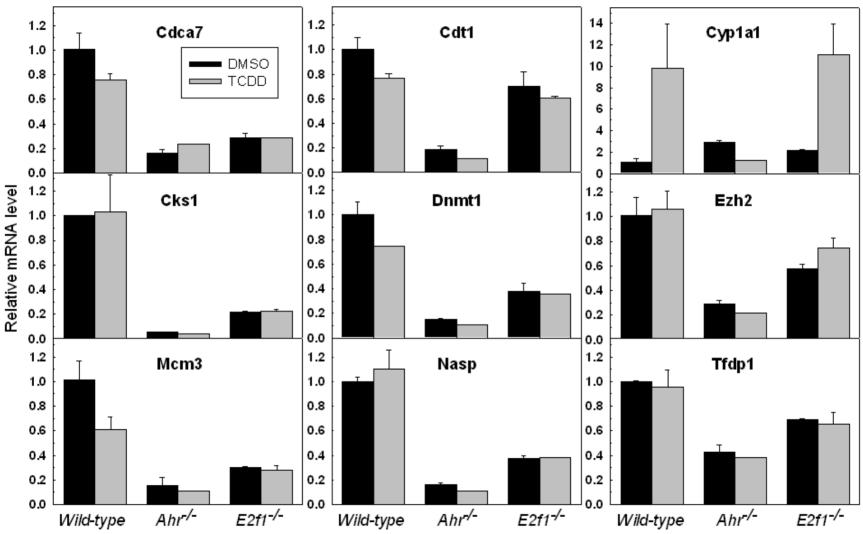
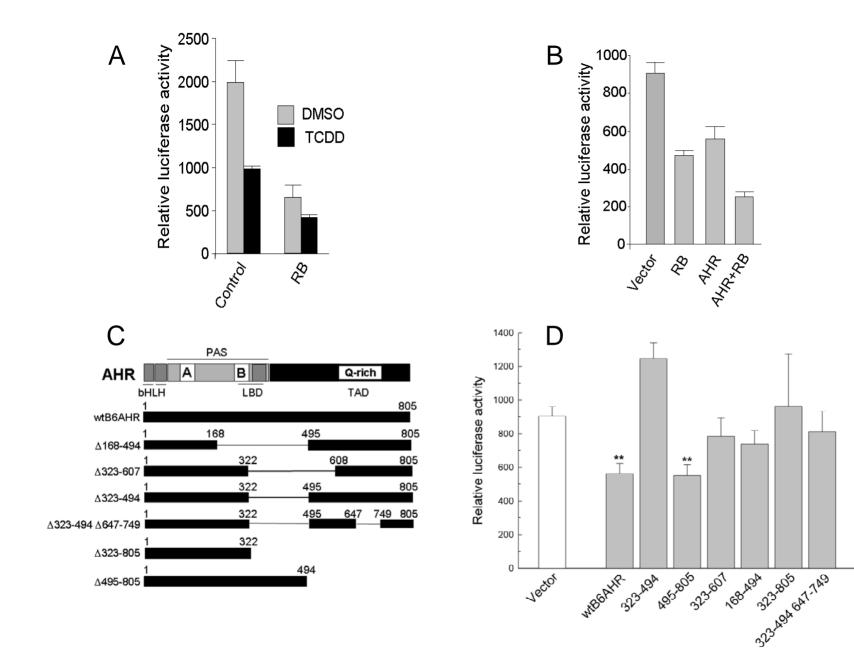
S-phase specific genes co-regulated by E2F1 and AHR (*Cyp1a1* included as control for AHR but not E2F regulation)



Marlowe et al. Supplemental Fig. S1

Supplemental Fig. S1. Several S-phase specific genes are co-regulated byE2F1 and AHR. Total cellular RNA was extracted from wild-type, $Ahr^{-/-}$ and $E2f1^{-/-}$ MEFs and mRNA levels for the genes indicated in each panel was determined by real-time RT-PCR and normalized to β -actin expression. Expression of *Cyp1a1*, an AHR but not an E2F1 target, is shown for comparison purposes.



Marlowe et al. Supplemental Fig. S2

Supplemental Fig. S2. The AHR PAS-B domain is involved in the AHR-E2F1 interaction. Transient expression assays of luciferase reporters were performed using Lipofectamine Plus (Invitrogen) on cells grown to 50-75% confluence in 24-well plates using protocols standardized previously (25). All transfection mixtures were brought to the same amount of total DNA by the addition of the appropriate amount of empty vector. To control for variations in transfection efficiency, cells were also co-transfected with a pCMVβgal plasmid (Clontech) and transfections were normalized to β -galactosidase expression. The results shown are the mean \pm SE of logtransformed results from multiple independent experiments. (A) Hepa-1 cells were cotransfected with the p3XE2FLUC reporter plus pRB, a RB expression plasmid {Puga, 2000 4549 /id} or an empty vector and treated with 5 nM TCDD or DMSO vehicle. (B) Saos-2 cells were co-transfected with the p3XE2FLuc reporter plus pRB, pcDNAI/B6AHR, both or reporter alone. In these cells, no ligand is needed to activate the AHR. (C) Schematic representation of the AHR protein, showing the approximate location of the bHlH domain, ligand-binding domain, PAS regions and Q-rich transcriptional activating domain. The regions deleted in the various truncation mutants tested for interaction with E2F are denoted by a thin line. (D). Saos-2 cells were transfected with the p3XE2FLuc reporter alone or co-transfected with the various AHR truncation mutants shown. Two asterisks denote p < 0.01.