Supplementary Figure S1. Effect of inhibition of *Nr1d1* expression on cellular senescence.
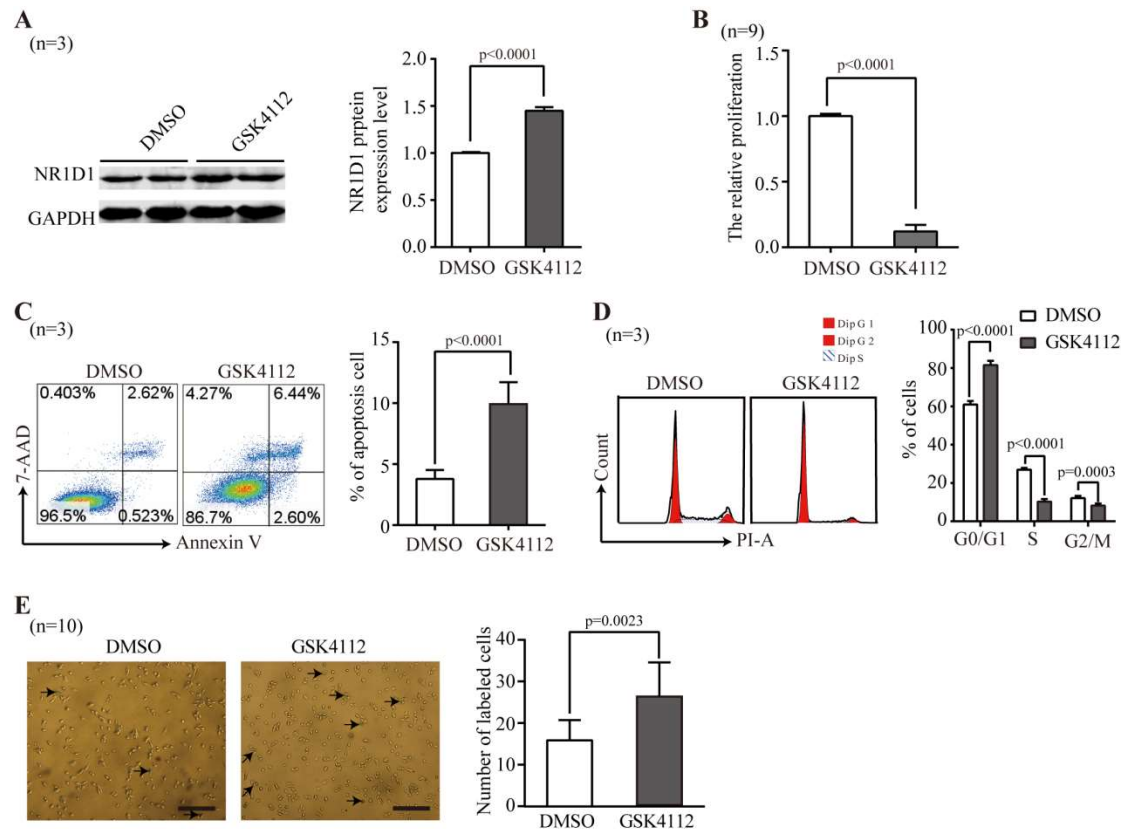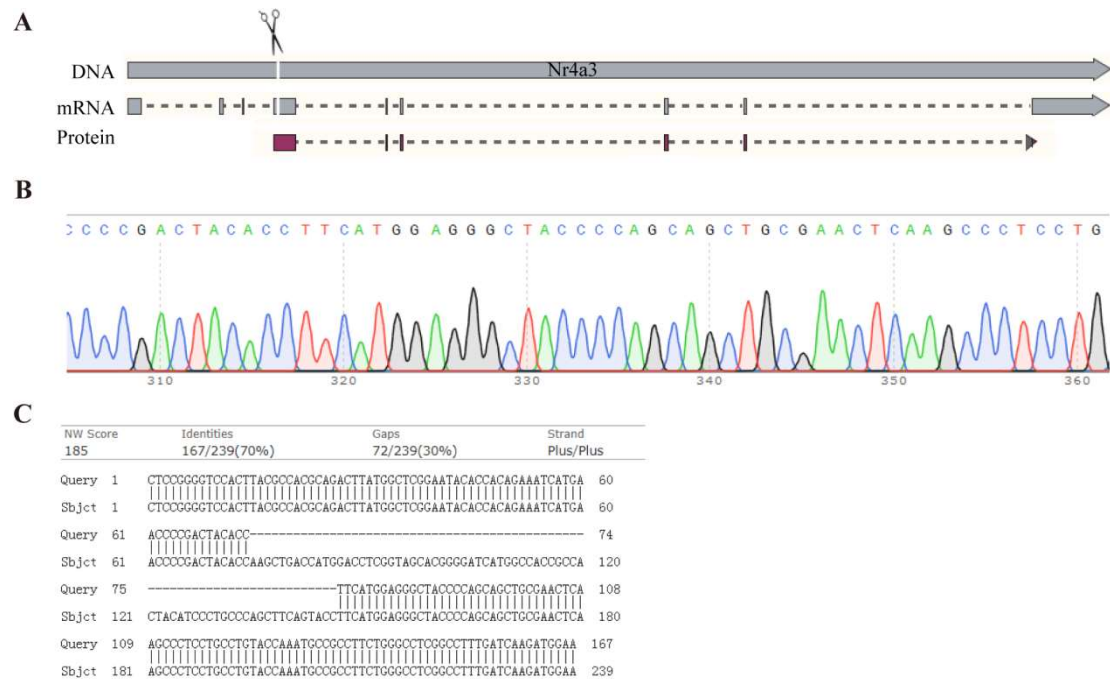
(A-E): MCM was treated with SR8278, and the control was treated with DMSO. (A) Immunoblotting of NR1D1 protein levels in MCM cells, data were from three independent experiments(n=3). Two-tail unpaired T test was applied for statistical analysis, $p < 0.05$ was considered significant. (B): MEM proliferation was detected by MTT assay, data were from three independent experiments, each experiment had 3 replicates(n=9). Two-tail unpaired T test was applied for statistical analysis, $p < 0.05$ was considered significant. (C): Apoptosis of MEM detected by annexin V-PE/7-AAD. The left panel is a representative flow cytometric scatter plot, and the right panel is a graph of apoptosis statistics, data were from three independent experiments(n=3). Two-tail unpaired T test was applied for statistical analysis, $p < 0.05$ was considered significant. (D): Cell cycle analysis in MCM performed by FCM, data were collected from three independent experiments(n=3), two-way ANOVA was applied for statistical analysis and $p < 0.05$ was considered significant. (E): Analysis of Senescence-associated beta-galactosidase staining in MCM. The image on the left is a representative field of view, scale bars: 50 μm. The graph on the right shows the number of cells that were stained blue in each random field of view. Each experiment was repeated 3 times, and 10 random fields of view were counted(n=10). Two-tail unpaired T test was applied for statistical analysis, $p < 0.05$ was considered significant. All data in bar graph presented as mean ± SD.

Supplementary Figure S2. Effect of promoting *Nr1d1* expression on cellular senescence.

(A-E): MCM was treated with GSK4112, and the control was treated with DMSO. (A) Immunoblotting of NR1D1 protein levels in MCM cells, data were from three independent experiments(n=3), two-tail unpaired T test was applied for statistical analysis, $p < 0.05$ was considered significant. (B): MEM proliferation was detected by MTT assay, data were from three independent experiments, each experiment had 3 replicates(n=9), two-tail unpaired T test was applied for statistical analysis, $p < 0.05$ was considered significant. (C): Apoptosis of MEM detected by annexin V-PE/7-AAD. The left panel is a representative flow cytometric scatter plot, and the right panel is a graph of apoptosis statistics, data were from three independent experiments(n=3), two-tail unpaired T test was applied for statistical analysis, $p < 0.05$ was considered significant. (D): Cell cycle analysis in MCM performed by FCM, data were collected from three independent experiments(n=3), two-way ANOVA was applied for statistical analysis and $p < 0.05$ was considered significant. (E): Analysis of Senescence-associated beta-galactosidase staining in MCM. The image on the left is a representative field of view, scale bars: 50 μm. The graph on the right shows the number of cells that were stained blue in each random field of view. Each experiment was repeated 3 times, and 10 random fields of view were counted(n=10), two-tail unpaired T test was applied for statistical analysis, $p < 0.05$ was considered significant.. All data in bar graph presented as mean ± SD.

A

DNA   Nr4a3

mRNA

Protein

B

C C C C G A C T A C A C C T T C A T G G A G G G C T A C C C C A G C A G C T G C G A A C T C A A G C C C T C C T G

310   320   330   340   350   360

C

NW Score	Identities	Gaps	Strand
185	167/239(70%)	72/239(30%)	Plus/Plus

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Query   1    CTCCGGGGTCCACTTACGCCACGCAGACTTATGGCTCGGAATACACCACAGAAATCATGA   60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct   1    CTCCGGGGTCCACTTACGCCACGCAGACTTATGGCTCGGAATACACCACAGAAATCATGA   60

Query   61   ACCCCGACTACACC---------------------------------------------   74
             ||||||||||||||
Sbjct   61   ACCCCGACTACACCAAGCTGACCATGGACCTCGGTAGCACGGGGATCATGGCCACCGCCA   120

Query   75   -----------------------TTCATGGAGGGCTACCCCAGCAGCTGCGAACTCA   108
                                    |||||||||||||||||||||||||||||||||
Sbjct   121  CTACATCCCTGCCCAGCTTCAGTACCTTCATGGAGGGCTACCCCAGCAGCTGCGAACTCA   180

Query   109  AGCCCTCCTGCCTGTACCAAATGCCGCCTTCTGGGCCTCGGCCTTTGATCAAGATGGAA   167
             |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct   181  AGCCCTCCTGCCTGTACCAAATGCCGCCTTCTGGGCCTCGGCCTTTGATCAAGATGGAA   239
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Supplementary Figure S3. Knockout of *Nr4a3*.
(A-C): Knockdown of *Nr4a3* gene in MCM cells by CRISPR/Cas9. (A): Strategies for the *Nr4a3* mutation. (B): Sequencing results of genes near the sgRNA locus after mutation.(C): The deletion of the target gene bases was detected by NCBI-BLAST section.