Supporting information

Plasmid Construction

pCETT2/AhRbHLH/ArntbHLH. All AhR and Arnt fragments used in this study were based on the human AhR and Arnt cDNA[1] generously provided by Patricia Harper and Allan Okey (Department of Pharmacology and Toxicology, University of Toronto). The AhRbHLH sequence encoding amino acids 20-90 of human AhR (GeneID: 196) was amplified from AhR cDNA[1] with oligonucleotides 1 and 2 (all oligonucleotides are listed in Table S1) and inserted between the Sal I and Xba I sites of pCETT2 to construct pCETT2/AhRbHLH. The ArntbHLH sequence encoding amino acids 82-149 of human Arnt isoform 1 (GeneID: 405) was constructed as described previously[2] and inserted between the EcoR I and Xho I sites of pCETT2 and pCETT2/AhRbHLH to generate pCETT2//ArntbHLH and pCETT2/AhRbHLH/ArntbHLH, respectively.

pCETT/AhRJun/ArntFos and pCETT2/AhRJun/ArntFos. In preparation for the cloning of AhRJun, a DNA fragment (pre-AhRJun) containing the sequence encoding amino acids 81-86 of human AhR followed by amino acids 296-332 of human JunD (GeneID: 3727) was assembled by self-priming PCR[3] using oligonucleotides 4-7. The final AhRJun fragment was then amplified using pre-AhRJunD and AhRbHLH (described above) as templates using oligonucleotides 1 and 7 as the 5' and 3' primers, respectively. This amplified fragment was digested with Sal I and Xba I, and then ligated into these restriction sites in pCETT and pCETT2 to construct pCETT//AhRJun and pCETT2/AhRJun, respectively.

We note that the AhRJun hybrid contains amino acids 20-86 of human AhR, which is in contrast to the AhRbHLH fragment constructed above that encodes amino acids 20-90 of human AhR. The ArntFos fragment was constructed in a similar manner to that of AhRJun and was previously named "ArntbHLH-Fos."[2] This fragment was digested with EcoR I and BamH I and inserted into these restriction sites in pCETT/AhRJun and pCETT2/AhRJun to generate pCETT/AhRJun/ArntFos and pCETT2/AhRJun/ArntFos, respectively. The EcoR I/BamH I-digested ArntFos fragment was also inserted between these restriction sites in pCETT and pCETT2 to generate pCETT//ArntFos and pCETT2//ArntFos, respectively. We also note that the

ArntFos hybrid contains amino acids 82-148 of human Arnt, which is in contrast to the ArntbHLH fragment constructed above that encodes amino acids 82-149 of human Arnt.

pCETT2/AhR(ΔL)Jun/ArntFos. The AhR(ΔL)Jun fragment was constructed in a similar fashion to that of AhRJun. A DNA fragment (pre-AhR(ΔL)Jun) was assembled by self-priming PCR[3] using oligonucleotides 8 and 5-7. The final AhR(ΔL)Jun fragment was then amplified with pre-AhR(ΔL)Jun and AhRbHLH as templates using oligonucleotides 1 and 7 as the 5' and 3' primers, respectively. This amplified fragment was digested with Sal I and Xba I and then ligated into these restriction sites in pCETT2 and pCETT2//ArntFos to generate pCETT2/AhR(ΔL)Jun and pCETT2/AhR(ΔL)Jun/ArntFos, respectively.

pGBKT7/AhRbHLH and pGADT7/AhRbHLH. The AhRbHLH fragment was amplified from AhR cDNA[1] with oligonucleotides 9 and 10 and inserted between the EcoR I and BamH I sites of pGBKT7 and pGADT7 to generate pGBKT7/AhRbHLH and pGADT7/AhRbHLH, respectively.

pGBKT7/ArntbHLH and pGADT7/ArntbHLH. The ArntbHLH fragment was amplified from the previously described NArnt (native Arnt) cDNA[4] with oligonucleotides 3 and 11 and inserted between the EcoR I and Pst I sites of pGBKT7 to generate pGBKT7/ArntbHLH; the EcoR I/Xho I-digested ArntbHLH fragment, described above, was inserted into these restriction sites in pGADT7 to generate pGADT7/ArntbHLH.

pGBKT7/AhRJun and pGADT7/AhRJun. The AhRJun fragment was amplified from the AhRJun fragment with oligonucleotides 9 and 12 and inserted between the EcoR I and BamH I sites of pGBKT7 and pGADT7 to generate pGBKT7/AhRJun and pGADT7/AhRJun, respectively.

pGBKT7/AhR(ΔL)Jun and pGADT7/AhR(ΔL)Jun. The AhR(ΔL)Jun fragment was amplified from the AhR(ΔL)Jun fragment with oligonucleotides 9 and 12 and inserted between the EcoR I and BamH I sites of pGBKT7 and pGADT7 to generate pGBKT7/AhR(ΔL)Jun and pGADT7/AhR(ΔL)Jun, respectively.

pGBKT7/ArntFos and pGADT7/ArntFos. The EcoR I/BamH I-digested ArntFos fragment was inserted into these restriction sites in pGBKT7 and pGADT7 to generate pGBKT7/ArntFos and pGADT7/ArntFos, respectively.

pET/AhRJun. The AhRJun sequence, optimized for expression in *E. coli*, was generated by gene assembly as described previously[2] using oligonucleotides 13-22. The final AhRJun fragment was digested with Nco I and Xho I and inserted into the multiple cloning site of pET28(a)+.

pET/ArntFos-SUMO. pET/ArntFos was previously constructed in our lab.[2] pET-SUMO was purchased from Invitrogen. The SUMO sequence contained in pET-SUMO was PCR-amplified using oligonucleotides 23-24. The amplified fragment was singly digested by Xho I and inserted into pET/ArntFos, placing the SUMO sequence at the 3' end of ArntFos. This method inserts the SUMO fragment in two possible orientations: the correct orientation was confirmed by sequencing.

#	Sequence
1	TGC**GTCGAC**CAGAAAACAGTAAAGCCAAT
2	TAT**TCTAGA**GGAGGATTTTAATGCAACA
3	ACT**GAATTC**AGCTCTGCGGATAAAGAGAG
4	AGCTTCTTTGATGTTGCATTAGAAGAAAAGGTTAAGACC
5	CAAAGAAGCGGTAGAAGCCAATTCGGTGTTTTGAGACTTCAAGGTCTTAACCTTTTCTTC
6	GCTTCTACCGCTTCTTTGTTGAGAGAACAAGTTGCTCAATTGAAGCAAAAGGTTTTGTCT
7	GTCC**TCTAGA**AACGTGAGACAAAACCTTTTGCTT
8	AGCTTCTTTGATGTTGCAGAAGAAAAGGTTAAGACC
9	TGC**GAATTC**CAGAAAACAGTAAAGCCAAT
10	TAT**GGATCC**GGAGGATTTTAATGCAACA
11	ATA**CTGCAG**GGATGTGTTGCCAGTTCCCC
12	GTCC**GGATCC**AACGTGAGACAAAACCTTTTGCTT
13	GCTA**CCATGG**GCCAGAAAACCGTGAAACCG
14	TTGCTCGGATTGCTTTTAATGCCTTCCGCCGGAATCGGTTTCACGGTTTT
15	AAGCAATCCGAGCAAACGTCATCGTGATCGTCTGAATACCGAACTGGATC
16	CACATCCTGCGGAAACGGCAGCAGGCTCGCCAGACGATCCAGTTCGGTAT
17	TTTCCGCAGGATGTGATTAATAAACTGGATAAACTGAGCGTGCTGCGTCTGAG
18	GCGCCACATCAAAAAAGCTTTTCGCACGCAGATAGCTCACGCTCAGACGCAGCACGCT
19	TTTTTGATGTGGCGCTGGAAGAAAAAGTGAAAACCCTGAAAAGCCAGAAT
20	ACACGCAGCAGGCTCGCGGTGCTCGCCAGTTCGGTATTCTGGCTTTTCAG
21	GAGCCTGCTGCGTGTGGAACAGGTGGCGCAGCTGAAACAGAAAGTGCTGA
22	GGTG**CTCGAG**ATGGCTCAGCACTTTCTGTT
23	aatt**ctcgag**GGCAGCGGCCTGGT
24	aatt**ctcgag**TTCTCTGTGAGCCTCAATAATATCGTTATCCTC

Table S1. Primers used in this study. All sequences are written from 5' to 3'. Restriction enzyme cleavage sites are indicated in bold.

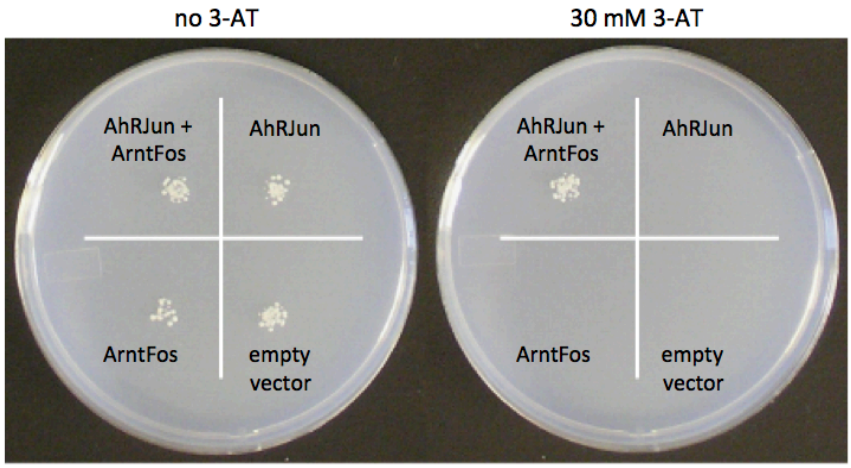


Figure S1. **Co-expression of AhRJun and ArntFos activates XRE1-controlled reporter gene in yeast.** The interaction between AhRJun and ArntFos was tested *in vivo* using the MY1H *HIS3* assay. pCETT/AhRJun/ArntFos, which co-expresses GAL4AD-AhRJun and ArntFos, was transformed into yeast strain YM4271[pHISi-1/XRE-6]. Other cells were transformed with pCETT/AhRJun, pCETT//ArntFos, or pCETT (empty vector). Cells were plated on SD/-H/-L plates with or without 30 mM 3-AT, and incubated at 30 °C for 5 days. Growth was observed for all transformants on the plate without 3-AT. Only cells co-expressing GAL4AD-AhRJun and ArntFos grew with 30 mM 3-AT.

Figure S2. Co-expression ofAhR and Arnt bHLH domains *without* the LZ is not sufficient to activate a XRE1-controlled reporter gene in yeast. The interaction between AhRbHLH and ArntbHLH was tested *in vivo* using the MY1H *HIS3* assay. pCETT2/AhRJun/ArntFos, which co-expresses GAL4AD-AhRJun and GAL4AD-ArntFos, was transformed into yeast strain YM4271[pHISi-1/XRE-6]. Other cells were transformed with pCETT2/AhRJun, pCETT2//ArntFos, or pCETT2 (empty vector). Cells were plated on SD/-H/-L plates with or without 30 mM 3-AT, incubated at 30 °C for 5 days. Growth was observed for all transformants on the plate without 3-AT. None of the transformants grew on the plate with 30 mM 3-AT.

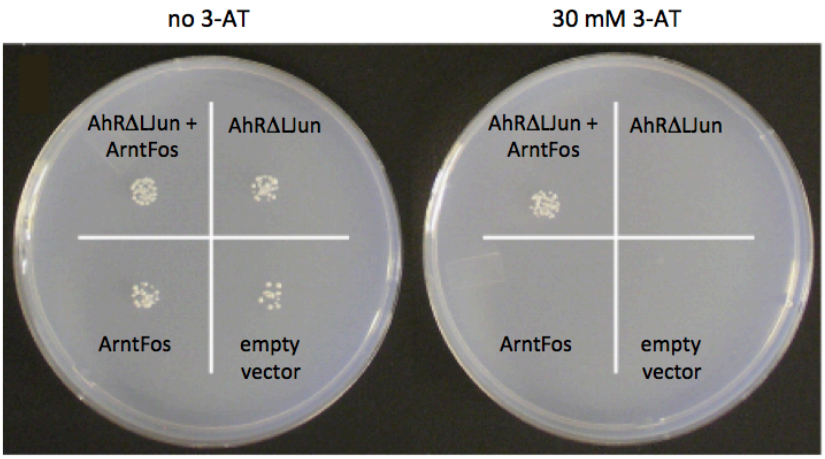


Figure S3. Disruption of the LZ heptad repeat register in AhRΔLJun does not affect the ability of AhRΔLJun/ArntFos to activate a XRE1-controlled reporter gene in yeast. The interaction between AhRΔLJun and ArntFos were tested *in vivo* using the MY1H *HIS3* assay. pCETT2/AhRΔLJun/ArntFos, which co-expresses GAL4AD-AhRΔLJun and GAL4AD-ArntFos, was transformed into yeast strain YM4271[pHISi-1/XRE-6]. Other cells were transformed with pCETT2/AhRΔLJun, pCETT2//ArntFos, or pCETT2 (empty vector). Cells were plated on SD/-H/-L plates with or without 30 mM 3-AT, and incubated at 30 °C for 5 days. Growth was observed for all transformants on the plate without 3-AT. Only cells co-expressing GAL4AD-AhRΔLJun and GAL4AD-ArntFos grew with 30 mM 3-AT.

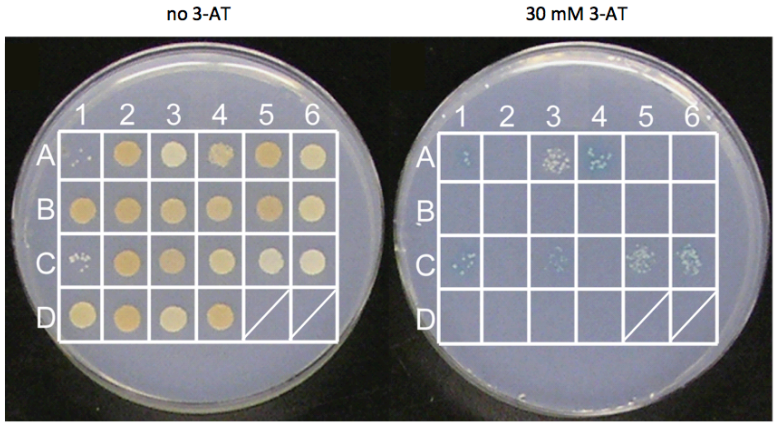


Figure S4. AhRJun and ArntFos are capable of forming both hetero- and homodimers in yeast. Protein/protein interactions were surveyed using the *in vivo* Y2H *HIS3* assay. Each spot represents yeast strain AH109 co-expressing proteins in different combinations, one fused to GAL4DBD and the other to GAL4AD. Cells were plated on SD/-H/-L plates with or without 30 mM 3-AT, and incubated at 30 °C for 5 days. Growth on the 30 mM 3-AT plate, along with development of blue color, indicated reporter gene activation. Details for the grid are given in Table S2, following.

Grid	Protein fused to GAL4DBD	Protein fused to GAL4AD	Growth in 3-AT	Description and interpretation
A1	AhRJun	ArntFos	+	Co-expression of AhRJun and ArntFos causes reporter gene activation, indicating that they heterodimerize in yeast.
A2	AhRJun	-	-	
A3	-	ArntFos	+[1]	
A4	ArntFos	AhRJun	+	
A5	ArntFos	-	-	
A6	-	AhRJun	-	
B1	AhR bHLH	Arnt bHLH	-	Co-expression of AhR bHLH and Arnt bHLH does not cause reporter gene activation. They are incapable of heterodimerizing in yeast.
B2	AhR bHLH	-	-	
B3	-	Arnt bHLH	-	
B4	Arnt bHLH	AhR bHLH	-	
B5	Arnt bHLH	-	-	
B6	-	AhR bHLH	-	
C1	AhR(ΔL)Jun	ArntFos	+	Co-expression of AhR(ΔL)Jun and ArntFos causes reporter gene activation, indicating that they heterodimerize in yeast.
C2	AhR(ΔL)Jun	-	-	
C3	ArntFos	AhR(ΔL)Jun	+	
C4	-	AhR(ΔL)Jun	-	
C5	AhRJun	AhRJun	+	Both AhRJun and ArntFos are capable of homodimerizing in yeast.
C6	ArntFos	ArntFos	+	
D1	AhRbHLH	AhRbHLH	-	Both AhR bHLH and Arnt bHLH are incapable of homodimerizing in yeast.
D2	ArntbHLH	ArntbHLH	-	
D3	AhR(ΔL)Jun	AhR(ΔL)Jun	-	AhR(ΔL)Jun is incapable of homodimerizing in yeast.[2]
D4	-	-	-	Double empty vector, negative control.

[1] Possibly a false positive signal, since blue color development was not observed.

[2] In contrast, Figure 3 (main text) shows AhR(ΔL)Jun capable of homodimerizing in yeast, but at weaker strength compared to AhRJun. The weaker signal may not have been detected under the serial dilutions of cells used for Figure S4.

Table S2. Legend for Figure S4. The letters A-D correspond to the rows in Figure S4, and the numbers correspond to the columns. The following information is provided for each spot on the grid: 1) Name of protein expressed with GAL4DBD, 2) Name of protein expressed with GAL4AD, 3) Whether there was growth on 30 mM 3-AT plate or not (positive or negative sign). A dash instead of protein name indicates that those cells were transformed with empty vectors.

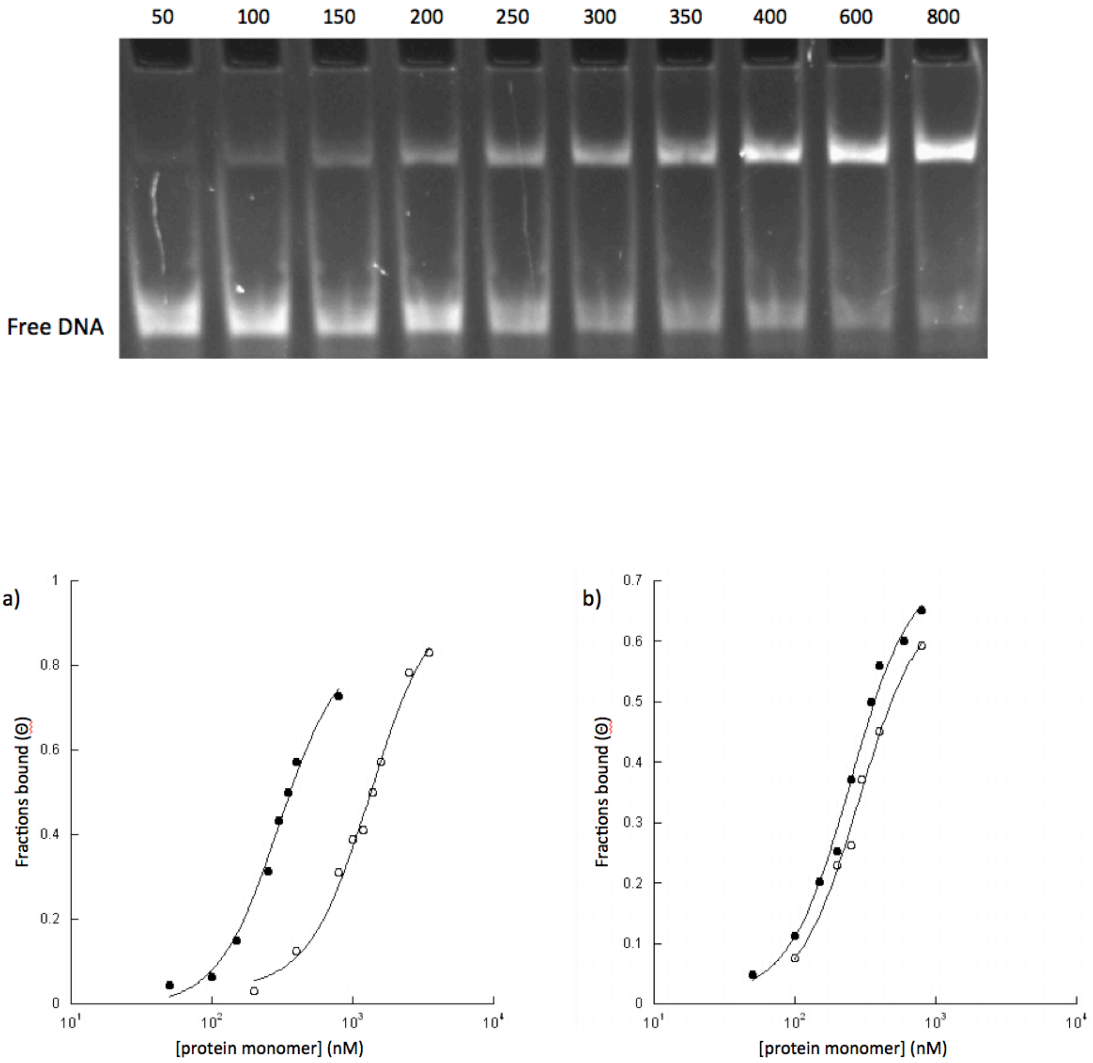
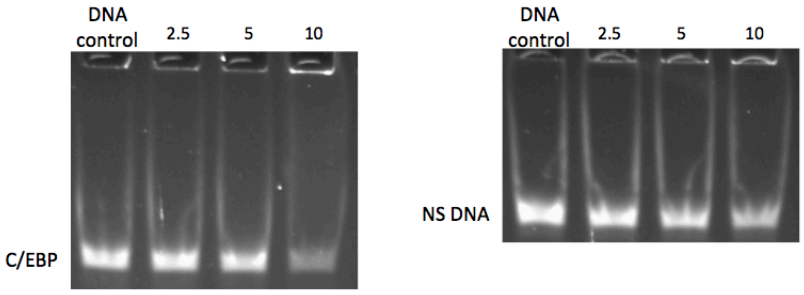
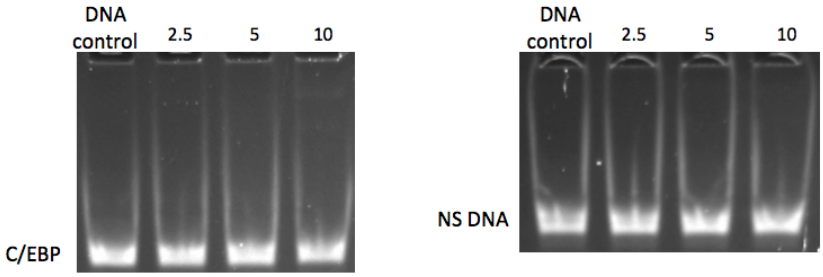


Figure S5. Investigation of protein:DNA interactions using quantitative EMSA. Top. Representative EMSA titration, showing mobility shift of the XRE1 DNA probe (Figure 1, main text) caused by a solution containing a 1:1 mixture of AhRJun:ArntFos. Total monomeric protein concentration for each reaction (nM) is indicated at the top of each well. **Bottom.** Representative protein:DNA binding curves. DNA probe containing XRE1 site (a) or Arnt E-box site (b) is shown titrated with equimolar AhRJun/ArntFos solution (black circles) or ArntFos alone (white circles).

a) AhRJun/ArntFos titrations



b) ArntFos titrations



c) AhRJun titrations

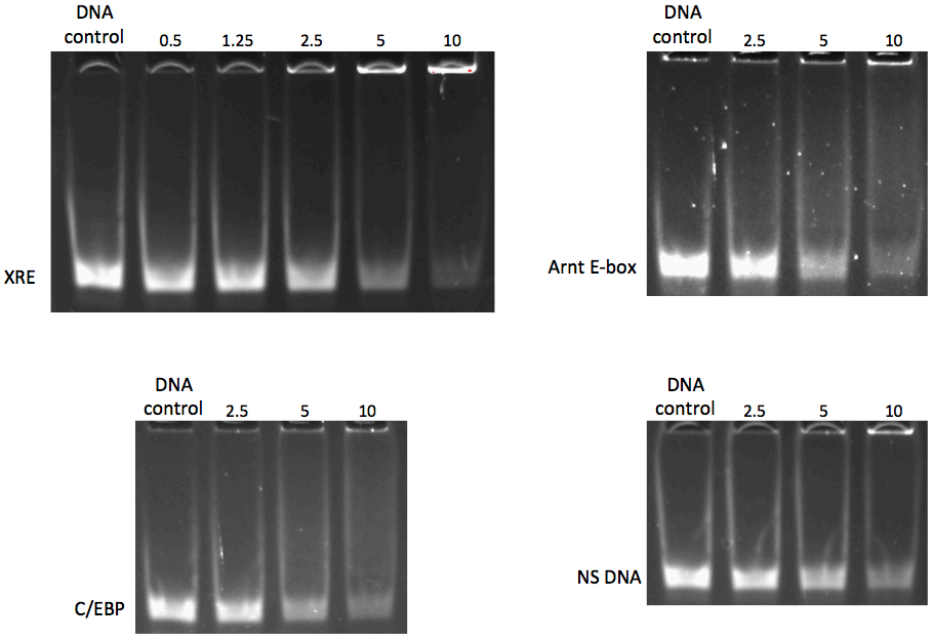


Figure S6. Qualitative EMSA for various protein:DNA combinations. a) Equimolar AhRJun/ArntFos solution against C/EBP or nonspecific (NS) DNA sites. **b)** ArntFos against C/EBP or NS-DNA sites. **c)** AhRJun against XRE1, Arnt E-box, C/EBP, or NS-DNA sites. Total monomeric protein concentration for each reaction (μM) is indicated at the top of each well.

References

1. J. M. Y. Wong, P. A. Harper, U. A. Meyer, K. W. Bock, K. Morike, J. Lagueux, P. Ayotte, R. F. Tyndale, E. M. Sellers, D. K. Manchester and A. B. Okey, *Pharmacogenetics*, 2001, **11**, 85-94.
2. G. Chen, A. T. De Jong and J. A. Shin, *Mol. BioSyst.*, 2012, **8**, 1286-1296.
3. P. J. Dillon and C. A. Rosen, *BioTechniques*, 1990, **9**, 298-300.
4. G. Chen and J. A. Shin, *Anal. Biochem.*, 2008, **382**, 101-106.