Supplementary Information

Repression of hepatocyte nuclear factor 4 alpha by AP-1 underlies dyslipidemia associated with retinoic acid

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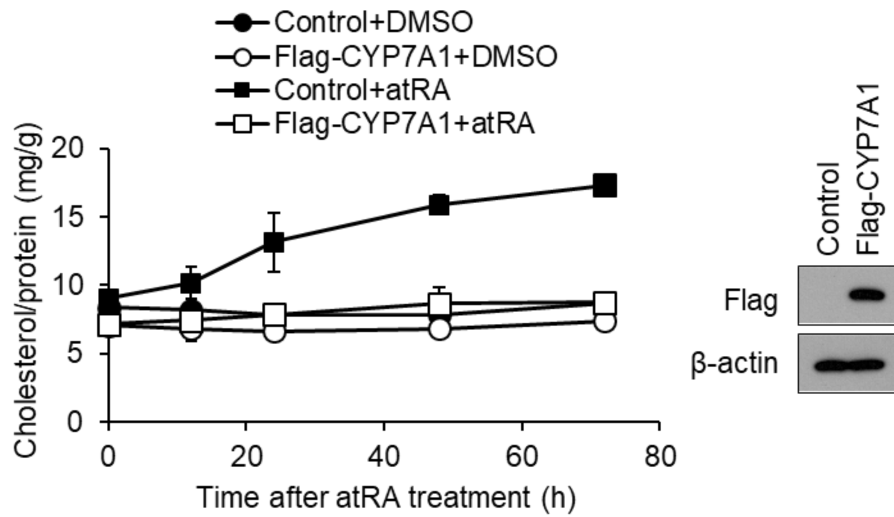
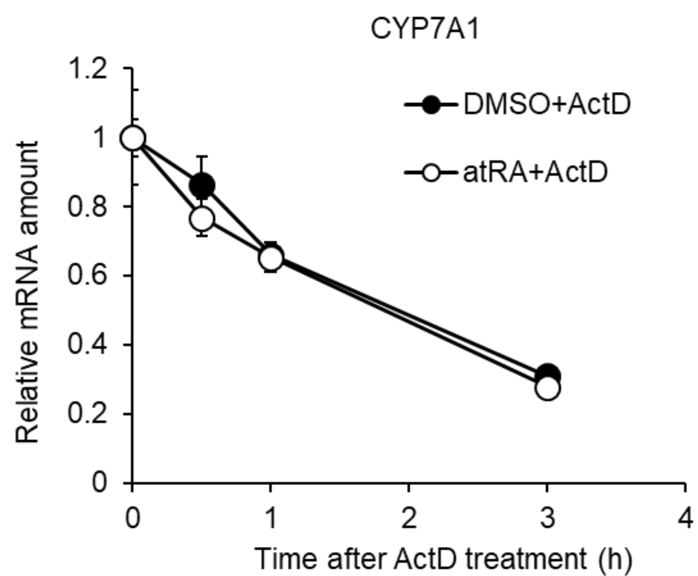
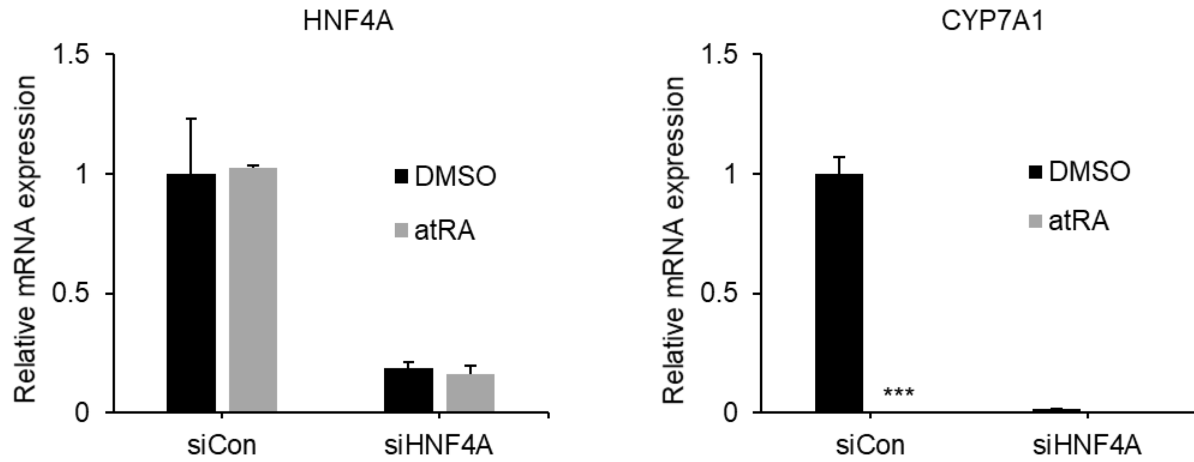


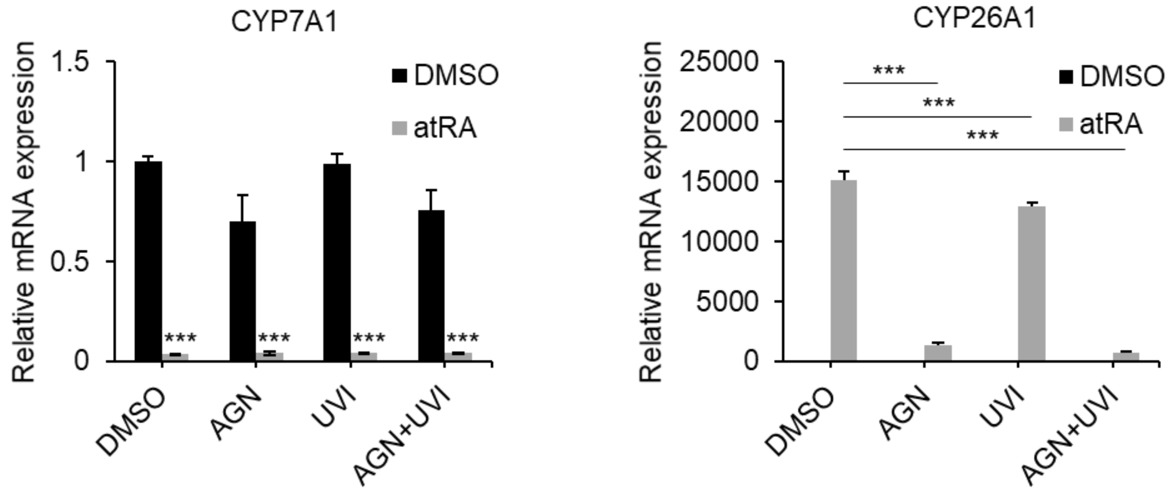
Supplemental Figure S1. *CYP7A1* overexpression abrogates atRA-induced cholesterol accumulation. Human hepatocytes overexpressing Flag-CYP7A1 were treated with atRA (1 μM) or DMSO for the time periods indicated, and cholesterol levels were measured. CYP7A1 overexpression was verified by western blot analysis.



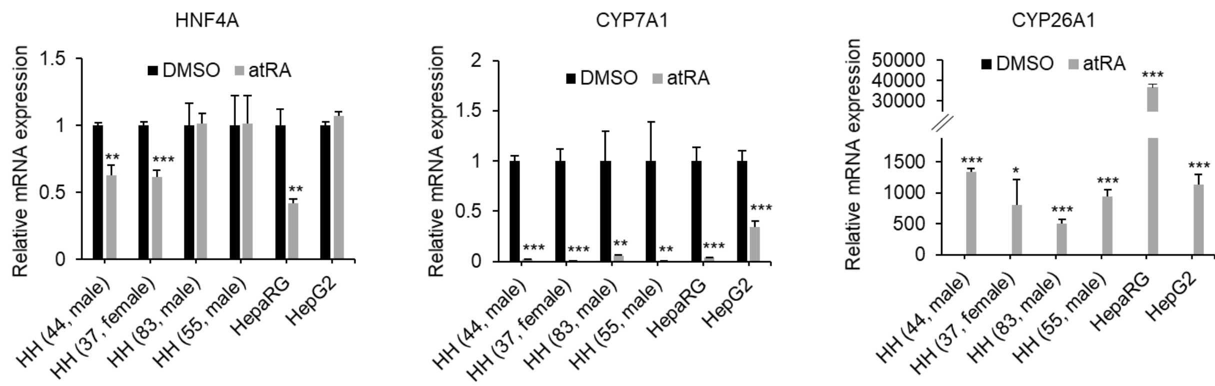
Supplemental Figure S2. *CYP7A1* mRNA stability is not affected by atRA. HepaRG cells were pretreated with atRA or DMSO for 1 h and treated with actinomycin D (ActD, 2 µg/ml) for 3 h. mRNA levels were determined by qPCR (n=3, mean±S.D.).



Supplemental Figure S3. HNF4α is a critical component in atRA-induced *CYP7A1* repression. Human hepatocytes were transfected with siRNA (50 nM) targeting *HNF4A* (siHNF4A) or control siRNA (siCon) for 48 h and treated with atRA (1 µM) or DMSO for 3 h. mRNA levels were determined by qPCR (n=3, mean±S.D.). ***, p<0.001 vs DMSO-treated group.

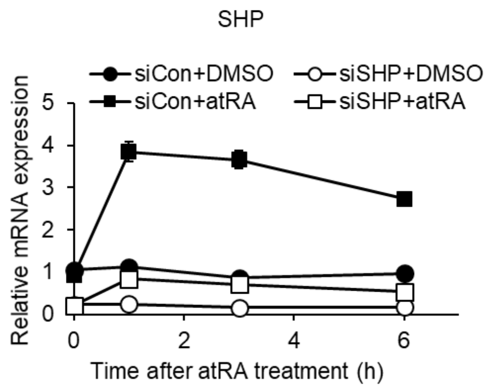


Supplemental Figure S4. RAR/RXR activation does not play a role in CYP7A1 repression. HepaRG cells were pretreated with AGN193109 (an RAR antagonist; 1 μM) and/or UVI3003 (an RXR antagonist; 10 μM) and treated with atRA (1 μM) for 3 h. mRNA levels were determined by qPCR (n=3, mean±S.D). ***, $p<0.001$.



Supplemental Figure S5. *HNF4A* expression is not repressed in all atRA-treated cells. Human hepatocytes (HH), HepaRG, HepG2 cells were treated with atRA (1 μM) or DMSO for 6 h. *HNF4A* mRNA levels were determined by qPCR (n=3, mean±S.D.). **, p<0.01; ***, p<0.001 vs DMSO-treated group.

A



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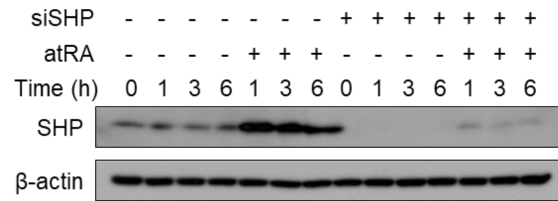

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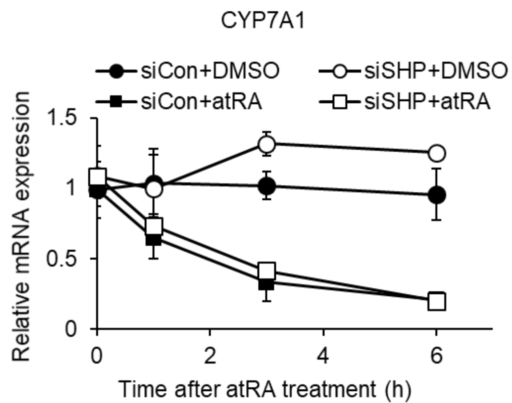
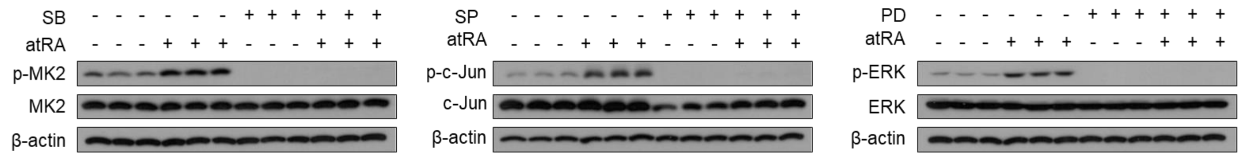
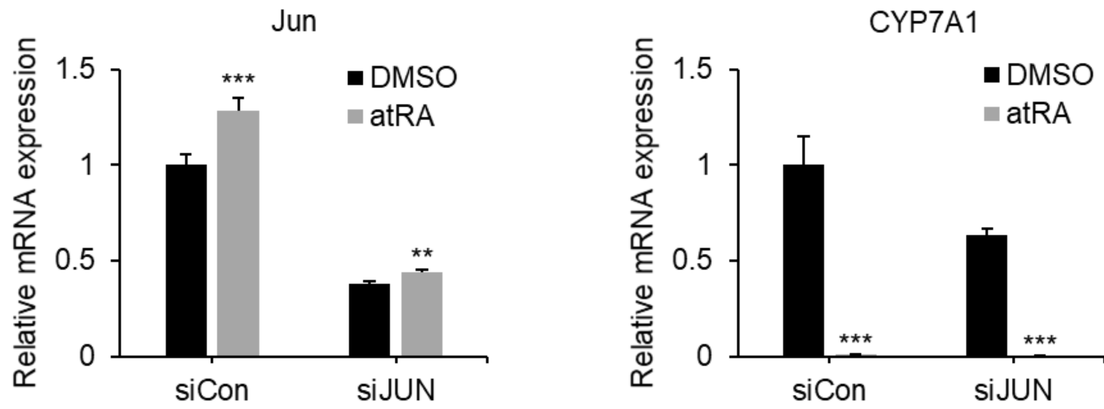


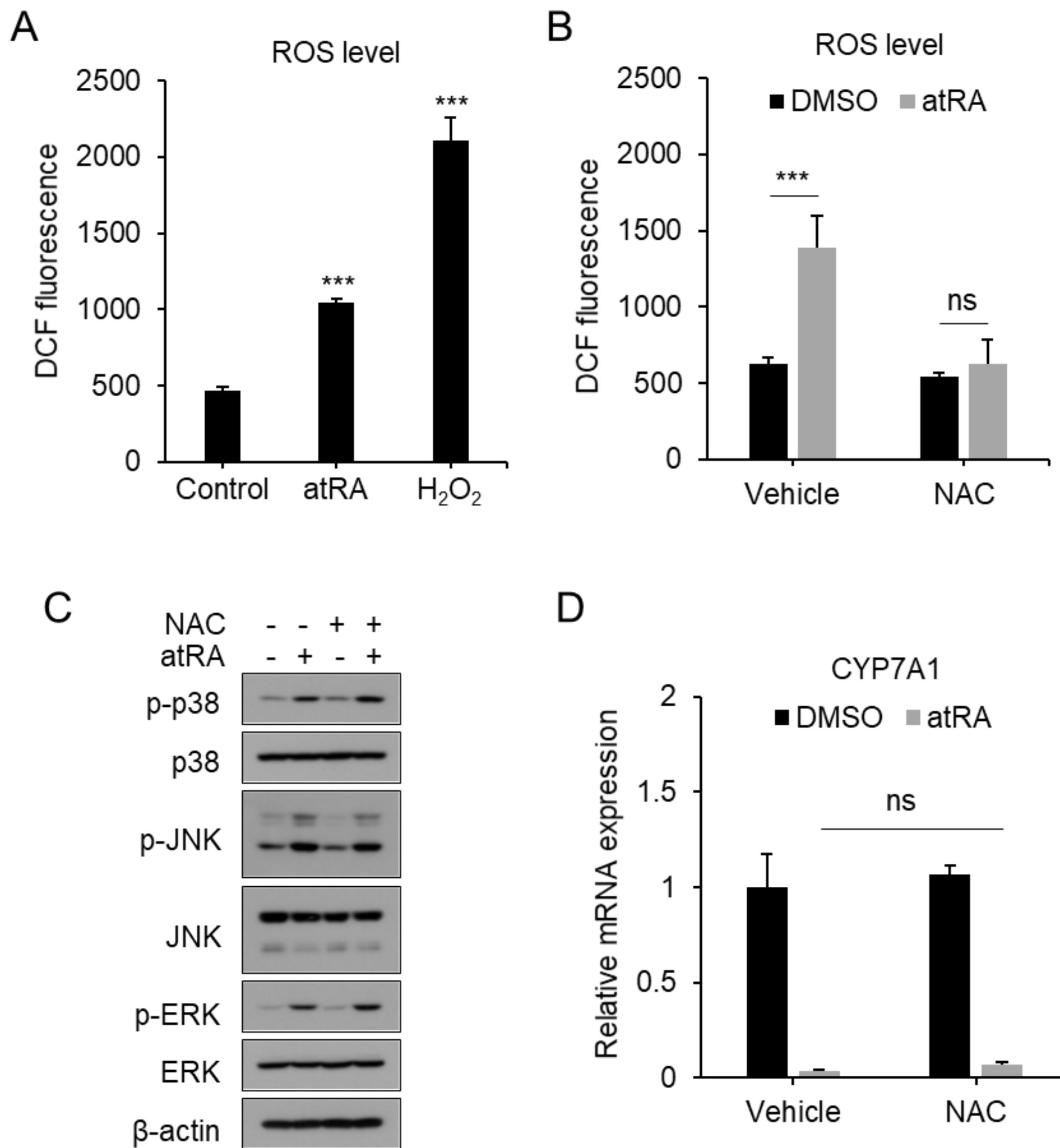
Supplemental Figure S6. atRA-induced SHP does not contribute to suppression of *CYP7A1*. Human hepatocytes were transfected with siRNA (50 nM) targeting *SHP* (siSHP) or control siRNA (siCon), followed by treatment with atRA (1 μM) or DMSO for the time periods indicated. mRNA and protein levels were determined by qPCR (n=3, mean±S.D.) (A, C) and western blot analysis (B), respectively.



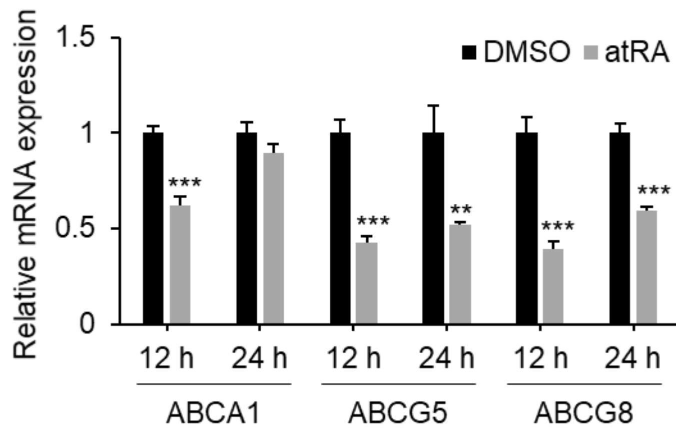
Supplemental Figure S7. Pharmacological inhibition of MAPK pathways. HepaRG cells were pretreated with SB203580 (SB, 50 μM), SP600125 (SP, 50 μM), or PD98059 (PD, 50 μM) for 1 h and treated with atRA (1 μM) for 3 h. Phosphorylation of downstream effector or signaling proteins (i.e., MK2 for p38, c-Jun for JNK, and ERK for ERK pathways) was detected by western blot analysis.



Supplemental Figure S8. atRA-mediated *CYP7A1* repression is maintained in *JUN* knocked-down cells . HepaRG cells were transfected with siRNA (50 nM) targeting JUN (siJUN) or control siRNA (siCon) for 48 h and treated with atRA (1 μM) or DMSO for 3 h. mRNA levels were determined by qPCR (n=3, mean±S.D.). **, $p<0.01$; ***, $p<0.001$ vs DMSO-treated group.



Supplemental Figure S9. atRA-mediated MAPK activation is ROS-independent. (A) HepaRG cells were treated with atRA (1 μM) or H_2O_2 (1 mM) for 1 h, followed by 2′,7′-dichlorofluorescin diacetate (DCFDA) treatment (25 μM) for 15 min. H_2O_2 was used as a positive control. Fluorescence levels were measured at 495/525 nm (n=6, mean±S.D.) ***, p<0.001 vs control group. (B-D) HepaRG cells were pretreated with N-acetylcysteine (NAC, 400 μM; a ROS inhibitor) for 1 h, and treated with atRA (1 μM) for 3 h. DCFDA (25 μM) was treated for 15 min, and fluorescence levels (B) were measured at 495/525 nm (n=6, mean±S.D.) ***, p<0.001 vs vehicle group; ns, not significant. Protein and mRNA levels were determined by western blot analysis (C) and qPCR (D) (n=3, mean±S.D.), respectively. ns, not significant.



Supplemental Figure S10. Altered expression of cholesterol transporters by atRA treatment. HepaRG cells were treated with atRA (1 μM) or DMSO for 24 h. ABCA1, ABCG5, and ABCG8 mRNA levels were determined by qPCR (n=3, mean±S.D.). **, $p<0.01$; ***, $p<0.001$ vs DMSO-treated group.