Supplementary Information

Structural insight into the ligand binding mechanism of aryl hydrocarbon receptor

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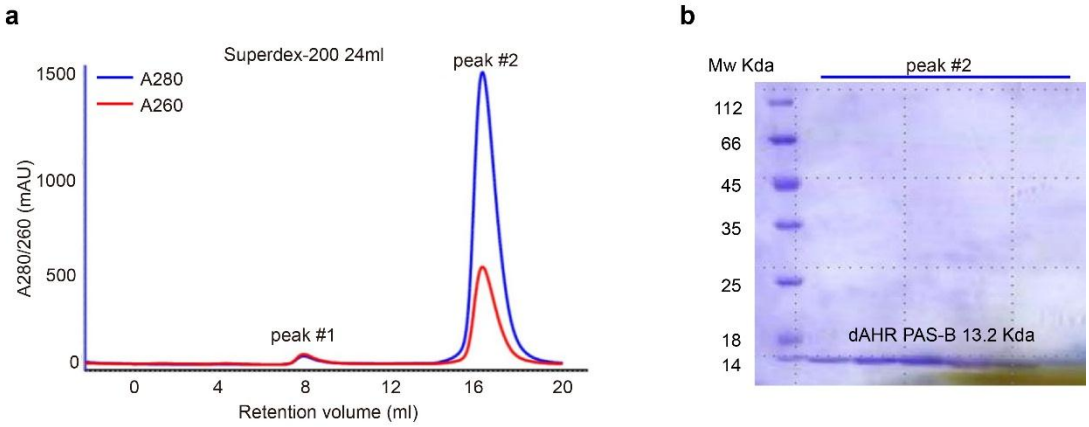
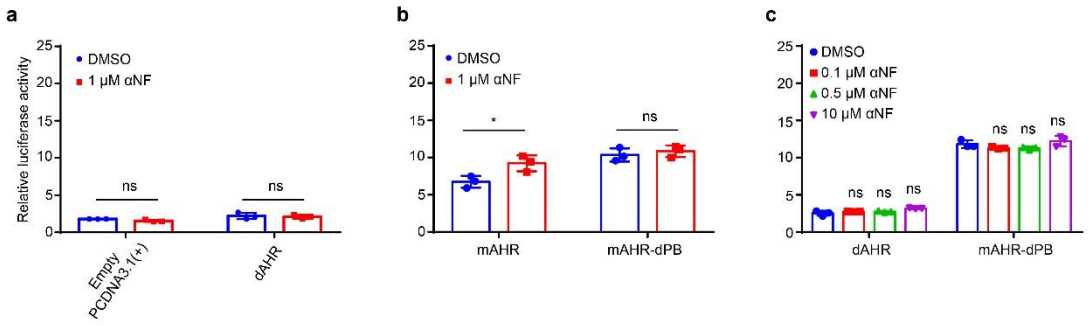
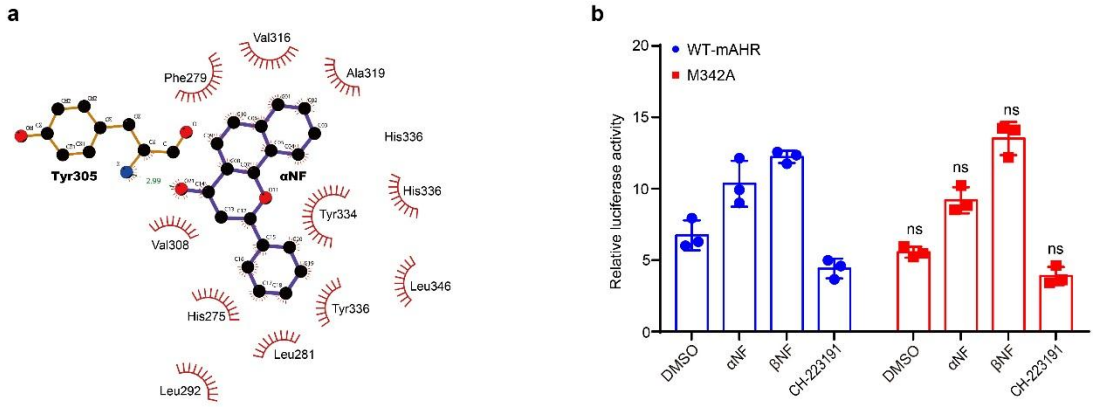


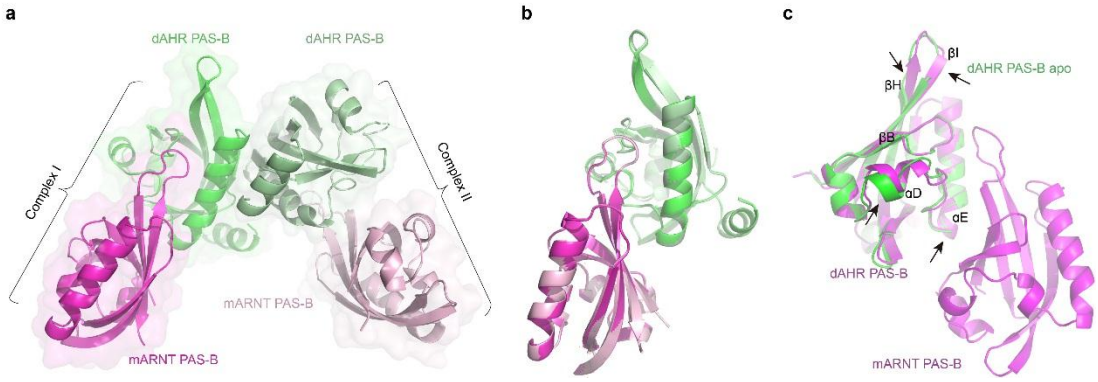
Supplementary Fig. 1 Recombinant expression and purification of dAHR PAS-B domain. a SEC profile of purified dAHR PAS-B. **b** SDS-PAGE analysis of dAHR PAS-B. These results are representative of at least three independent experiments. Source data are provided as a Source Data file.



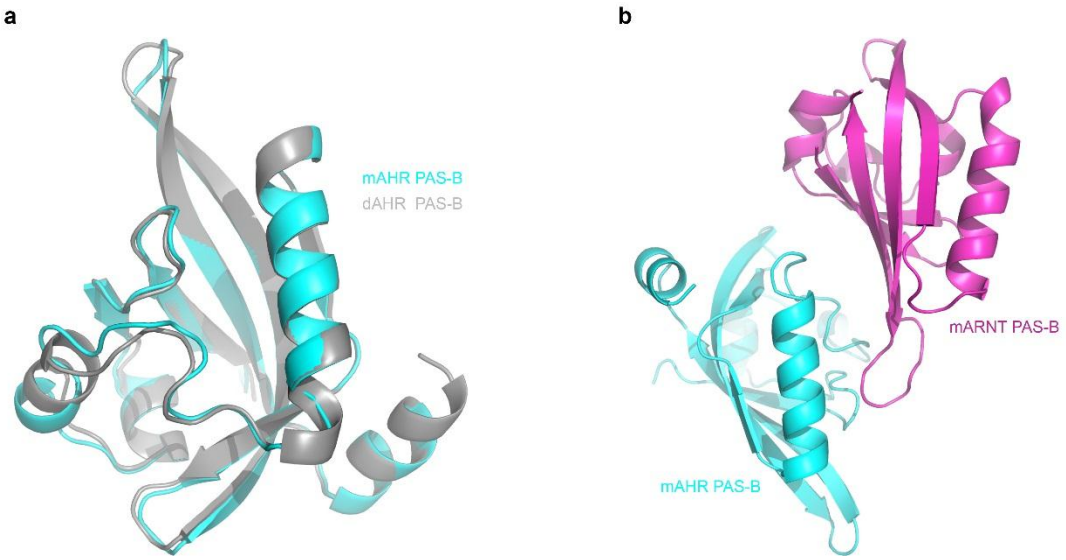
Supplementary Fig. 2 Luciferase gene reporter assays performed for dAHR and mAHR-dPB. a dAHR shows little or no transcriptional activity in HEK293T cells. **b** mAHR-dPB has a higher basal activity than mAHR, and the presence of αNF does not impact its transcriptional activation. **c** Different concentrations of aNF have no impact on the activity of mAHR-dPB or dAHR. n = 3 biological repeats for each group. Data are shown as mean ± SD. *p*-values were calculated using a two-way ANOVA with Tukey's multiple comparisons test and presented in the Source Data file. *: p<0.05, ns: not statistically significant ($p > 0.05$). Source data are provided as a Source Data file.



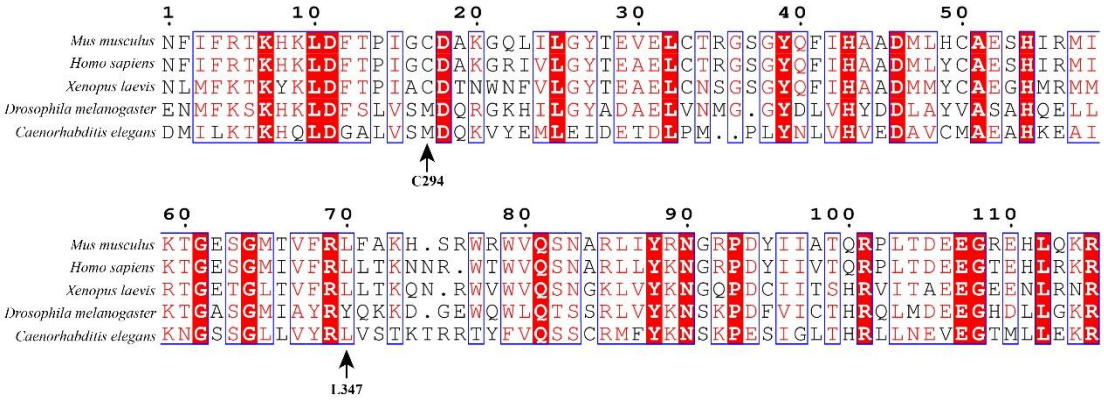
Supplementary Fig. 3 The binding of αNF to AHR PAS-B. a LigPlot representation of the interactions between αNF and dAHR PAS-B. **b** DRE luciferase reporter assay examining the effect of the M342A mutation on the transactivation of full-length mAHR in 293T cells. The ligands βNF (agonist), αNF (partial agonist) and CH-223191 (antagonist) were supplied at 500 nM, 1 μM and 1 μM, respectively. n = 3 biological repeats for each group. Data are shown as mean ± SD. *p* values were calculated using a multiple two-sided *t* test with the WT mAHR group treated by the same ligand as a control and presented in the Source Data file. ns: not statistically significant ($p > 0.05$). Source data are provided as a Source Data file.



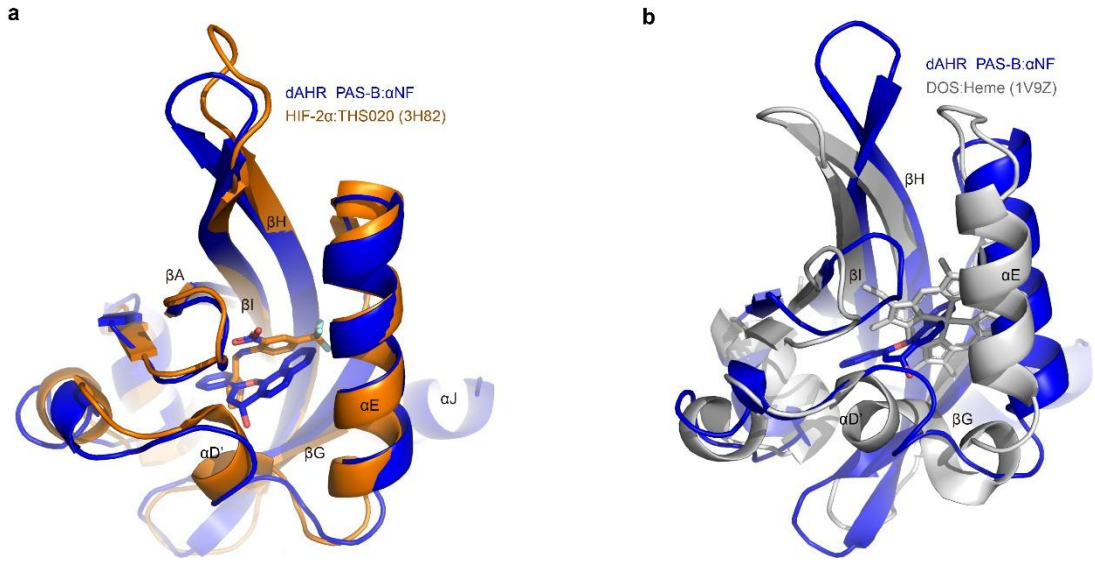
Supplementary Fig. 4 Crystal structure of dAHR PAS-B:mARNT complex. a The asymmetric unit of the complex crystal. **b** Superimposition of the two heterodimer complexes (complex I and complex II) in the ASU. **c** Comparison of apo (green) and ARNT-bound (magenta) dAHR PAS-B structures. Conformational differences are indicated by black arrows.



Supplementary Fig. 5 Structure model mAHR PAS-B and ARNT PAS-B heterodimer. a mAHR PAS-B model (cyan). The model was generated by the program Modeller with apo dAHR PAS-B structure (gray) used as the single template. **b** mAHR-ARNT PAS-B heterodimer model. The model was generated by roughly overlaying the mAHR PAS-B onto the dAHR PAS-B:mARNT structure.



Supplementary Fig. 6 Multiple sequence alignment of AHR PAS-B domains from different species.



Supplementary Fig. 7 Ligand binding positions of different PAS proteins. a Comparison of ligand binding poses of dAHR (blue) and human HIF-2α (orange, PDB code 3H82). **b** Ligand binding poses of dAHR and *Escherichia coli* DOS (grey, PDB code 1V9Z).

Supplementary Table 1 Data collection and refinement statistics.

	dAHR PAS-B apo	dAHR PAS-B:αNF	dAHR PAS-B:ARNT
Data collection			
Resolution (Å)	31.6-2.6 (2.7-2.6)	30.2-2.4 (2.5-2.4)	44.16-2.0 (2.1-2.0)
Wavelength (Å)	1.58	0.975	0.975
Space group	$P2_12_12_1$	$P4_12_12$	$C2_1$
a, b, c (Å)	34.3, 46.8, 81.7	60.2, 60.2, 121.0	136.0, 55.6, 77.1
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 103.1, 90
Unique reflections	4172 (255)	9195 (851)	36452 (3021)
Redundancy	10.7 (2.2)	23.2 (16.1)	6.8 (6.1)
Completeness (%)	95.1 (55)	99.9 (99.8)	96.8 (96.3)
Mean I/sigma (I)	17.7 (1.4)	16.3 (2.4)	16.5 (2.6)
Refinement			
R-work / R-free	0.21 (0.23) /0.26 (0.29)	0.23 (0.31) /0.27 (0.36)	0.18 (0.23) / 0.22 (0.28)
NO. of non-hydrogen atoms	915	1896	3990
Macromolecules	904	1806	3554
Ligands	0	42	0
Solvent	11	48	436
Protein residues	114	226	432
RMS (bonds)	0.007	0.004	0.006
RMS (angles)	0.51	0.70	0.83
Clash score	6.8	5.6	10.1
Ramachandran favored (%)	98	97	97
Ramachandran outliers (%)	1.8	0	2.8
Rotamer outliers (%)	2.2	1.1	2.8
Average B-factor	38.5	44.1	31.8