cancers

MDPI

Supplementary Materials

High-Throughput Drug Screening Revealed That Ciclopirox Olamine Can Engender Gastric Cancer Stem-like Cells

Table S1: Primers used for real-time PCR analysis

Gene	Primer Forward (5′ → 3′)	Primer Reverse (5′ → 3′)
ARNT2	AAGAACCGGGAGTGGATGTTG	CTGTTGCTGAAGTTGCTTGACG
β-catenin	CAATGGCTTGGAATGAGACTGC	GGATCATCCTGGTGATATCCAAG
c-Jun	AGTGAGTGACCGCGACTTTT	GATGCCTCCCGCACTCTTAC
C-MYC	GCGACTCTGAGGAGGAACAAG	TGGGCTGTGAGGAGGTTTG
CNOT3	GGCTCACGAATACCATCGACA	GCTTATCCTTGTCGCCCTTCT
FOXA1	AAGGGCATGAAACCAGCGAC	GCCTGAGTTCATGTTGCTGAC
FOXA2	ATGCACTCGGCTTCCAGTAT	GTTGCTCACGGAGGAGTAGC
FOXC2	CTACAGCTACATCGCGCTCATCA	ACTGGTAGATGCCGTTCAAGGTG
GATA6	AAGCGCGTGCCTTCATCA	CATAGCAAGTGGTCTGGGCA
GLI1	GAAGTCATACTCACGCCTCGAA	CAGCCAGGGAGCTTACATACAT
GLI2	AGCAGCAGCAACTGTCTGAGTGA	GACCTTGCTGCGCTTGTGAA
Hes1	ACACGACACCGGATAAACCA	ATGCCGCGAGCTATCTTTCT
HIF-1α	GGCGCGAACGACAAGAAAAAGATA	GGCCTTATCAAGATGCGAACTCAC
HMGA1	GTGCCAACACCTAAGAGACCT	TCTGCTGGTTTTCCGGCTC
HMGA2	CCCAAAGGCAGCAAAAACAA	GCCTCTTGGCCGTTTTTCTC
HOXA10	CCCTTCCGAGAGCAGCAAA	TCTTCCGACCACTCTTTGCC
HOXA5	GCGCAAGCTGCACATAAGTC	CGGAGAGGCAAAGAGCATGT
ISL1	CTGCTTTTCAGCAACTGGTCA	TAGGACTGGCTACCATGCTGT
KLF4	CAGAGGAGCCCAAGCCAAAG	TTTCTCACCTGTGTGGGTTCG
KLF5	AAGGAGTAACCCCGATTTGG	CAGCCTTCCCAGGTACACTT
MEIS2	TCCAGCATCTCACACATCCG	GAAAACCTGCTCGATTTGACTGG
NANOG	GAACCTCAGCTACAAACAGGTGAA	TTCTGCGTCACACCATTGCT
OCT1	CAAAATGGCGGACGGAGGA	GTTCATTCTTGAGTCTGCTGCTG
OCT4	TGCAGCAGATCAGCCACAT	ACACTGGTCCCCCTGAGAAA
RelA	GCTGCATCCACAGTTTCCAGA	CCCCACGCTGCTCTTCTAT
SOX4	GACTTCGAGTTTGCTCCCCT	TAACTCGCCTTCTTGCTGGG
SOX9	CGGAGGAAGTCGGTGAAG	CTGGGATTGCCCCGAGTGCT

STAT3	GAGGACTGAGCATCGAGCA	CATGTGATCTGACACCCTGAA
TCF4	TTTGGAAGAAGCGGCCAAGAGG	TTGGGGAGGTAGGGGCTCGT
TEAD2	TTTGGGGTGTGCCCAGATG	TCCTCACTGCCTTCCTCACT
ZNF273	AGCCTAGAAATGGGACCACTG	GCTGTGAAGTGTCCAGGCAT
18S	CGCCGCTAGAGGTGAAATTC	CATTCTTGGCAAATGCTTTCG

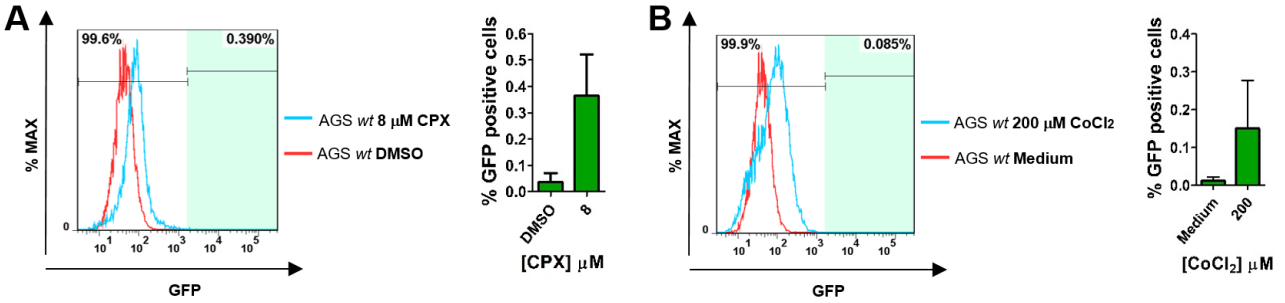


Figure S1: Evaluation of the percentage of GFP positive cells by flow cytometry. Flow cytometry analysis of GFP expression in AGS wt cells **(A)** after 48 h of treatment with 8 µM CPX and **(B)** after 48 h of treatment with 200 µM $CoCl_2$. wt corresponds to the parental cell line (AGS). DMSO or medium were used as a negative control. Results are mean ± SD of at least three independent experiments.

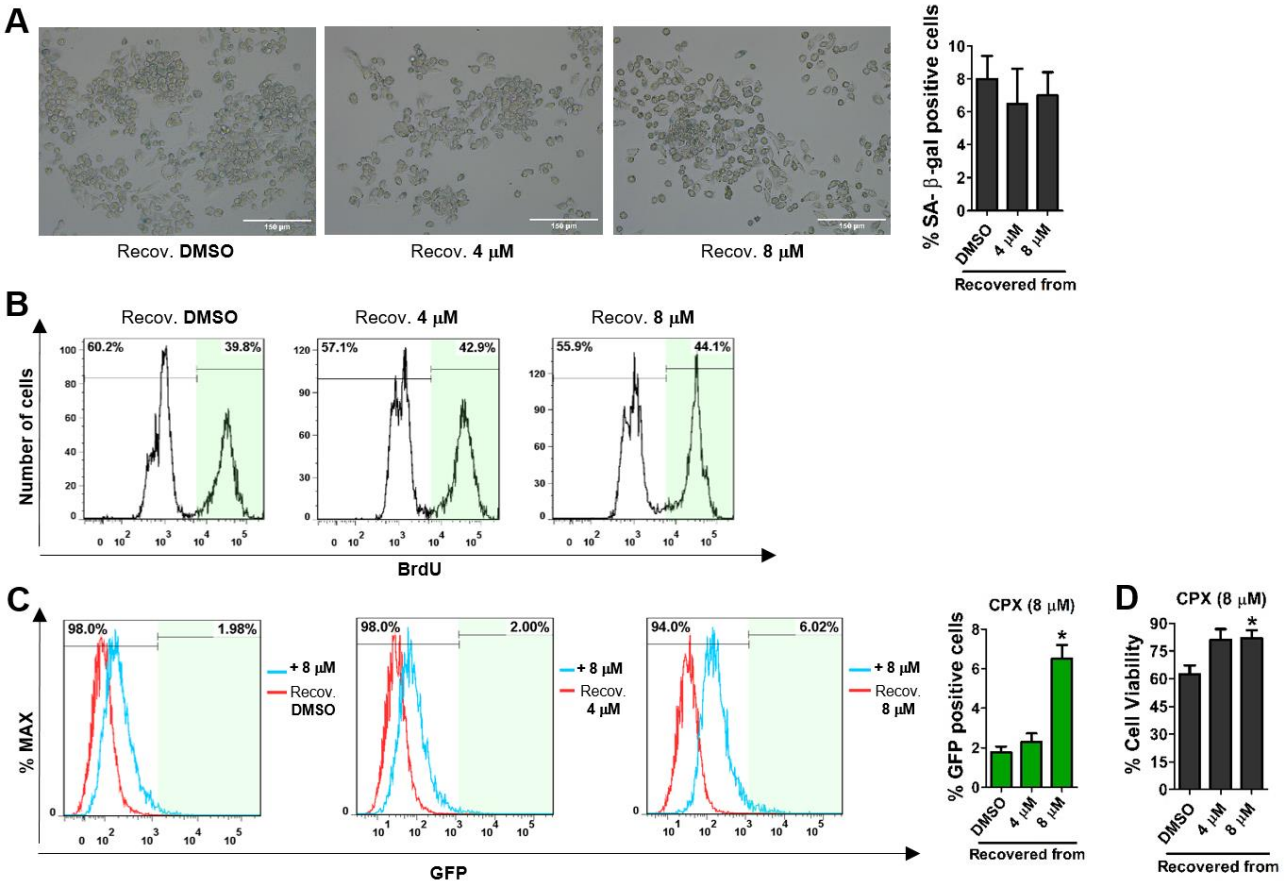


Figure S2: CPX-induced cellular senescence is reversible and cells regain the proliferative ability. **(A)** Percentage of SA-β-gal positive cells in Kato III SORE6− cells 4 weeks after recovery from treatment with DMSO, 4 or 8 μM CPX for 48 h, following representative images of SA-β-gal marking. Scale bar = 150 μm. **(B)** Representative flow cytometry analysis of the percentage of BrdU positive cells in AGS SORE6− cells 4 weeks after recovery from treatment with DMSO, 4 or 8 μM CPX for 48 h. **(C)** Percentage of cell viability after 48 h of treatment of AGS SORE6− and Kato III SORE6− recovered cells with 4.5 μg/mL (IC50) of 5-FU. **(D)** Percentage of GFP-positive cells, following the respective flow cytometry analysis, and (E) cell viability in Kato III SORE6− recovered cells after new treatment with 8 μM CPX for 48 h. DMSO was used as a negative control. Results are mean ± SD of at least three independent experiments. Significant differences * $p \leq 0.05$.

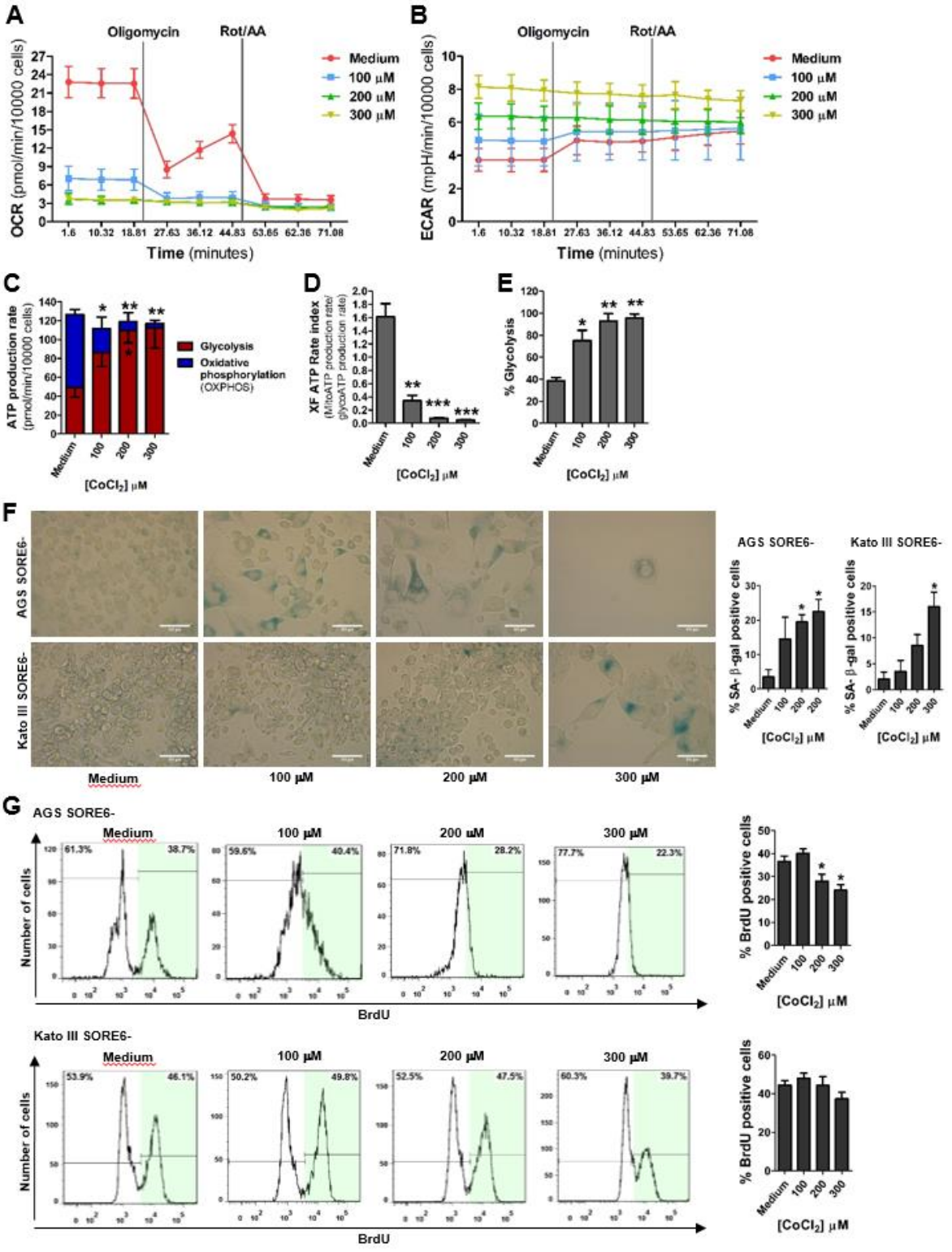


Figure S3: $CoCl_2$ treatment inhibits ATP production by OXPHOS and induces cellular senescence with consequent loss of proliferative activity. (A) Representative real-time measurements of the kinetic profile of the OCR, **(B)** representative real-time measurements of the kinetic profile of the ECAR, **(C)** metabolic flux analysis showing quantification of mitochondrial ATP production and glycolytic ATP production, **(D)** ATP production rate index calculated from the data showed in H (i.e., mitochondrial ATP production rate/glycolytic ATP production rate) and **(E)** percentage of gycolysis following treatment with 100, 200 or 300 μM $CoCl_2$ for 48 h. **(F)** Percentage of SA-β-gal positive cells in AGS SORE6− and Kato III SORE6− cells treated with 100, 200 or 300 μM $CoCl_2$ for 48 h, followed by representative images of SA-β-gal marking. Scale bar = 150 μm. **(G)** Percentage of

BrdU positive cells in AGS SORE6− and Kato III SORE6− cells treated with 100, 200 or 300 μM $CoCl_2$ for 48 h, followed by representative flow cytometry analysis. Medium was used as a negative control. Results are mean ± SD of at least three independent experiments. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

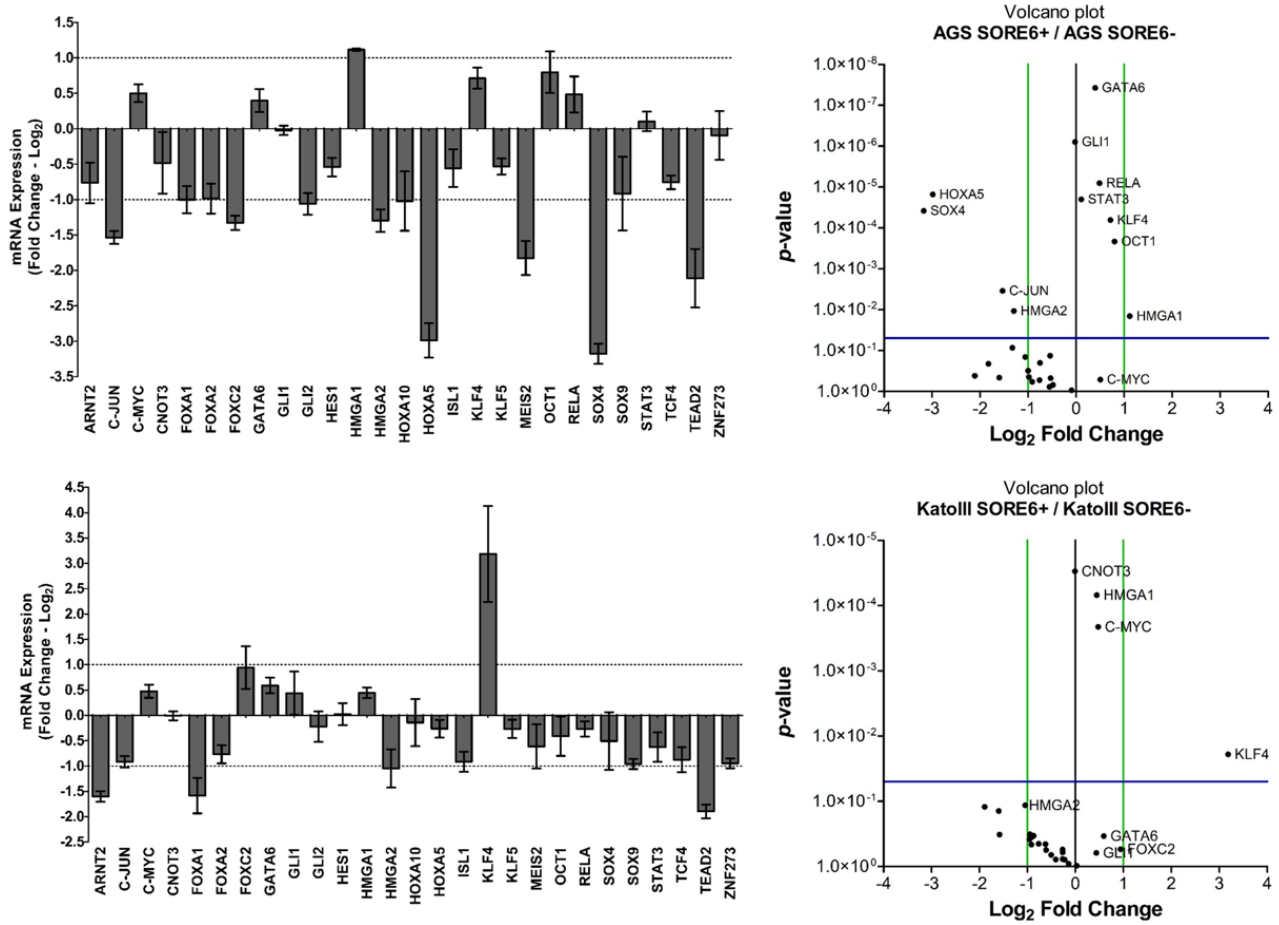
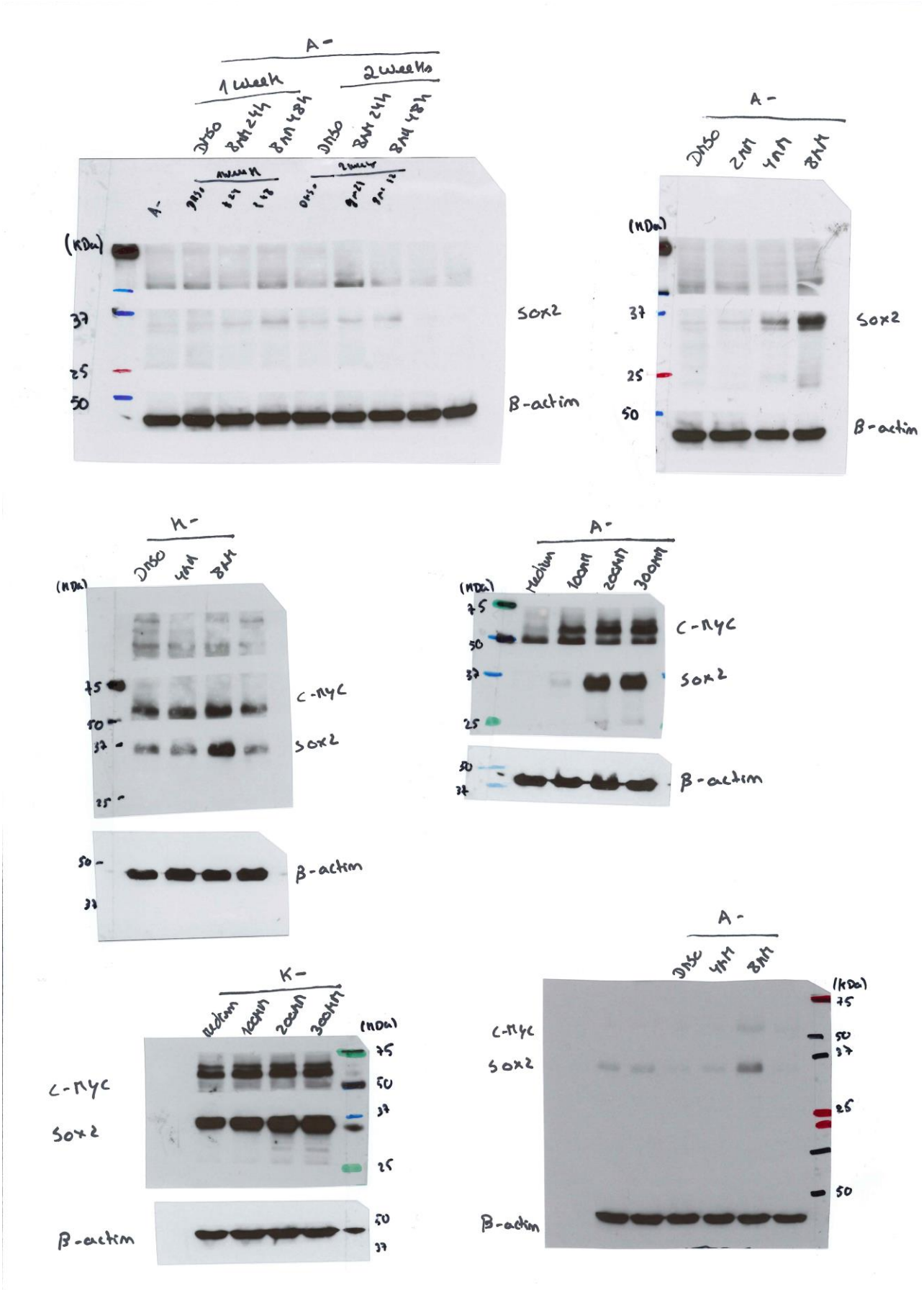


Figure S4: Comparison of the expression of relevant transcription factors between SORE6+ and SORE6− cells. Real-time PCR analysis of mRNA levels of the transcription factors ARNT2, C-JUN, C-MYC, CNOT3, FOXA1, FOXA2, FOXC2, GATA6, GLI1, GLI2, HES1, HMGA1, HMGA2, HOXA10, HOXA5, ISL1, KLF4, KLF5, MEIS2, OCT1, RELA, SOX4, SOX9, STAT3, TCF4, TEAD2 and ZNF273 in AGS SORE6+ and Kato III SORE6+ cells in comparison to AGS SORE6− and Kato III SORE6− cells. Followed by the volcano plot (SORE6+ compared to SORE6− cells) where the horizontal blue line represents the threshold of statistical significance ($p = 0.05$) and the green lines corresponds to the fold change cut-off ≥ 2. Results were normalized to 18S expression. Results are mean ± SD of at least three independent experiments.

UNCROPPED WESTERN BLOT FIGURES


A-
1 week
2 weeks
DMSO
8nM 24h
8nM 48h
(KDa)
37
25
50
Sox2
β-actin
A-
DMSO
2nM
4nM
8nM
(KDa)
37
25
50
Sox2
β-actin
K-
DMSO
4nM
8nM
(KDa)
75
50
37
25
c-Myc
Sox2
50
37
β-actin
A-
Medium
100nM
200nM
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