Supplemental Figure S1. Sequence alignment of the Arg-Cys region of AhRs from diverse species. Conserved Arg residues are indicated in blue and Cys residues in yellow.

\_\_\_\_\_

Species	Protein	Sequence
		* **
Mouse (C57)	$AhR^{\mathtt{b}}$	NASFME <mark>rc</mark> fr <mark>cr</mark> l <mark>rc</mark> lldns
Guinea Pig	AhR	NSPLME <mark>rC</mark> FV <mark>Cr</mark> L <mark>rC</mark> LLDNS
Rat (RN)	AhR	NTAFME <mark>rc</mark> fr <mark>cr</mark> lr <mark>c</mark> lldns
Human	AhR	NSPLME <mark>rc</mark> fi <mark>cr</mark> lrclldns
Hamster	AhR	SASFLE <mark>rc</mark> fi <mark>crlrc</mark> lldns
Rabbit	AhR	NSSFME <mark>rC</mark> FI <mark>CrlrC</mark> lldns
Seal	AhR	NSSCME <mark>r</mark> SFV <mark>CrlrC</mark> lldns
Whale	AhR	NSSSME <mark>rc</mark> fV <mark>crlrc</mark> lldns
Dolphin	AhR	NSSSME <mark>rc</mark> fv <mark>cr</mark> lr <mark>c</mark> lldns
Chicken	AhR	 NSSFME <mark>r</mark> nfi <mark>Cr</mark> lr <mark>c</mark> lldns
Tern	AhR	NSSFME <mark>r</mark> nfi <mark>cr</mark> lr <mark>c</mark> lldns
Trout	AhR	NSSFLE <mark>r</mark> nfV <mark>Cr</mark> fr <mark>C</mark> lldns
Fundulus	AhR1	nssfle <mark>r</mark> nfv <mark>crfrc</mark> lldns
Fundulus	AhR2	SSSFLE <mark>r</mark> sfv <mark>cr</mark> fr <mark>c</mark> lldns
Zebrafish	AhR1a	NSTCLE <mark>r</mark> nfi <mark>cr</mark> lr <mark>c</mark> lldst
Zebrafish	AhR1b	NSSFLE <mark>r</mark> nfV <mark>CrfrC</mark> LLDNS
Zebrafish	AhR2	NSSFLE <mark>r</mark> SFC <mark>Cr</mark> F <mark>rC</mark> LLDNS
Tomcod	AhR	NSSFLE <mark>r</mark> SFV <mark>Cr</mark> f <mark>rC</mark> LLDNS
Lamprey	AhR	nssfle <mark>r</mark> qfi <mark>crfrc</mark> lldns

## Supplemental Figure S2.

Hsp90 binding to an AhR containing a D371A mutation is similar to that of wild-type (wt) AhR. D371A and wtAhR are indicated with an asterisk. Hsp90 binding to other AhR mutants are also shown, but are not included in this manuscript.

COS-1 cells grown in 100 mm culture plates were transiently transfected with 8  $\mu$ g of wtAhR or the indicated mutant AhR expression vector and 20  $\mu$ l of Lipofectamine 2000 (Invitrogen). Twenty four hours after transfection, cells were rinsed with PBS, plates were scraped and cells lysed on ice for 30 min using MEG-RIPA buffer (25 mM MOPS pH 7.5, 10% v/v glycerol, 1 mM EDTA, 1% v/v Igepal CA-630, 0.5% w/v sodium deoxycholate, 0.1% w/v SDS, 0.5% v/v protein inhibitor cocktail (Sigma)). Lysates were centrifuged at 14,000xg for 10 min, and 400  $\mu$ l aliquots of supernatant (0.8-1.2 mg protein) were incubated with antihsp90 antibody 3G3 or control IgM bound to Affigel 10 substrate (BioRad) (prepared as previously described (21,23)) for 1 h at 4°C with shaking. Samples were washed three times with MEGRIPA. Proteins were resolved by SDS-PAGE and detected by Western blotting as described (20) using anti-AhR M20 antibody (Santa Cruz Biotechnology), anti-hsp90 antibody 3G3 and anti- $\beta$ -actin antibody (Santa Cruz Biotechnology). Results are representative of three independent experiments.

