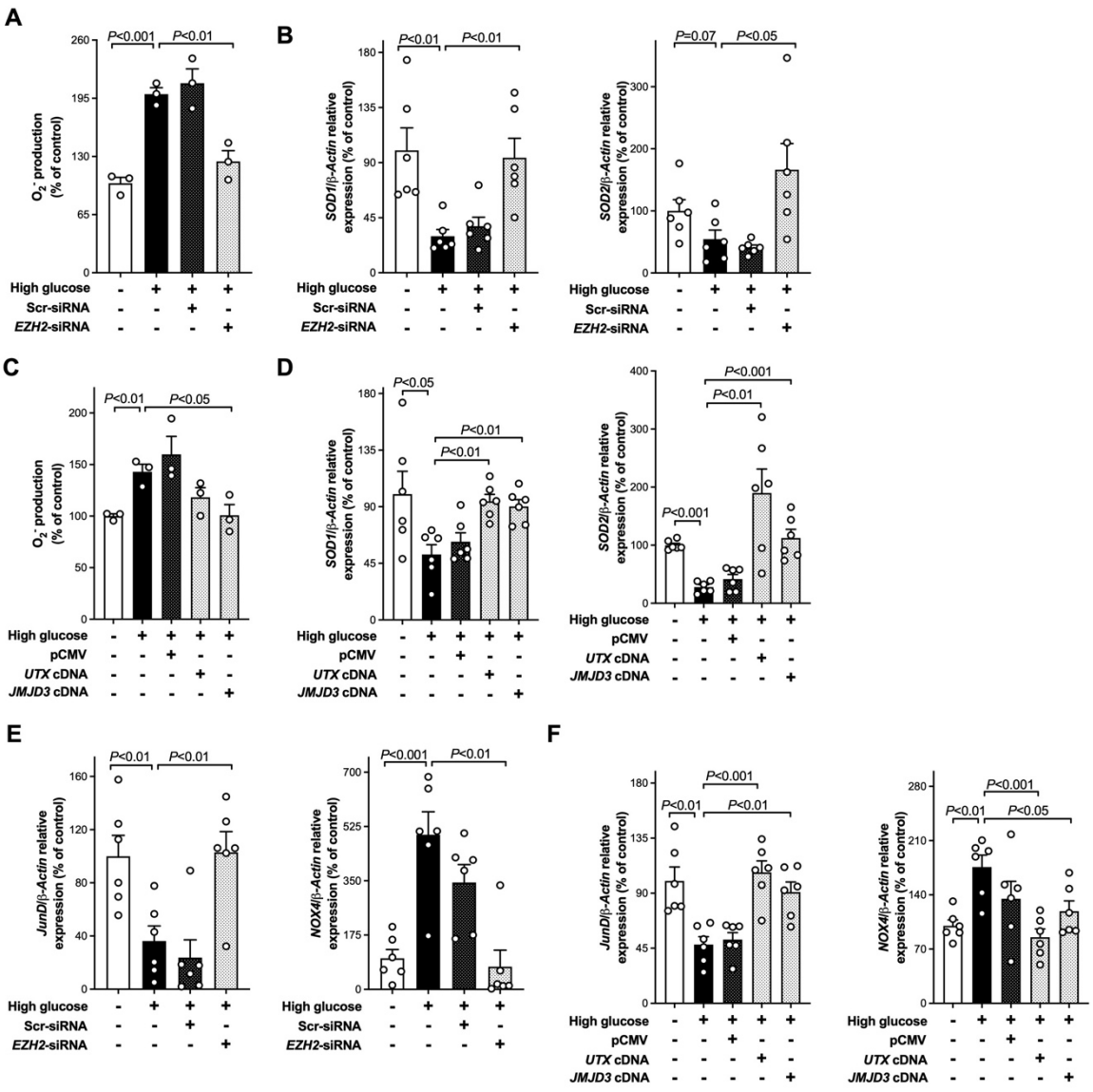

Fig. S5 Reprogramming of EZH2, UTX and JMJD3 expression levels blunts glucose-induced oxidative stress. **(A, C)** O_2^- production in the absence and in the presence of EZH2-siRNA, UTX cDNA, and JMJD3 cDNA, respectively (n=3/group). **(B and D-F)** Gene expression of *SOD1, SOD2*, *JunD* and *NOX4* in HAEC exposed to normal (5mmol/l) or high glucose in the presence and in the absence of EZH2-siRNA, UTX or JMJD3 overexpressing vectors. Scramble-siRNA and pCMV were used as controls for siRNA-mediated knockdown and vector-based overexpression, respectively (n=6/group).

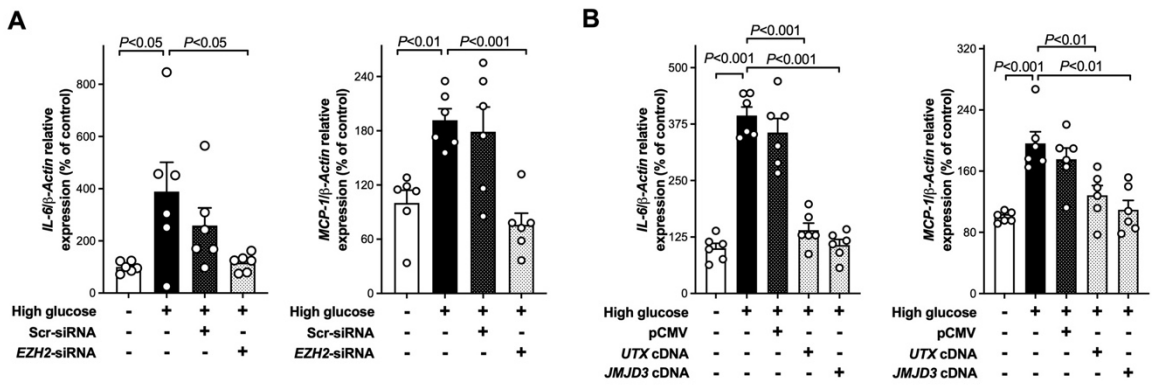

Fig. S6 Reprogramming of EZH2, UTX and JMJD3 expression abolishes glucose-induced inflammation. RT-qPCR showing *IL-6* and *MCP-1 gene expression* in HAEC exposed to normal (5 mmol/l) or high glucose (25 mmol/l) in the presence and in the absence of **(A)** EZH2 siRNA, and **(B)** UTX and JMJD3 overexpressing vectors. Scramble-siRNA and pCMV were used as controls for siRNA-mediated knockdown and vector-based overexpression, respectively (n=6/group).

Table S1. Primers used in RT-qPCR experiments.

	Primer sequences (5′ → 3′)	
Gene	**Forward**	**Reverse**
β-Actin	GTTGTCGACGACGAGCG	GCACAGAGCCTCGCCTT
ALDH1	CCACTCACTGAATCATGCCA	GCACGCCAGACTTACCTGTC
ALDH2	ACAATGGCAAGCCCTATGTC	ACAGGTTCATGGCGTGTGTA
CAT	AGTCAGGGTGGACCTCAGTG	CTGGAGAAGTGCGGAGATTC
EZH1	ACCATCGGATTGGGATCTTT	GCATCAGCTTGGCTGTACCT
EZH2	GGACTCAGAAGGCAGTGGAG	CCCTTCTCAGATTTCTTCCCA
GPX1	TTGACATCGAGCCTGACATC	ACTGGGATCAACAGGACCAG
ICAM-1	TCACACTGACTGAGGCCTTG	GGCTGGAGCTGTTTGAGAAC
IL-6	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
JunD	ATCGACATCGACACGCAGGAG	CTCCGTGTTCTGACTCTTCAGG
UTX	AATTCTGGGAGGAGGAGGAA	ATACGACACGCACAAAAGCA
JMJD3	AGACAGGGCACACCAAACTC	AGTCCTTTCACAGCCAATTCC
UTY	GAGCTGGAATGCAATGGTG	TGAGGTCAGGAGTTCAAGACAAG
MCP-1	AGCAAGTGTCCCAAAGAAGC	TGGAATCCTGAACCCACTTC
NOX4	GGATAAGGCTGCAGTTGAGG	AACCAAGGGCCAGAGTATCA
SOD1	CCACACCTTCACTGGTCCAT	CTAGCGAGTTATGGCGACG
SOD2	GCTCCGGTTTTGGGGTATCTG	GCGTTGATGTGAGGTTCCAG
TNFα	CAGCGCTGAGTCGGTCACCC	AGCCGCATCGCCGTCTCCTA
VCAM-1	TAAAATGCCTGGGAAGATGG	GGTGCTGCAAGTCAATGAGA
JunD	AATAACTCCTTGGCGCCTCC	CCAAGCGCTTTCAGCAACTT
NOX4	GATAAAGAAACTGGCGGCTG	GTAACGAAATTTGAGCCGGA
SOD1	AATCCTTGGCCCGAAAACCC	CAGCCAGCCCAGGAACGCAG
SOD2	TAAGCCGACCTTGGGACCTA	CAAAACATGACTGCCAGGGC

Table S2. Primers used in ChIP-qPCR assays.

	Primer sequences (5′ → 3′)	
Gene	**Forward**	**Reverse**
JunD promoter	AATAACTCCTTGGCGCCTCC	CCAAGCGCTTTCAGCAACTT
NOX4 promoter	GATAAAGAAACTGGCGGCTG	GTAACGAAATTTGAGCCGGA
SOD1 promoter	AATCCTTGGCCCGAAAACCC	CAGCCAGCCCAGGAACGCAG
SOD2 promoter	TAAGCCGACCTTGGGACCTA	CAAAACATGACTGCCAGGGC