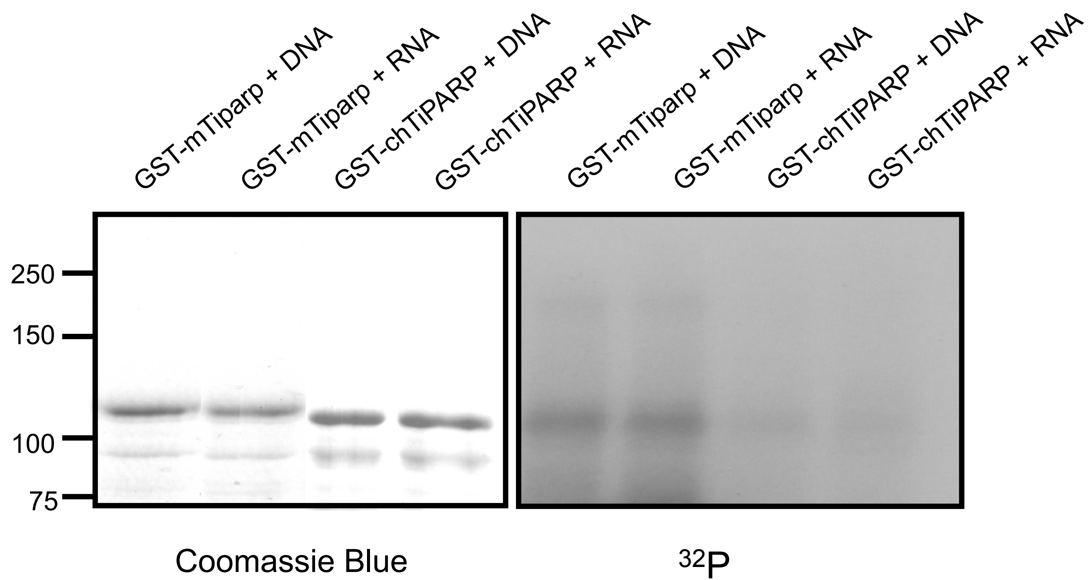
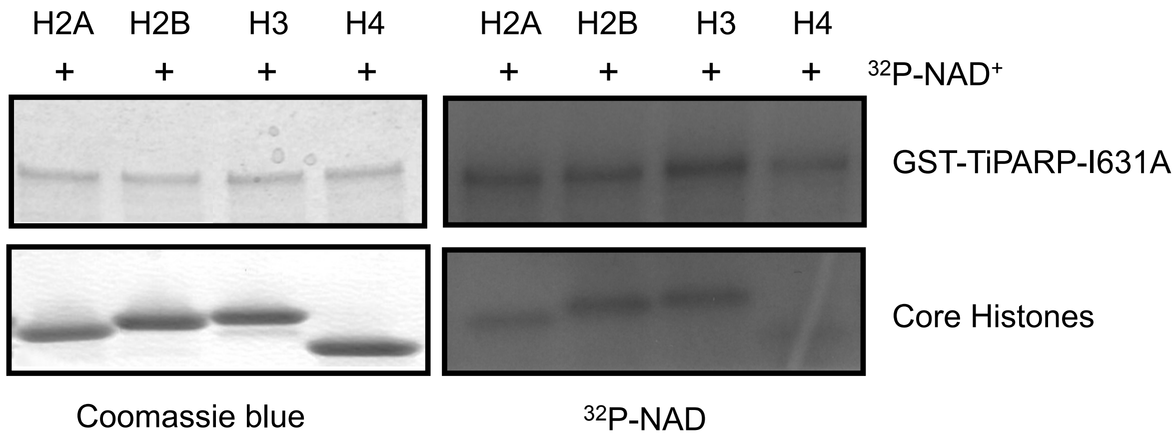
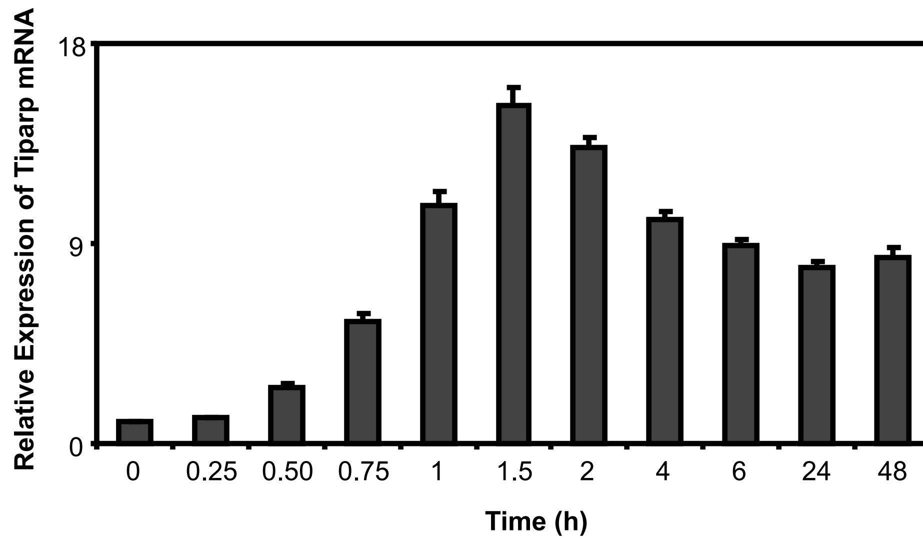


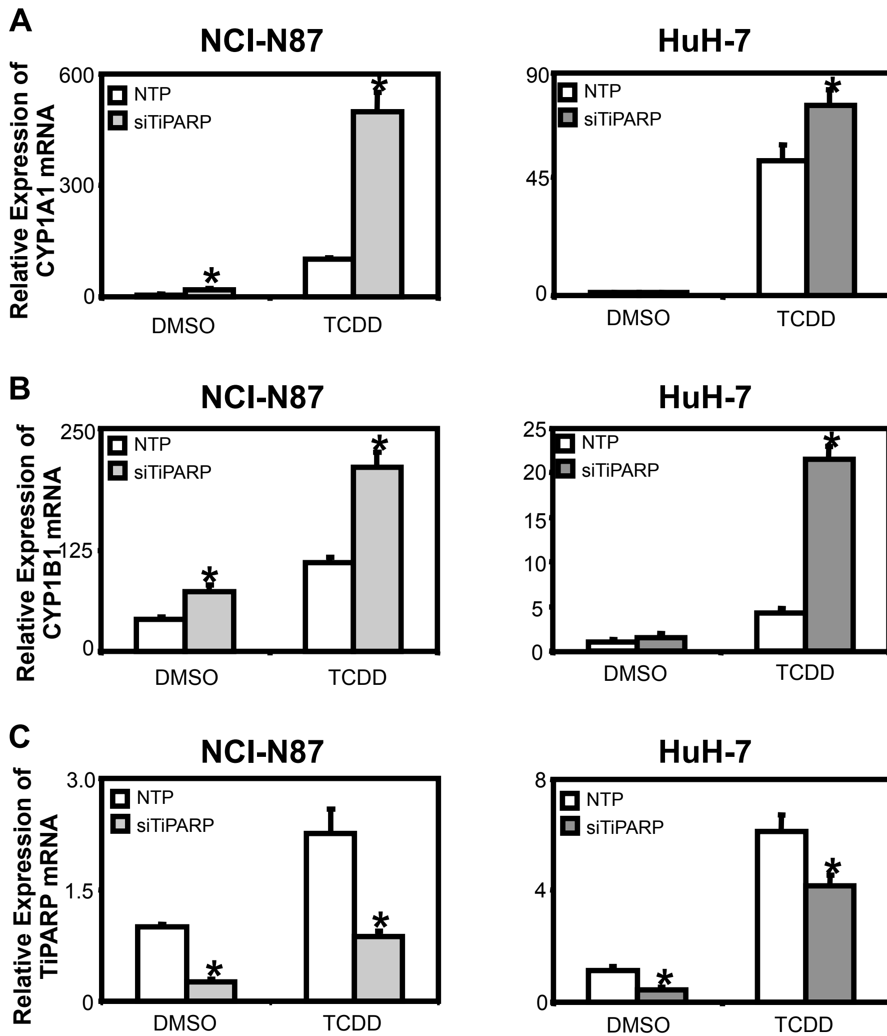
S1. Auto-ADP-ribosylation of mouse Tiparp (mTiparp) and chicken TiPARP (chTiPARP) incubated with DNA or RNA. Mouse GST-tagged Tiparp (GST-mTiparp) and chicken GST-tagged TiPARP (GST-chTiPARP) were incubated with ^{32}P-NAD^{+} and activated DNA or DNAse-treated RNA and analyzed by SDS-PAGE and autoradiography.



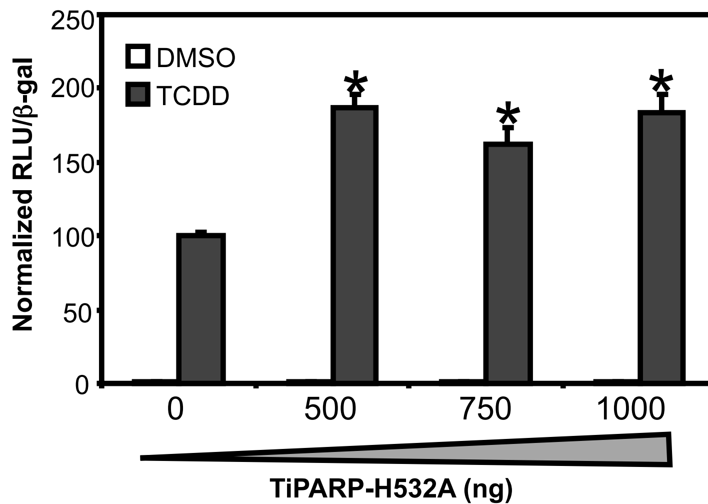
S2. Hetero-ADP-ribosylation of core histones by TiPARP catalytic mutant I631A. GST-TiPARP-I631A was incubated with ^{32}P-NAD^{+} and histones H2A, H2B, H3 or H4 and analyzed by SDS-PAGE and autoradiography.



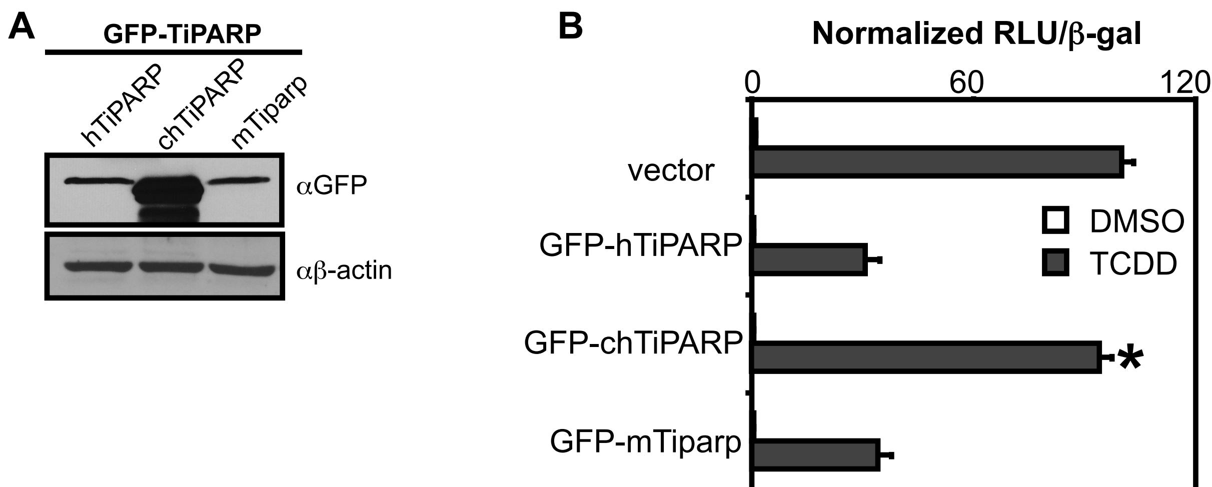
S3. Temporal analysis of TCDD-induced Tiparp mRNA expression levels in Hepa1c1c7 cells. Hepa1c1c7 cells were treated with 10 nM TCDD at the times indicated. Data were presented as means ± S.E.M. of three independent replicates.



S4. Pooled RNAi-mediated TiPARP knockdown in NCI-N87, and HuH-7 cells. Cell lines were transfected with siRNA pool (containing four different siRNAs) targeting against TiPARP for 48 h. Cells were then treated with 10 nM TCDD and DMSO (control) for 24 h and RNA was isolated and reverse transcribed as described in the Materials and Methods. Changes in (A) CYP1A1; (B) CYP1B1; and (C) TiPARP mRNA expression was then determined using qPCR. Data were normalized to non-targeting pool (NTP) DMSO (control) and to β-actin levels of each cell line. Results shown were means ± S.E.M for at least four independent experiments. mRNA expression levels for each cell line significantly ($P < 0.05$) different than NTP transfected cells were denoted with an asterisk.



S5. Dose-response of TiPARP H532A catalytic mutant in HuH-7 cells. HuH-7 cells were plated and co-transfected with varying amounts of pcDNA-TiPARP-H532A construct (0-1000 ng) and pCYP1A1-luc. Transfected cells were treated with TCDD for 24 h and reporter gene assays were performed as described in Materials and Methods. Results shown were means ± S.E.M. of two independent replicates. Luciferase activity significantly (*P* <0.05) different than empty vector transfected cells was denoted with an asterisk.



S6. (A) Overexpression of GFP-tagged human (hTiPARP), chicken (ChTiPARP) and mouse TiPARP (mTiparp) in HuH-7 cells. HuH-7 cells were transfected with pEGFP-TiPARP construct for 48 h. Proteins were detected by Western blot using αGFP antibody. **(B) The effect of overexpression of GFP-tagged human, chicken and mouse TiPARP on *CYP1A1*-Luc.** HuH-7 cells were transiently co-transfected with pEGFP-TiPARP construct and pCYP1A1-luc as described in Materials and Methods. Results shown were means ± S.E.M. of at least two independent replicates. Luciferase activity significantly (*P* <0.05) different than GFP-hTiPARP transfected cells was denoted with an asterisk.

A

Normalized RLU/β-gal

0 60 120

vector

GFP Zn Catalytic 1-657 *

GFP Zn WWE Catalytic 33-657 *

GFP Zn WWE Catalytic 103-657 *

GFP Zn WWE Catalytic 200-657 * #

GFP Zn WWE Catalytic 218-657 *

GFP Zn WWE Catalytic 225-657 *#

GFP WWE Catalytic 245-657 #

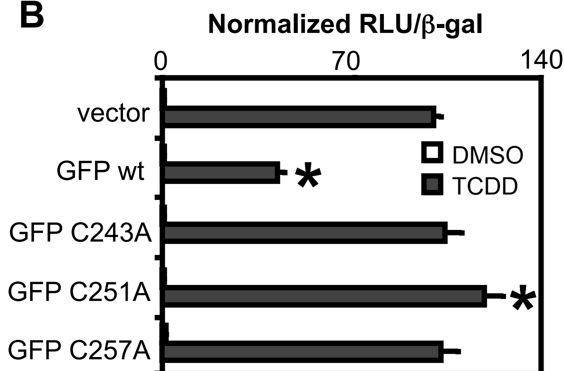
GFP WWE Catalytic 275-657 #

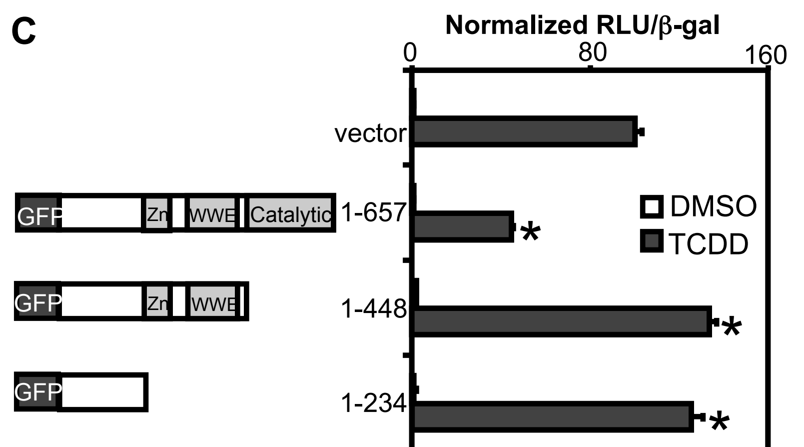
GFP WWE Catalytic 328-657 #

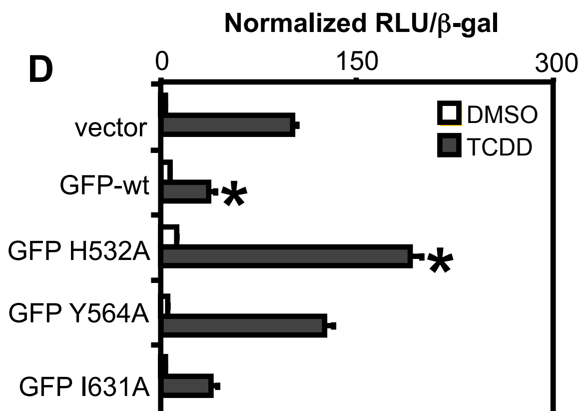
GFP Catalytic 445-657 #

DMSO

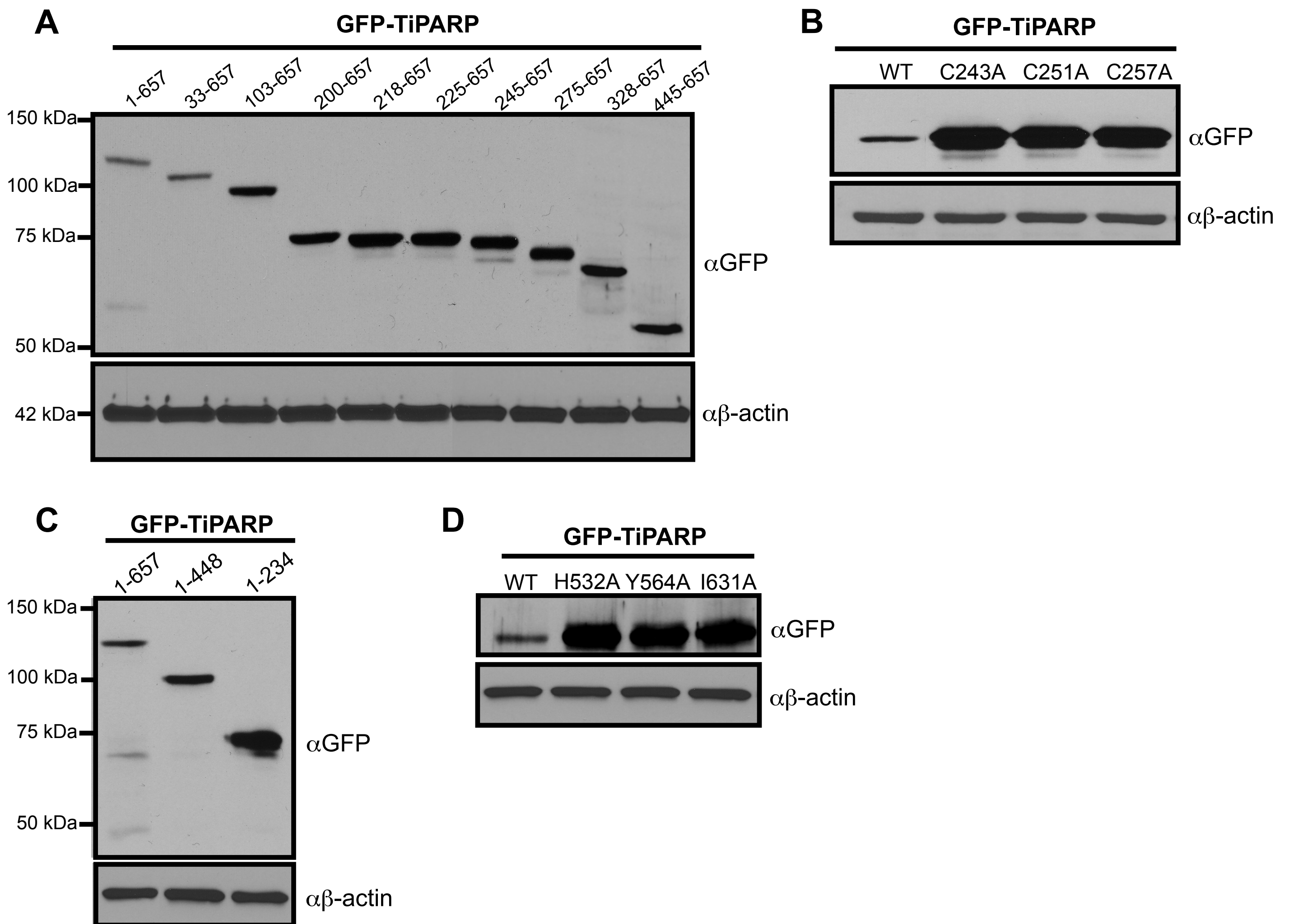
TCDD



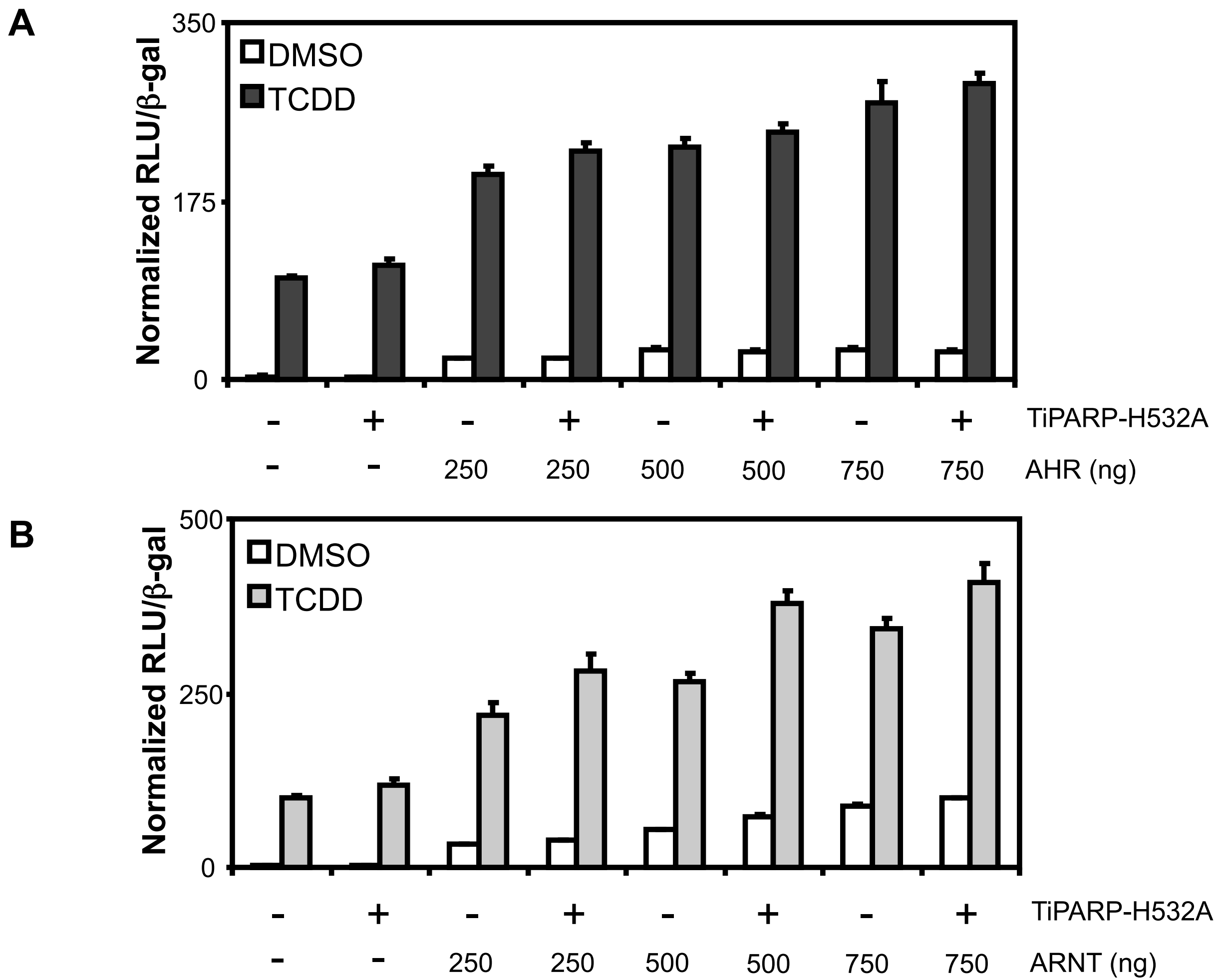




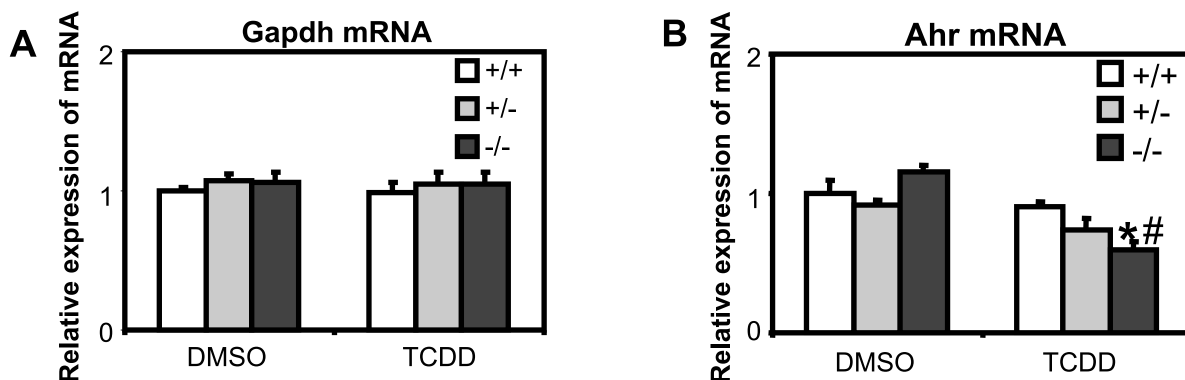
S7. The effect of GFP-tagged TiPARP N-terminal truncations (A), zinc finger point mutants (B), C-terminal truncations (C), catalytic point mutants (D) on reporter gene activity. Full-length GFP-TiPARP (1-657, wt), GFP-TiPARP truncation or point mutant constructs were co-overexpression with pCYP1A1-luc in HuH-7 cells and treated with TCDD. Data were presented as means ± S.E.M from three independent experiments and significance analyzed by one-way ANOVA and Tukey's multiple comparisons test. Reporter gene activity significantly different than (*P* < 0.05) TCDD-treated empty vector or TiPARP (1-657) was denoted with an asterisk or pound sign, respectively.



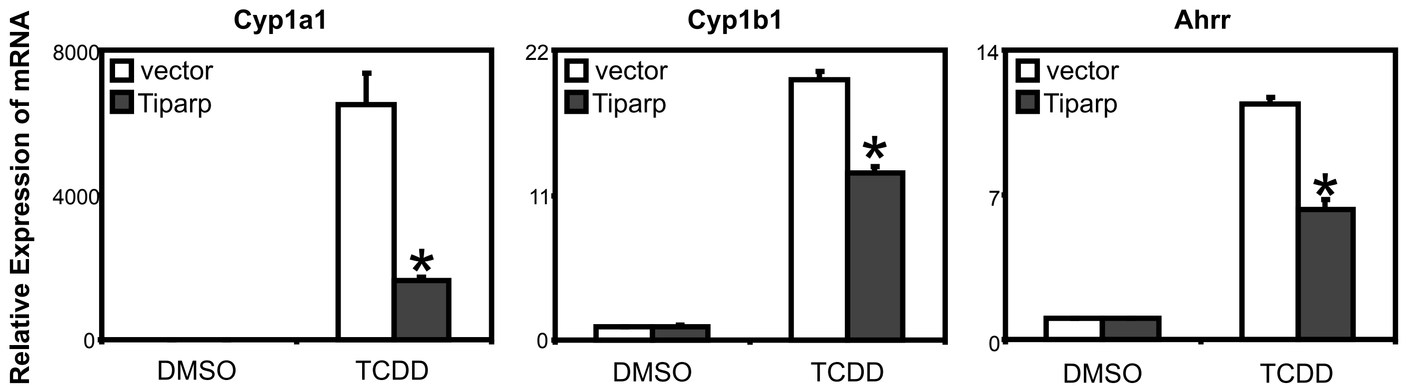
S8. Overexpression of GFP-tagged TiPARP N-terminal truncations (A), zinc finger point mutants (B), C-terminal truncations (C), catalytic mutants (D). HuH-7 cells were transfected with pEGFP-TiPARP constructs for 48 h. Proteins were detected by Western blot using αGFP antibody. Representative blots of three independent replicates.



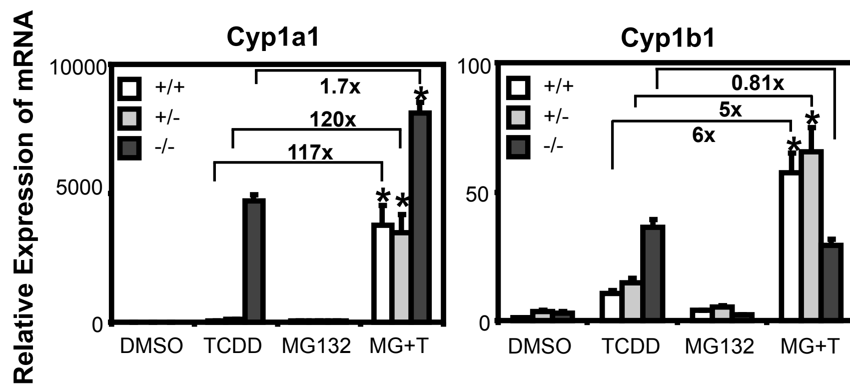
S9. Ectopic AHR and ARNT rescue was not observed with TiPARP catalytic mutant H532A. HuH-7 cells were co-transfected with pCYP1A1-luc, TiPARP-H532A (200 ng) and **(A)** AHR (250-750 ng) or **(B)** ARNT. Results were shown as means ± S.E.M from two independent experiments.



S10. Expression of Gapdh mRNA (A) and Ahr mRNA (B) in *Tiparp*-wildtype (+/+), het (+/-) and -null (-/-) mouse embryonic fibroblasts. MEF lines were treated with DMSO or TCDD for 24 h and RNA was isolated as described in Materials and Methods. Data were normalized to wildtype DMSO-treated cells and to β-actin levels. Results shown were means ± S.E.M for three independent experiments. mRNA expression levels significantly ($P < 0.05$) different than genotype-matched DMSO-treated cells are denoted with an asterisk. mRNA expression levels significantly ($P < 0.05$) different than treatment-matched wildtype (+/+) cells were denoted with a pound sign.



S11. Genetic Complementation of *Tiparp*$^{-/-}$ MEFs with mouse Tiparp. *Tiparp*$^{-/-}$ MEFs were transfected with 2 µg pcDNA-mTiparp or pcDNA (vector). Following 24 h transfected cells were treated with TCDD for 24 h and RNA was isolated and reverse transcribed as described in Materials and Methods. Data presented were representative of means ± S.E.M. of three independent experiments. mRNA expression significantly (P<0.05) different than treatment-match and vector transfected cells was denoted with an asterisk.



S12. TiPARP is a labile negative regulator of AHR. *Tiparp*$^{-/-}$ MEFs were treated with 10 nM TCDD or 25 mM MG-132 (MG) alone or in combination. The expression levels of CYP1A1 and CYP1B1 were determined by qPCR as described in Materials and Methods. Data presented were representative of means ± S.E.M. of three independent experiments. Gene expression levels significantly different (P < 0.05) than TCDD alone within each genotype were denoted with an asterisk.