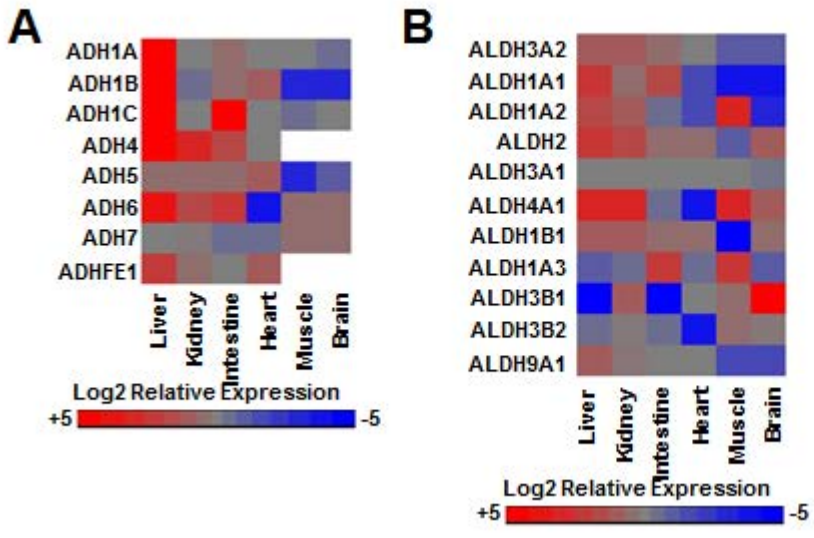
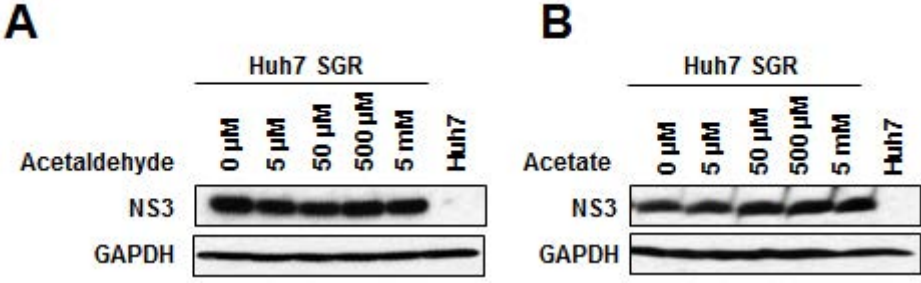
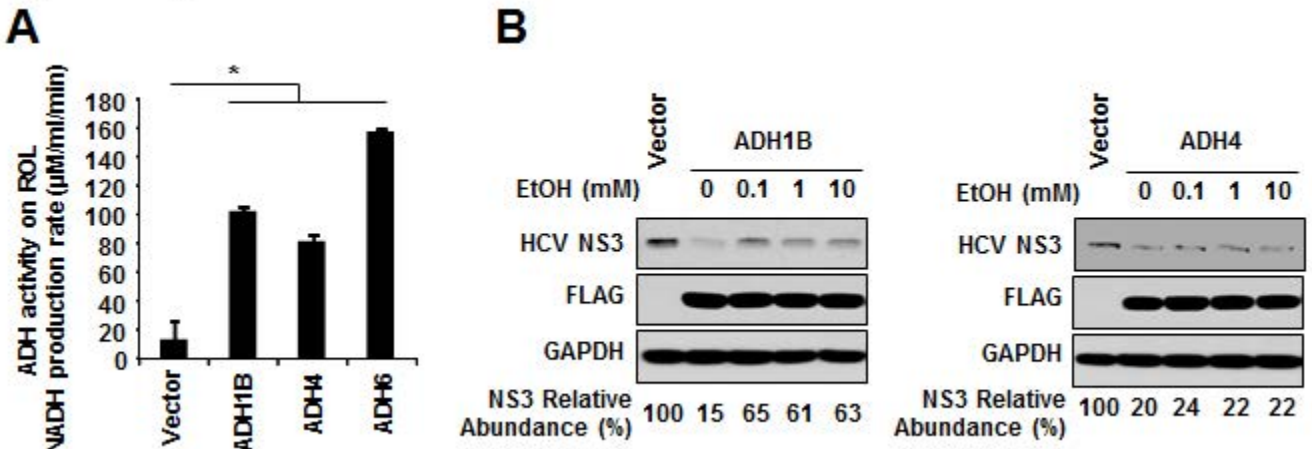
Supplemental Figure 1



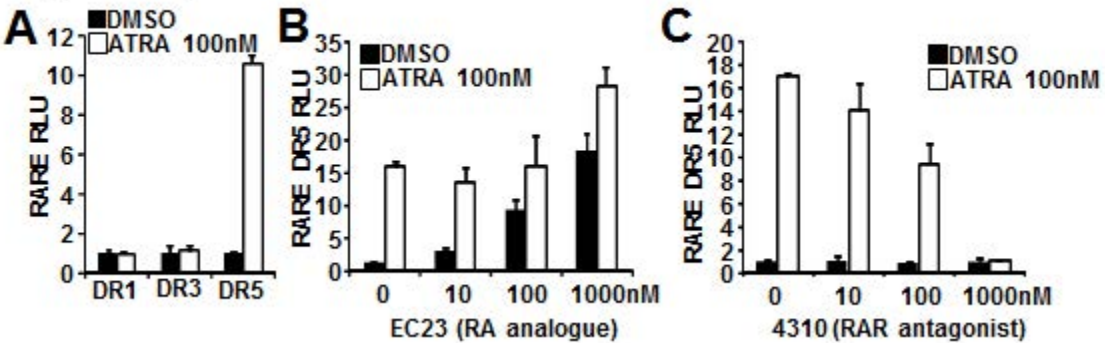
Supplemental Figure 2



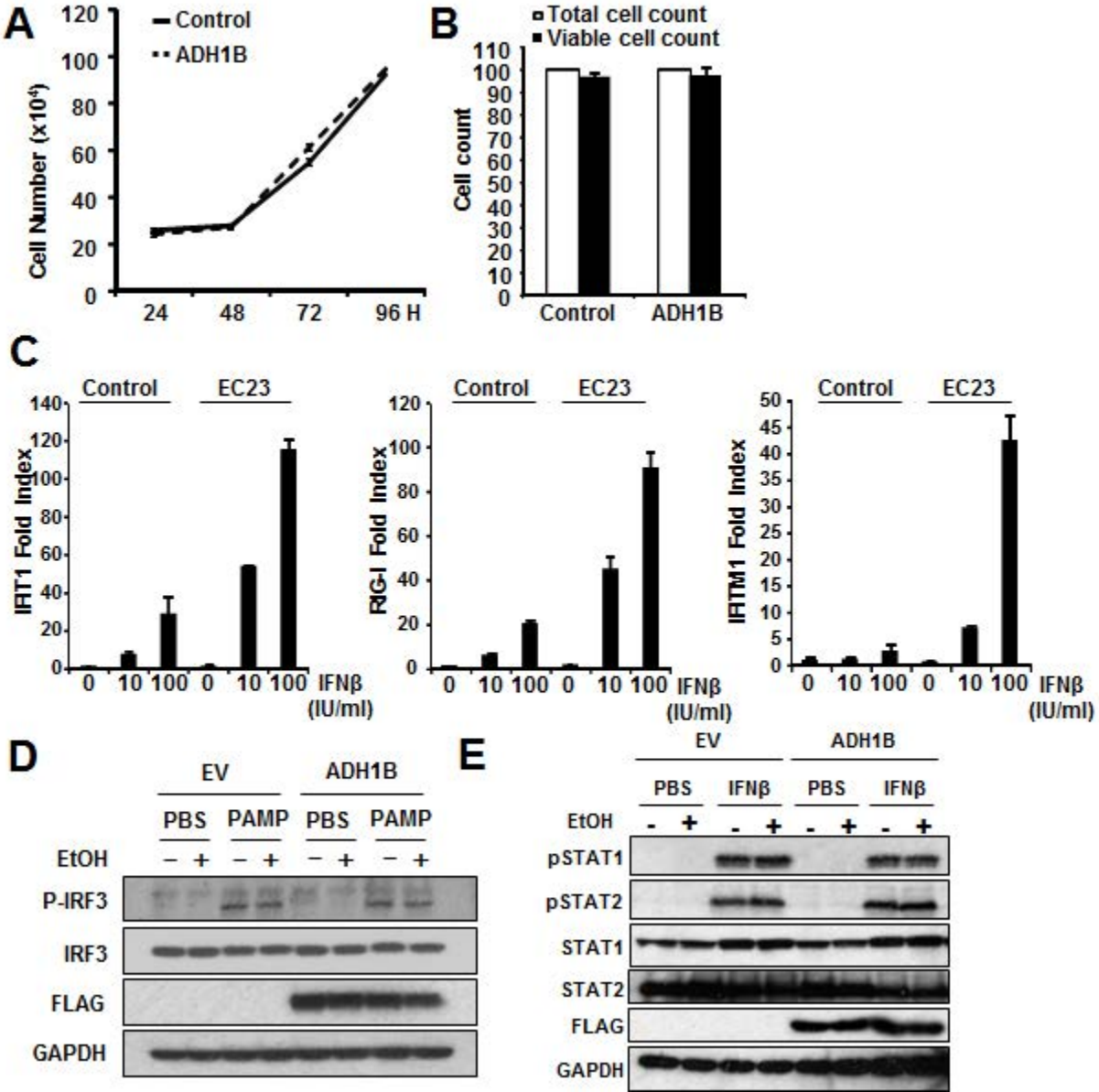
Supplemental Figure 3



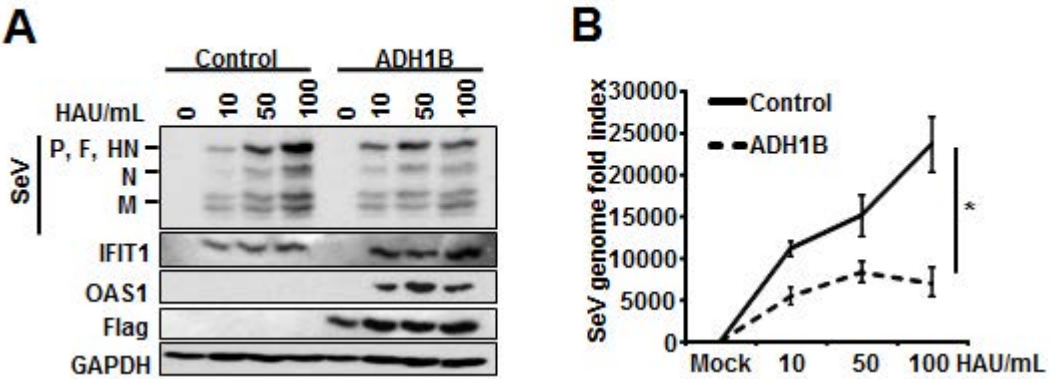
Supplemental Figure 4



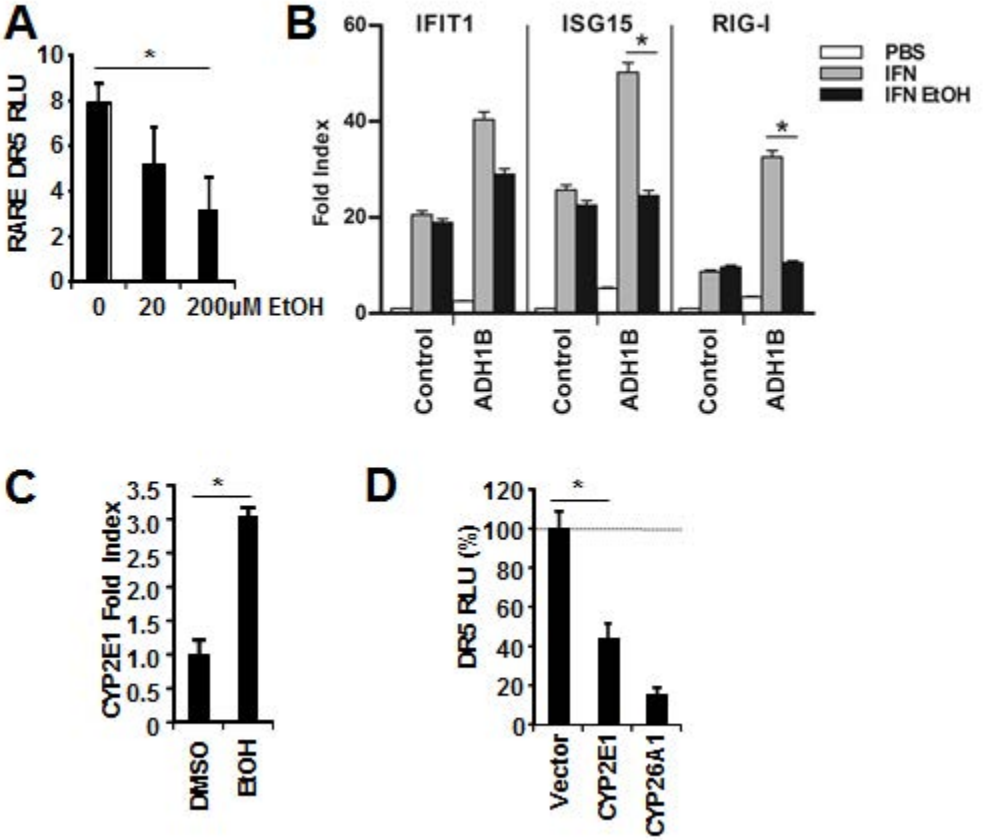
Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7



Supplemental Information

Antibodies: The following antibodies were used: mouse anti-HCV NS5A;9E10 (a gift from C. Rice, Rockefeller University), rabbit anti-HCV NS3 (Abcam), rabbit anti-ADH1B (Abcam), rabbit anti-ADH4 (Aviva System Biology), ADH6 (Aviva System Biology), rabbit anti-ADHFE1 (GeneTex), rabbit anti-CYP2E1 (Abcam), rabbit anti-ALDH1A1 (Abcam), rabbit anti-ALDH2 (Abcam), rabbit anti-ALDH4A1 (GeneTex), mouse anti-GAPDH (GeneTex), Flag M2 (Sigma-Aldrich), IFIT1 (a gift from G. Sen, Cleveland Clinic), mouse anti-OAS1 (Kineta), mouse anti-IFITM1 (ProSci), rabbit anti-MX1 (Aviva System Biology), rabbit anti-RIG-I (Cell Signaling), rabbit anti-pIRF3 (Cell Signaling), mouse anti-IRF3 (Biolegend).

Bioinformatics: Bioinformatics: ISG gene regulatory region analysis: The ExPlain^{TM} v3.1 (www.biobase-international.com/explain) from BIOBASE Corporation(1) was used to scan for potential binding sites of RAR-RXR. A list of 446 ISGs was formed based on the data set included in previous publications(2-4) and uploaded into ExPlain. Match function was used to scan for possible binding sites of RAR-RXR in the [-5000, 500] regions around best-supported TSS for the 446 ISGs using position-based weight matrix V$RXRRAR_01 (M02272) with minimized false negatives (minFN) options. To obtain the background occurrence of RAR-RXR in human genome, 500 human genes were randomly selected and scanned using Match with the same parameters. All 446 ISGs were used as enrichment background to minimize any enrichment biases.

Nucleic Acid, Plasmid and Luciferase reporter assay: In vitro transcription of HCV genome or subgenomes was carried out using MEGAscript T7 transcription kit (Life Technologies). HCV PAMP RNA was generated similarly to previously described(5). Cellular RNA extraction was carried out using the RNAeasy kit (QIAGEN). QPCR was conducted using SYBR green-based one-step RT-qPCR method (Life Technologies). The relative gene expression was normalized to GAPDH values to obtain fold increases. The primer sequences for RT-qPCR and expression vector construction are available upon request. RT-qPCR array for the evaluation of Retinoic Acid target genes or ISG expression was conducted using Retinol Metabolism Panel (BioRad) or Type I IFN response panel (QIAGEN) a two-step RT-QPCR. The obtained delta-CT values were analyzed with RT2 Profiler PCR Array Data Analysis version 3.5 and converted to a heat map after exclusion of redundancy, unrelated genes, or genes not expressed in the cells tested. The relative amplitude effect in log2 scale are listed in Table 2. The transient expression or lentiviral expression constructs were generated in pEF Myc/His Version C (Life Technologies) or pCDH-CMV-MCS-EF1-Puro (System Bioscience). All plasmids used in this study were propagated in DH5α under Ampicillin selection and purified with ZR plasmid miniprep or ZymoPure Plasmid Midiprep kit (Zymo Research). Luciferase based reporter assays were carried out with a RARE sequence driven firefly luciferase reporter construct with Dual Luciferase Assay kit (Promega)(6). The inducibility of RARE luciferase (Firefly) was normalized with the value from co-transfected CMV promoter driven Renilla luciferase to determine Relative Luciferase Unit (RLU).

References for Supplemental Information

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Supplemental Figure 1. Identification of liver dominant ADH/ALDH isoforms. A-B. Meta-analysis of ADH and ALDH expression: Nextbio was used to obtain the expression profiles of ADH and ALDH in public datasets. In Nextbio, high-quality public microarray dataset from GEO and other genomic data repositories were selected, normalized and pre-calculated for cross-study comparisons. Relative expression of a gene (fold-change) was calculated as the ratio between its expression in the selected tissue and its median expression across 52 tissue groups that are derived from 1067 arrays. For ADH/ALDH expression analysis, 11 liver (GSE3526, GSE1133, GSE2361, GSE7307), 4 kidney (GSE1133, GSE2361, GSE7307), 3 small intestine (GSE2361, GSE7307), 2 heart (GSE2361, GSE7307), 2 smooth muscle (GSE1133), 2 whole brain (GSE1133) tissue samples were analyzed. Relative expression of multiple microarray probes for the same gene were consolidated using the median ratio. **C.** The relative expression abundance of flag-tagged ADH1B or CYP2E1 in Huh 7 cells were compared to that of freshly isolated primary human hepatocytes (PHH). The Huh7 cells were ectopically expressed with the indicated EtOH metabolizing enzymes for 24 hours. Then cell lysates were subjected to immunoblotting analysis for a relative abundance comparison with the cell lysates of freshly isolated PHH.

Supplemental Figure 2. The effect of EtOH metabolic byproduct on HCV replication. A-B. Huh7 cells stably harboring HCV subgenomic replicon (JFH1 strain) were treated with acetaldehyde (**A**) or acetate (**B**) at indicated concentration for 48 hours. Cell lysates were then subjected to IB analysis for the detection of HCV protein (NS3). Naïve Huh7 lysates were used as a negative control for HCV.

Supplemental Figure 3. Distinct enzymatic activity of liver dominant ADH isoforms for the catabolism of ROL and its susceptibility to EtOH inhibition. A. Cell lysate expressing indicated ADH were incubated with NAD (3mM) in the presence or absence of ROL (100μM) to assess NAD-NADH conversion rate (μMoles/min/ml) via detection of 340/440nm fluorescent. **B.** Huh7 cells harboring HCV SGR was transduced with a control vector, ADH1B (Left Panel), or ADH4 (Right Panel) for 24 hours. Then cells were treated with the indicated concentration of EtOH for 24 hours followed by IB analysis of the indicated protein. The signal intensity of HCV NS3 was quantified with ImageJ software and the value was normalized to the signal from control vector transfected cells that were treated with PBS. The values are shown in %.

Supplemental Figure 4. RA regulation of gene transcription through the activation of RARE DR5. A-C. Huh7 cells were co-transfected with indicated RARE luciferase and renilla luciferase reporter for 24 hours. Cells were then treated with ATRA treatment alone at 100nM (**A**), co-treated with EC23 (**B**), or 4310 (**C**) at the indicated concentration for 36 hours. Cell lysates are then subjected to dual luciferase assay.

Supplemental Figure 5. RA augment both basal and induced expression of ISG in hepatocytes. A-B. Huh7 cells stably expressing ADH1B or control vector are monitored for the rate of cellular proliferation every 24 hours up to 96 hours (**A**) or subjected to trypan blue staining to determine the viable cells (**B**) at 72 hours. **C.** Freshly isolated primay human hepatocytes were treated with vehicle (DMSO) or EC23 for 16 hours followed by IFNβ treatment for 8 hours at indicated dose. The RNA was subjected to

RT-qPCR for the detection of indicated ISG. **D-E.** Huh7 cells were transfected with either ADH1B or control vector for 24 hours. 8 hours after expression vector transfection, cells were treated with EtOH (25mM) for 16 hours followed by HCV–PAMP RNA transfection (2μg/5x10^5cells)(**D**) or IFN-β (100IU/ml)(**E**). 16 hours after RNA transfection(**D**) or 1 hours after IFN-β treatment, cell lysates were subjected to IB analysis of the indicated protein expression analysis.

Supplemental Figure 6. ADH-ALDH decreases cellular susceptibility to RNA virus infection. A-B. Huh7 cells stably expressing ADH1B or control vector were infected with Sendai Virus (SeV) at indicated infection titer for 24 hours. RNA and protein extracted were subjected to IB analysis (**A**) or QPCR (**B**) for the detection of viral product. *p<0.01.

Supplemental Figure 7. Two distinct EtOH metabolizing pathways impair RA-mediated gene regulation. A. Huh7 cells were co-transfected with an ADH1B expression vector, a RARE DR5 luciferase, and renilla luciferase reporter followed by treatment with ROL (1μM) and the indicated concentration of EtOH for 36 hours. Cell lysates were subjected to dual luciferase assay. *p<0.01. **B.** Huh 7 cells stably expressing ADH1B or control vector were treated with PBS or EtOH (25mM) for 40 hours prior to IFNβ (50IU/ml) treatment for 8 hours. Extracted RNA was used for RT-qPCR analysis of indicated ISGs expression. *p<0.01. **C.** Freshly isolated primary human hepatocytes were cultured in the presence of either DMSO (vehicle) or EtOH 10mM for 24 hours followed by QPCR analysis of CYP2E1 expression change. GAPDH

was used for the normalization and the calculation of fold index. *p<0.01. **D.** Huh7 cells were cotransfected with indicated CYP expression vectors, RARE DR5 luciferase, and renilla luciferase reporter for 36 hours. Cell lysates were subjected to dual luciferase assay. CYP26A1 that catabolize RA serves as a positive control. *p<0.01.