Supplementary Methodology

Plasmid transfection and overexpression

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EPO levels were quantified in HepG2 cell-culture supernatants and mouse serum, using commercial ELISA kits specific for human (Novus biologicals) and mouse (R&D Systems) EPO, according to the manufacturer's instructions.

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65 °C for 6h. The purified DNA was amplified using specific primers for the EPO and BMAL1 promoters. GAPDH primers were used as a negative control DNA region to check the non-specific amplification and specificity of the antibodies used in the assay. The primer sequences used in the ChIP assay were as follows: EPO h-RRE1 forward 5'- AGTGTCTGGGTTTGCAGAGTAC-3' and reverse 5'-GTCTGGAAGCCCAGAAGCTTTA-3', EPO h-RRE2 forward 5'-TGGACTGTGTGCTCTGTGCA-3' and reverse 5'-TGGAGCAGCTGGGACTACAG-3' and BMAL1 forward 5'-TTGGGCACAGCGATTGGTG-3', reverse 5'-TAAACAGGCACCTCCGTCCC -3' and GAPDH forward 5'- CGGTGCGTGCCCAGTTG-3' and reverse 5'- GCGACGCAAAAGAAGATG-3'.

Primers for Electrophoretic mobility shift assay (EMSA)

The oligonucleotide sequence containing h-RRE1 (5'-TTCCTCAACATGGTCAACATTGTTTC-3' and 5'-GAAACAATGTTGACCATGTTGAGGAA-3') and h-RRE2 (5'-TCACCTGAGGTCAGGAGTTCAAGACCG-3' and (5'-CGGTCTTGAACTCCTGACCTCAGGTGA-3') response elements present on human *EPO* promoter were used. Mutant EPO oligonucleotides mut h-RRE1 (5'-TTCCTCAACA*GCCGAT*ACATTGTTTC-3' and (5'-GAAACAATGTATCGGCTGTTGAGGAA-3') and mut h-RRE2 (5'-TCACCTG*CTTGAC*GG*CTTGAC*AGACCG-3' and (5'-CGGTCTGTCAAGCCGTCAAGCAGGTGA-3') were generated whereby the mutations were done in the core motif.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from cells, kidneys, and livers using the TRIzol reagent (Ambion, Invitrogen), and 1 μg of RNA was reverse transcribed as per the manufacturer's instructions using the Verso cDNA Synthesis Kit (Thermo Scientific). The cDNA was then subjected to SYBR green (Thermo Scientific)-based qRT-PCR with gene-specific primers. Using the formula $2^{-\Delta\Delta Ct}$, the relative abundance of the genes was calculated.

Immunoblot analysis

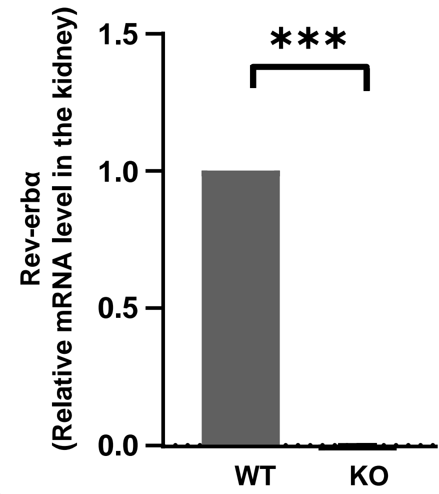
For immunoblot analysis, cells were subjected to lysis using mammalian cell lysis buffer for 15 minutes on ice, and protein concentrations in the samples were quantified by Bradford reagent (Sigma-Aldrich). Furthermore, the protein was resolved with SDS-polyacrylamide gel electrophoresis (PAGE) followed by transfer to a polyvinylidene difluoride membrane (PVDF; Immobilon-P, Millipore). The membranes were blocked with 5% BSA (Merck Millipore) and incubated overnight at 4°C with primary antibodies. Membranes were then washed three times with TBS (Tris-HCL-buffer saline) containing 0.1% Tween 20 (Sigma-Aldrich), followed by 60 minutes at room temperature incubation with suitable HRP-conjugated secondary antibodies (Santa Cruz Biotechnology) and visualization with chemiluminescent HRP substrate Luminata Forte (Millipore).

A

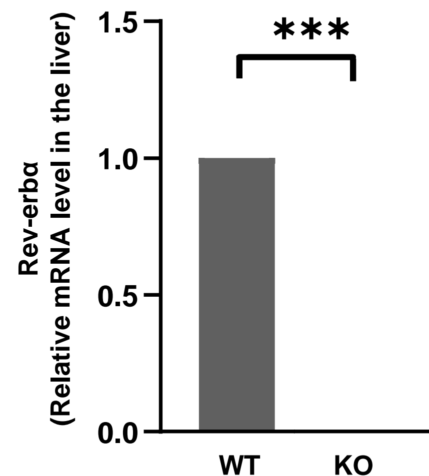
Other blood parameters

Parameter (Units)	WT	KO
MCV (fL)	46.43 ± 2.1	46.35 ± 1.3
MCH (pg)	15.23 ± 0.4	15.43 ± 0.3
MCHC (g/dL)	32.95 ± 2.2	33.30 ± 0.5
RDW (%)	13.53 ± 2.3	13.08 ± 2.0
MPV (fL)	4.25 ± 0.1	4.40 ± 0.1
PDW	15.83 ± 0.3	16.23 ± 0.6
PCT	0.38 ± 0.1	0.34 ± 0.2
Lymphocytes (%)	80.05 ± 3.8	79.35 ± 2.4
Monocytes (%)	2.88 ± 0.4	3.40 ± 0.8
Granulocytes (%)	17.08 ± 3.4	17.25 ± 1.7

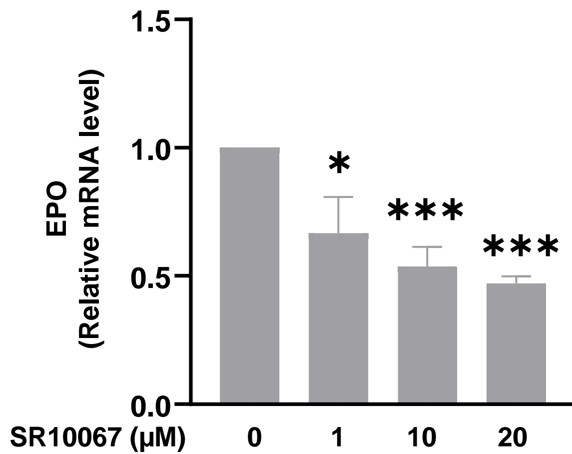
B

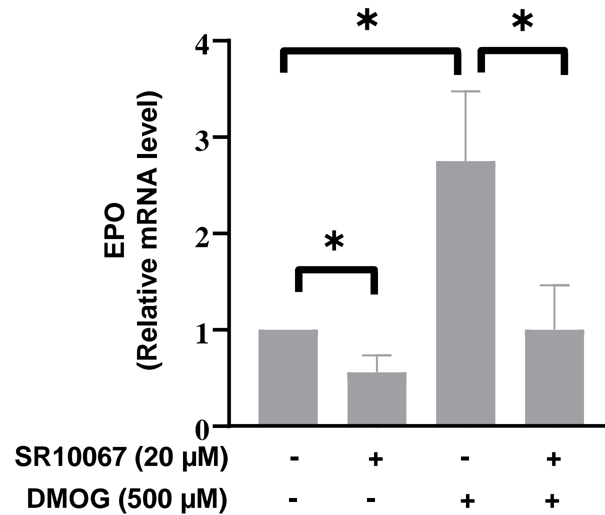


C



Supplementary Figure 1: Different blood parameters in Rev-erbα knockout and wild type mice. A) Rev-erbα KO and WT mice showing the values for different blood parameters; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT). **B and C)** Relative expression of Rev-erbα in the B) kidney and C) liver of WT and KO mice determined by qRT-PCR. Values are mean ± SD (n = 4). ***$p < 0.001$ as indicated.

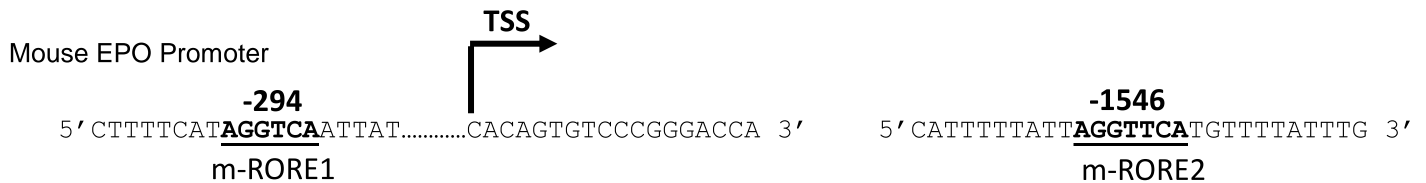




Supplementary Figure 2: Rev-erbα ligand SR10067 supresses EPO expression. A) HepG2 cells were treated with an increasing concentration of SR10067 for 24 h and expression of EPO is measured with qRT-PCR. **B)** HepG2 cells were treated with DMOG (500 µM) or SR10067 (20 µM) for 24 h, and EPO expression was evaluated by qRT-PCR Data are representative and mean ± SD from three independent experiments. *p < 0.05, ***p < 0.001, compared to control or as indicated.

Supplementary Figure 3

A



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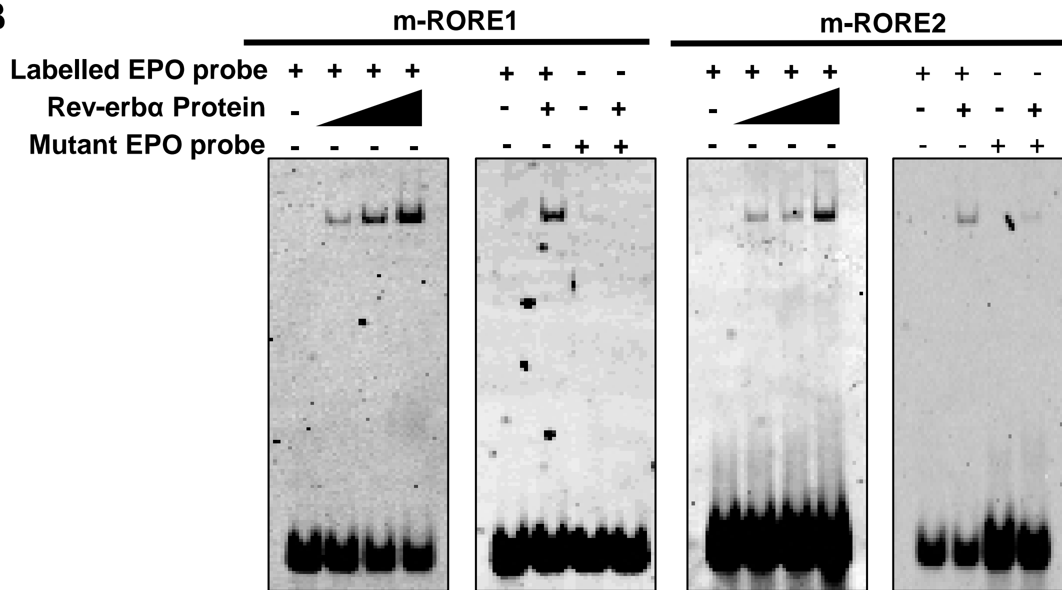
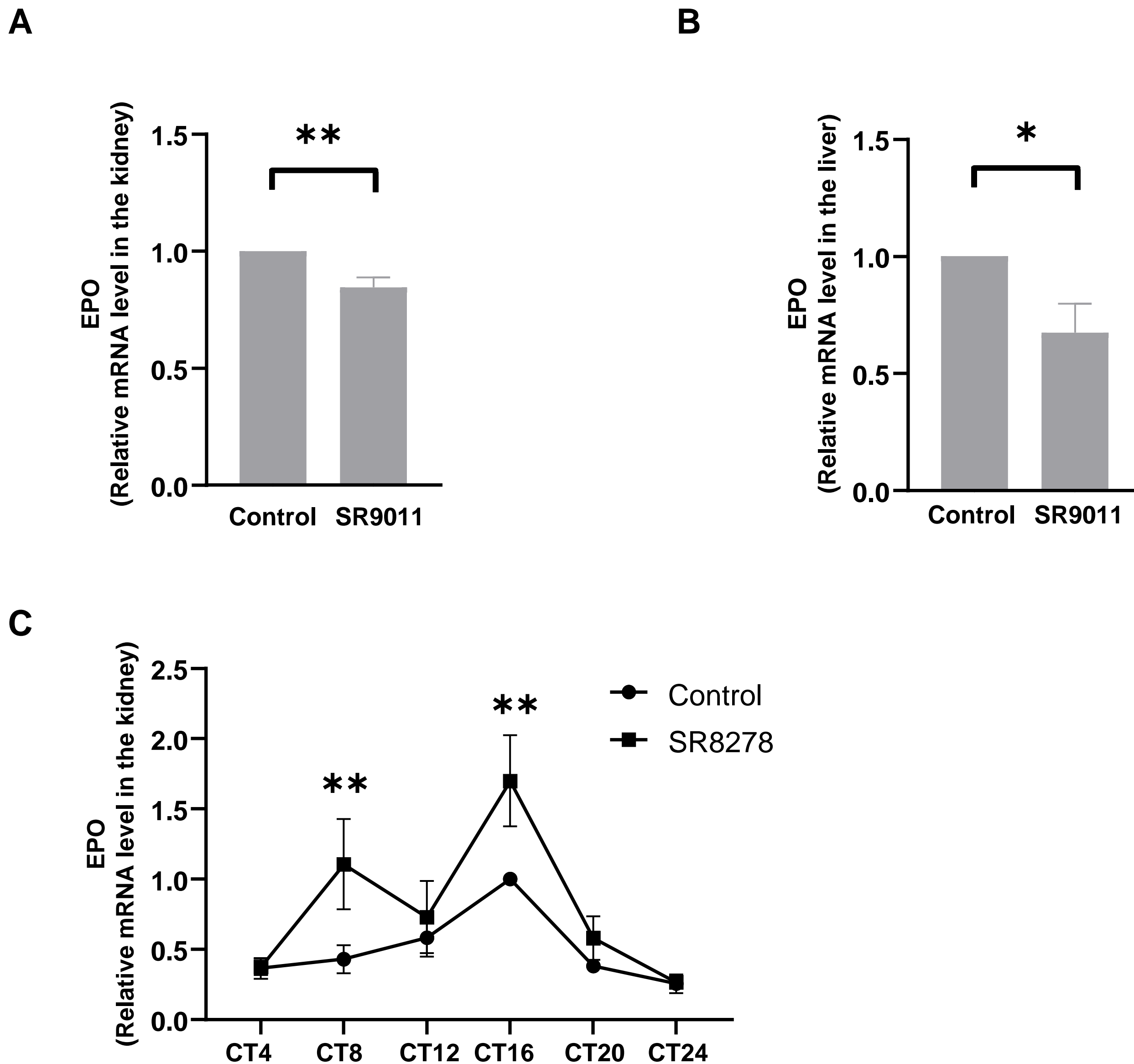


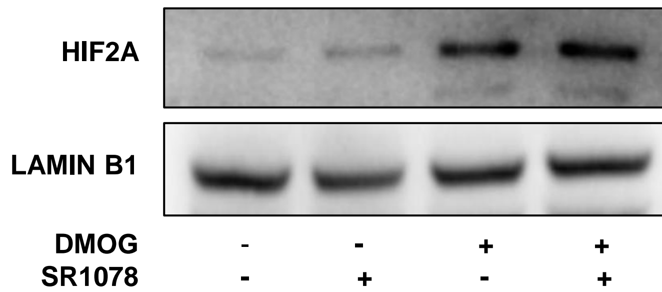
Supplementary Figure 3: Rev-erbα binds to mice EPO promoter: A) Pictorial representation of Rev-erbα binding sites on mice EPO gene promoter, 294 bp to 299 bp (m-RORE1) and 1546 bp to 1552 bp (m-RORE2) upstream of the TSS. **B)** EMSA with Rev-erbα protein on mice epo promoter by using **a)** WT (containing m-RORE1: CCCAACTTTTCAT**AGGTCA**ATTATCCTT and AAGGATAATTGACCTATGAAAAGTTGGG) and mutant (Mut m-RORE1:CCCAACTTTTCAT**CTTGAC**ATTATCCTT and AAGGATAATGTCAAGATGAAAAGTTGGG) fluorescent probes **b**) WT (containing m-RORE2: CATTTTTATT**AGGTTCA**TGTTTTATTTG and CAAATAAAACATGAACCTAATAAAAATG) and mutant (Mut m-RORE2:CATTTTTATT**CTTGGAC**TGTTTTATTTG and CAAATAAAACACAGGTTGAATAAAAATG) fluorescent probes.



Supplementary Figure 4: Effect of Rev-erbα on EPO production under *in-vivo* condition. C57BL/6 mice were treated with the Rev-erbα agonist SR9011 for three alternative days and the relative mRNA expression of EPO was assessed in both the **A)** kidney and **B)** liver (n=3). C57BL/6 mice were treated with the Rev-erbα antagonist SR8278 (25mg/kg); samples were collected at indicated time points and the relative mRNA expression of EPO was measured in kidney. *$p < 0.05$, **$p < 0.01$, as compared to the control.

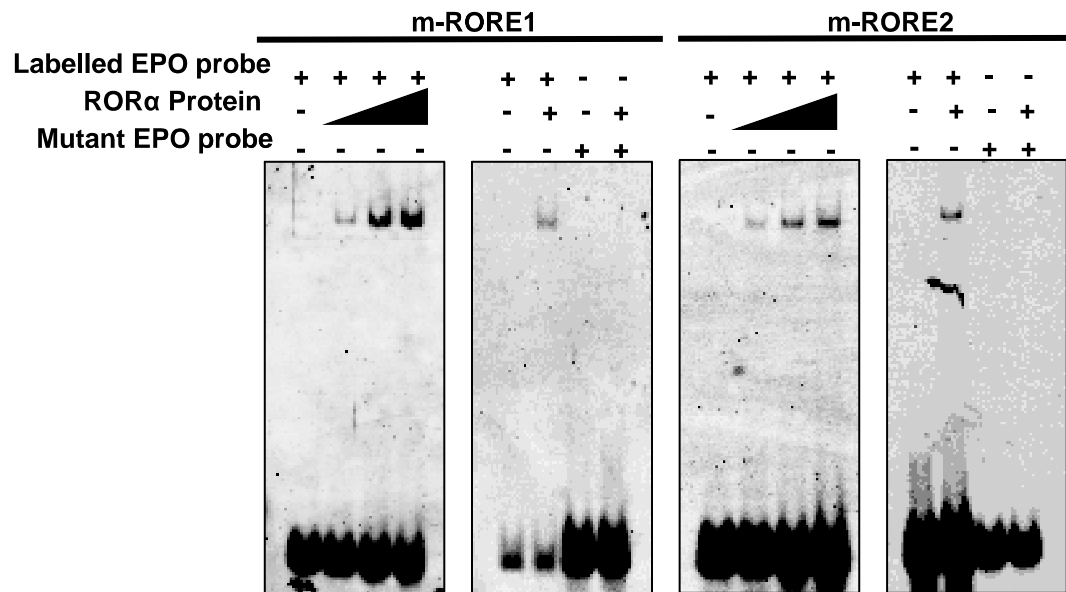
Supplementary Figure 5

A

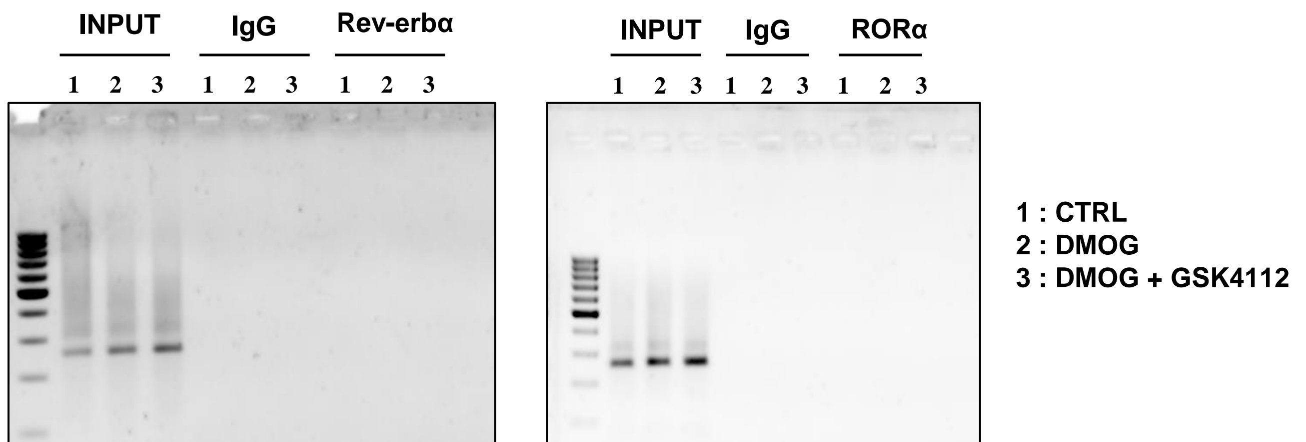


B

Supplementary Figure 5: Effect of RORα on HIF2α translocation: A) Immunoblot analysis of HIF2α in the nuclear extract of HepG2 cells treated with DMOG, SR1078, or their combination for 24 hours. **B)** Image analysis of HIF2α protein translocation by confocal microscopy of HepG2 cells treated with DMOG or SR1078 or combination for 24 h.



Supplementary Figure 6: RORα binds to mice EPO promoter: EMSA with RORα protein on mice EPO promoter by using **a)** WT (containing m-RORE1: CCCAACTTTTCAT**AGGTCA**ATTATCCTT and AAGGATAATTGACCTATGAAAAGTTGGG) and mutant (Mut m-RORE1:CCCAACTTTTCAT**CTTGAC**ATTATCCTT and AAGGATAATGTCAAGATGAAAAGTTGGG) fluorescent probes **b**) WT (containing m-RORE2: CATTTTTATT**AGGTTCA**TGTTTTATTTG and CAAATAAAACATGAACCTAATAAAAATG) and mutant (Mut m-RORE2:CATTTTTATT**CTTGGAC**TGTTTTATTTG and CAAATAAAACACAGGTTGAATAAAAATG) fluorescent probes.



Supplementary Figure 7: Representative Semi-quantitative PCR gel showing the amplified product for the ChIP experiment, to evaluate the specificity of isotype IgG, Rev-erbα, and RORα antibodies used. GAPDH primers were used as negative DNA region control.

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