MATERIALS AND METHODS

Primary Murine Hepatocyte Isolation

Anesthetized mice were perfused with 8 ml Buffer-I (5 mM dextrose, 116 mM NaCl, 760 μM NaH₂PO₄, 5.3 mM KCl, 26 mM NaHCO₃, 10 mM HEPES, 500 μM EGTA pH 7.2) over 1 min followed by 25 ml Buffer-II (0.2 mg/ml type-I collagenase (Worthington), 5.3 mM KCl, 116 mM NaCl, 5 mM dextrose, 26 mM NaHCO₃, 1.6 mM MgSO₄, 900 μM CaCl₂, 48 μg/ml trypsin inhibitor pH 7.2) over 4 min. Hepatocytes were dissociated, centrifuged (500 g, 1 min) and resuspended in media-I (DMEM, 10% FBS, 2.5% DMSO, 10 nM dexamethasone, 100 IU/ml penicillin, 100 μg/ml streptomycin). Viability was assessed via trypan blue staining and 10⁶ cells/well were seeded into type-I collagen-coated 6-well plates (BD Bioscience). After 4 h incubation, non-adherent cells were aspirated and fresh media-I was added. After overnight incubation, media was replaced with long-term hepatocyte culture media (Hepatozyme-SFM (Invitrogen)/2.5% DMSO/10 nM dexamethasone/100 IU/ml penicillin and 100 μg/ml streptomycin). Cells were cultivated for 5 d before treatment.

Characterization of ΔH1-AHR (murine):

Expression Constructs

Plasmid constructs pcDNA3-mAhRWt, pcDNA3-ARNT-HA, pEYPFmAhRWt, pCI-XAP2, pGudLuc 6.1 were generated previously. The mAhR mutational constructs, pcDNA3-mAhR ΔH1 (Δ43-51) and pEYPF-mAhR ΔH1 were generated using loop-out primer pairs 5’-CACAGAGACCGGCTGGGAGGACTGCTGCCCTTCCC-3’ and 5’-GGGAAGGGCAGCAGTCCTCCCAGCCGGTCTCTGTG. Each mutagenesis was performed
using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) and pcDNA3mAhR and pEYFPmAhR constructs respectively. Plasmid DNA was prepared using a plasmid midi-prep kit (Qiagen, Chatsworth, CA).

**Transient Transfections and Luciferase Assays**

Transfections in Cos-1 cells were carried out using LipofectAMINE PLUS (Invitrogen) as specified by the manufacturer. Luciferase reporter assays were performed in 6-well dishes using cells that were 70-90% confluent at the time of transfection. Each well was transfected with a total of 1.5 μg of total DNA. The following amounts of DNA were added to the wells: 0-100 ng pcDNA3-mAhR or 0-100 ng of pcDNA3-mAhRΔH1 along with 200 ng of the AhR-driven Luciferase reporter plasmid pGUDLUC 6.1, 200 ng of pDJMβ-gal constitutively expressed, β-galactosidase and empty pcDNA3 vector to total 1.5 μg per well. After 18 h, transfected cells were treated for 6 h with carrier solvent, or 10nM TCDD. Treated cells were lysed using 1X Lysis Buffer (Promega, Madison, WI). Luciferase assays were carried out using a Turner TD-20e luminometer (Turner Designs, Sunnyvale, CA). Luciferase readings were normalized using β-galactosidase enzyme activity assay.

**Real-Time RT-PCR**

BP8 cells were transfected in 6 well plates using LipofectAMINE PLUS reagent. Each well was transfected with 2 μg of either pcDNA3-mAhR ΔH1, pcDNA3-mAhR WT, control plasmid or was mock transfected. 24 h after cells were treated with 10nM TCDD or DMSO for 4 h. mRNA was isolated using Tri-reagent and cDNA synthesized using the cDNA Achieve Kit (Applied
Biosystems, Foster City, CA). Real Time RT-PCR was carried out using the rat CYP1A1 primers 5′-CCTCTTTGGAGCTGCTGGTTTG
5′-GCTGTGGGGATGCTGAA and the β-actin, control primers 5′-GAGGCCCAGAGCAAGAGAG and 5′-GGCTGGGGTGTGAAGGT. Real time reactions were carried out using SYBR-Green supermix (Biorad).

**Immunoprecipitations**

Cos-1 cells were transfected for 8 h in 100 mm plates with 4 μg of pcDNA3-ARNT-HA and 5 μg of pcDNA3-mAhR, pcDNA3-mAhR ΔH1 or pcDNA3 control plasmid. 24 h after transfection cells were treated with 10 nM TCDD for 45 min and then trypsinized. Cells were washed with PBS and lysed in MENG buffer (25 mM MOPS, 2 mM EDTA, 0.02% sodium azide, pH 7.4) plus 20 mM sodium molybdate, 500 mM NaCl, 1% NP40 and protease inhibitors. Cell lysates were then incubated for 1 h with Anti-Rabbit IgG agarose that was pre-bound to anti-HA rabbit polyclonal antibody (Santa Cruz Biotechnology). The pellet was then washed 4X with MENG buffer. The precipitated proteins were then denatured at 95°C in sample buffer and resolved using TSDS-PAGE (8% acrylamide) and transferred to PVDF membrane.

**Antibodies and Blotting**

Membranes were blocked at room temperature in 10 mM Na_2PO_4, 150 mM NaCl, 0.5% Tween 20, 3% BSA, pH 7.4 for 1 h. HA-Tagged ARNT proteins were probed using rabbit anti-HA polyclonal antibody (Sigma) at 0.2 μg/ml. mAhR and mAhRΔH1 proteins were detected using mouse monoclonal antibody RPT1 (Affinity Bioreagents) at 1 μg/ml. Primary antibodies were detected with their cognate biotinylated or horseradish peroxidase conjugated rabbit anti-mouse
or goat anti-rabbit secondary antibodies (Jackson Immunoresearch). Biotin-conjugated and horseradish peroxidase secondary antibodies were detected using $^{125}$I-streptavidin (Amersham Biosciences) or the West Pico Chemiluminescent substrate kit, respectively. Autoradiographs were visualized using Biomax MS film (Kodak) and Chemiluminescence was visualized using X-Omat Blue film (Kodak).

**Radio-ligand Binding Assay**

The mAHR photoaffinity ligand 2-Azido-3-$[^{125}\text{I}]$iodo-dibromodibenzo-$p$-dioxin ($^{125}$I N$_3$Br$_2$DpD) was synthesized as described earlier (Perdew, 2004). Cytosol from transiently transfected Cos-1 cells was diluted to 0.5 mg/ml and incubated with the photo-affinity ligand; 2-azido-3-$[^{125}\text{I}]$iodo-7,8-dibromodibenzo-$p$-dioxin (2176 Ci/mMol) in borosilicate tubes for 30 min at room temperature. The samples were then placed on ice for 5 min, dextran-coated-charcoal was added to a final concentration of 1%, and then the samples were incubated for a further 20 min. The samples were photolysed at >302nm at a distance of 8cm for 4 min using two UV lamps (Dazor Mfg. Corp. St. Louis, MO. The samples were spun 30,000Xg for 10 min at 4°C and the supernatant was added to sample buffer and resolved on TSDS-PAGE (8% -acrylamide). The gel was dried and visualized using autoradiography.

**Flourescent Microscopy**

Cos-1 cells were transfected with 0.750 μg of pEYFPmAHR or pEYFPmAHRΔH1 and pCI/XAP2 in 30-mm glass bottom dishes (MatTek Corp., Ashland, MA). 18 h after transfection cells were visualized prior to and following treatment with 10 nM TCDD or carrier solvent. After transfection and treatment, cells were visualized by fluorescence microscopy.
FIGURE LEGENDS

Figure S1 Identification of Genes Regulated by AHR in a DNA-binding Independent Manner

(a) SV40 immortalized AHR-null mouse hepatocytes were co-transfected with a combination of GFP and WT-AHR/A78D-AHR/control vector in a ratio of 1:3, using Lipo2000 transfection reagent. Phase contrast (top) and fluorescence (bottom) microscopy were performed 24 h post-transfection. (b) Venn diagram representation of microarray data analysis for changes in gene expression. Gray area represents genes altered by WT- and A78D-AHR expression but not in control vector transfected AHR-null mouse hepatocytes.

Figure S2 Characterization of the Heterodimerization AHR Mutant (ΔH1-AHR)

(a) Immunoprecipitation-Western blot analysis of whole-cell lysate from Cos-1 cells transiently transfected with WT-AHR or ΔH1-AHR along with HA-tagged ARNT. Protein immunoprecipitated with anti-HA antibody was probed for AHR with the RPT1 anti-AhR antibody, and for HA-ARNT with anti-HA antibody. (b) Luciferase reporter assay to assess transcriptional activity of WT-AHR and ΔH1-AHR on DRE-driven GUD-LUC6.1 reporter in transiently transfected Cos-1 cells. After 18 h of transfection, cells were treated with 10 nM TCDD for 6 h. Data represents mean and standard deviation of triplicate measurements. (c) Real-time PCR to measure Cyp1a1 mRNA induction in BP8 cells transiently transfected with WT-AhR or ΔH1-AHR. After 24 h of transfection, cells were treated with 10 nM TCDD for 4 h. Data represents mean and standard deviation of triplicate measurements. (d) Autoradiograph (top) demonstrating binding of the photo-affinity radioligand (2-azido-3-[125]iodo-7,8-dibromodibenzo-p-dioxin) to WT-AHR and ΔH1-AHR present in cytosolic lysate obtained from
transiently transfected Cos-1 cells. Expression of WT-AHR and ΔH1-AHR in transfected Cos-1 cells was analyzed by Western blot (bottom). (e) Fluorescence microscopy of Cos-1 cells transiently transfected with YFP-tagged versions of WT-AHR (top) or ΔH1-AHR (bottom), before and after 1 h treatment with 10 nM TCDD.
Figure 1S

(a) Phase Contrast and Fluorescence images of Blank, GFP, WT, and A78D conditions.

(b) Venn diagram illustrating the overlap of A78D, WT, and GFP conditions.
Figure S2:

**Figure S2:**

- **a**: Table showing IP: α HA and INPUT: 10% for WT-AHR, ΔH1-AHR, HA-ARNT, and Control IgG. WB: α AHR and WB: α HA are shown.

- **b**: Bar graph showing RLU/Gal for Control, WT-AHR, ΔH1-AHR under CONTROL and TCDD conditions.

- **c**: Bar graph showing CYP1A1/Δ Gal for Control, WT-AHR, ΔH1-AHR under CONTROL and TCDD conditions.

- **d**: Images showing Ligand Binding and 10% Input WB:α AHR for Control, WT-AHR, and ΔH1-AHR.

- **e**: Images showing WT-AHR and ΔH1-AHR under Control and TCDD conditions.