TCDD (n = 46)

CCATAG**TCGCGTGA**CAGGAAT
GATTCA**TTGCGTGC**GAGTGAG
GATTCA**TCGCGTGA**GAAGATG
AAAATA**TCGCGTGA**GAATCTT
AGGGCC**TAGCGTGA**CAAGGTC
AGTGGT**TCGCGTGC**GAATCTT
GGGCCC**TTGCGTGA**CATAATG
AGCTTA**TCGCGTGC**GAATCTT
GATTCA**TCGCGTGC**AAAGGTG
GATTCA**TCGCGTGA**GAGCGTG
CAAGAT**TCGCGTGC**AAACGAC
GGGCCA**TCGCGTGA**CAATCTG
CAAGAT**TCGCGTGA**CAACGCA
CCTCAA**TCGCGTGC**GATGAAT
GCCGCA**TCGCGTGC**GAGTGAA
GGCCGA**TAGCGTGA**GAACCGA
TTCTGA**TCGCGTGC**GAGCGGC
TTCAGA**TTGCGTGC**GAGCGGC
CACTGT**TCGCGTGA**GAGGCCC
CAAGAT**TCGCGTGC**AAAGGTA
TTCAGA**TTGCGTGC**GAGCGGC
CCCAGA**TTGCGTGC**GAGGAAT
CATTGC**TCGCGTGC**GAATCTT
GGGCCA**TCGCGTGC**CACTGAG
GGGCCA**TAGCGTGA**CAATCGG
ATACAA**TCGCGTGC**GAATCTT
CCGATG**TTGCGTGC**GAATCTT
CGATAG**TTGCGTGC**GAATCTT
CCGATG**TTGCGTGC**GAATCTT
CACGAA**TAGCGTGG**AATCTTG
CTAATA**TCGCGTGA**GAATCTT
TTCCGG**TTGCGTGC**GAGCGGC
GACAAA**TCGCGTGC**GAATCTT
GGGCCT**ATGCGTGA**CAAAATG
TCTAAT**ATGCGTGC**GAATCTT
TGTAAT**GTGCGTGC**GAATCTT
AAAAAT**GTGCGTGC**GAATCTT
CACCGT**GTGCGTGC**GAATCTT
TCAAGA**TTCCGTGA**CAAGATA
TCAAGA**TTCCGTGC**GAAGACC
TCAAGA**TTCCGTGC**GAACAGC
TCAAGA**TTCCGTGC**AAAGCGG
TCAAGA**TTCCGTGC**GAACGTA
TCAAGA**TTCCGTGC**GAACATC
TCAAGA**TTCCGTGC**GAAGGCG
GGAGCA**TTGCGTGC**GAATCTT

YH439 (n = 28)

TGTAAT**GTGCGTGC**GAATCTT
CCCATG**TTGCGTGC**GAATCTT
TGTAAT**GTGCGTGC**GAATCTT
TTCAAC**TCGCGTGC**GATCGGC
GGGCCA**TCGCGTGC**GAACAGG
GATGTG**TTGCGTGA**GAATCTT
CAAGAT**TCGCGTGC**GAGGATT
TCGGGT**ATGCGTGC**CAAGGCC
CAGGGC**CTGCGTGC**GACGATA
TAAAGC**TAGCGTGC**GAATCTT
GGCCCA**TTGCGTGC**CAAATGA
TGTAAT**GTGCGTGC**GAATCTT
CTACCT**TTGCGTGC**GAATCTT
GGCCCA**TGCCGTGA**CAAGGGA
GGGCCA**TAGCGTGA**CAATACG
TGTAAT**GTGCGTGC**GAATCTT
CCCCCA**TTGCGTGA**CATGAAT
GGGCCC**TCGCGTGC**GAACATG
TCCGCA**TTGCGTGC**GTAGGCC
TCAAGA**TTCCGTGC**GAACATC
GGCCCA**TTGCGTGC**CAAATGA
TCAAGA**TTCCGTGC**GAAGCCC
GAGGTA**TCGCGTGC**GAATCTT
CAAGAT**TCGCGTGA**GAAGCTC
TGTAAT**GTGCGTGC**GAATCTT
TTCCGT**TTTCGTGC**GAACGGC
ACAGGA**TAGCGTGC**GGCCCTG
GATTCA**TCGCGTGA**CATAAGG

βNF (n = 38)

GCCCAT**ATGCGTGC**GGATGAA
TCCTAG**TTGCGTGC**GACGGCC
GACCGA**TCGCGTGA**GAATCTT
CCAACG**TTGCGTGC**GAATCTT
TCATGG**TCGCGTGC**GGAGGCC
AACGAC**TTGCGTGC**GAATCTT
CCCAAA**TCGCGTGA**GAATCTT
CAAAGT**ATGCGTGA**GAATCTT
ATTCGT**GTGCGTGA**CAAGCGG
CCTCAA**TCGCGTGC**TAAGAAT
TCATGG**ACGCGTGC**GGTGGCC
GCGGCT**ATGCGTGC**GAATCTT
ATCATC**TAGCGTGC**GAATCTT
TCAAGA**TTCCGTGC**GAAGGCT
GCCTAG**TTGCGTGA**CAATGAA
CTACCG**TAGCGTGC**GAATCTT
CAAGAT**TCGCGTGA**GAACCCT
TCAAGA**TTCCGTGC**GAAACGT
TGGGCG**TAGCGTGC**GAATCTT
CCCTGC**TAGCGTGC**GAATCTT
TTCAGA**TCGCGTGA**GAAGGGC
TTCAGG**TTGCGTGC**GAGTGGC
TTCAGG**TTGCGTGC**GAGTGGC
AGGGCC**TTCCGTGA**GAATGCG
TCAAAA**TCGCGTGC**GAAGGCC

Supplemental Figure S1: Compilation of all DNA sequences isolated in the ligand-dependent DNA selection and amplification studies.

Continued on next page.

GCGGCT**ATGCGTGC**GAATCTT
CCTGGA**TTGCGTGC**GAATCTT
TCAAGA**TTCCGTGC**GAAGCAC
CAATTG**TTGCGTGC**GAATCTT
CGAACA**TTGCGTGC**GAATCTT
TCAAGA**TTCCGTGA**GAGTATC
CCCAAA**TAGCGTGC**AACGAAT
GCCTCA**TAGCGTGA**GAAGGAA
GATTCG**TAGCGTGC**GAAGTTG
GGCCGA**TCGCGTGC**GATAGGA
GGGCCT**ATGCGTGA**CAAAATG
GGCCCA**TCGCGTGA**CGCTCGA
CGACCA**TGGCGTGA**CAGAATC

3MC (n = 30)

GGGCCC**TTGCGTGT**AAACGAG
CAAGAT**TCGCGTGA**GAAGGGG
TCATGA**TTCCGTGA**CAACAGG
CAAGAT**TCGCGTGA**GAAGGGG
ATTCGA**TTGCGTGC**CAATCGG
TCAACT**TGCCGTGC**GAAGGCC
TCAAGA**TTCCGTGC**GAAGAGT
ATTCGA**TAGCGTGC**GAACTGG
GGCCTA**TAGCGTGC**GAATCTT
TCAAGA**TTCCGTGC**GATGACG
TCAAGA**TTCCGTGA**CAAACCC
ATTCGA**TTGCGTGC**CAATCGG
TTCCGA**TCGCGTGA**GAAGGGC
CAAGAT**TCGCGTGA**CAATGGG
TTCAGC**TTGCGTGC**GAATCTT
CAAGAT**TCGCGTGC**GAACCGT
CTAGGA**TCGCGTGC**GAGGCCC
TCGTGA**TCGCGTGC**CGCGGCC
TCGTGA**TCGCGTGC**CGCGGCC
GCCAGG**TTGCGTGC**GAATCTT
ACATCC**TTGCGTGC**GAATCTT
GGCCTA**TAGCGTGC**GAATCTT
ATTCCG**TCGCGTGC**AAATGGG
CCCACA**TCGCGTGC**GAGGAAT
TCCGGT**TTGCGTGC**GAGGGCC
GGCCCA**TCGCGTGC**GAAAAGA
TTCAGA**TCGCGTGA**GAGGGGC
GAGATA**TTGCGTGC**GAATCTT
AGGGCC**TTGCGTGC**GATGAGA
TCAAGA**TTCCGTGC**GAAGAAT

IR (n = 27)

TGACTG**TCGCGTG**AGAATCTT
ACAAGA**TCGCGTG**CGAATCTT
CTATCA**TTGCGTG**CGAATCTT
ACAAGA**TCGCGTG**CGAATCTT
CCTTGG**TCGCGTG**CGAGGCCC
ACAAGA**TCGCGTGC**GAATCTT
GGGCCA**TCGCGTGC**AGAGTCG
TACACA**TCGCGTGC**GAATCTT
CCCGCC**TCGCGTGA**GAATCTT
TCAAGA**TTCCGTGA**GAAGGGT
TTCAGG**TTGCGTGA**CAATGGC
GCACAT**ATGCGTGC**GAATCTT
CCCCAA**TAGCGTGC**CAAGAAT
CGGCAT**ATGCGTGC**GAATCTT
CACGAA**TGTCGTGA**GAATCTT
CAATAT**ATGCGTGC**GAATCTT
TCCACG**TTGCGTGC**GAAGGCC
GCCTGT**TCGCGTGC**GAGCGAA
CAAGAT**TCGCGTGC**GAAGCTG
TCATGC**TTGCGTGC**GAAGGCC
CTAACA**TCGCGTGC**GAGGCCC
TCAAGA**TTCCGTGC**GAGCGAA
TTCCTA**TCGCGTGA**CACGGGC
TGTAAT**GTGCGTGC**GAATCTT
CGCGTT**ATGCGTGC**GAATCTT
GGCCCC**TTGCGTGA**CAAATGA
CGCCAA**TCGCGTGC**GAATCTT

LK (n = 27)

ACCGAG**TTGCGTGC**GAATCTT
TCAAGA**TTCCGTGC**GAACGGG
CAAGGG**TAGCGTGC**GAATCTT
GCCCAG**TCGCGTGC**AAATGAA
TCAAGA**TTCCGTGA**GAATGCT
TCAAGA**TTCCGTGA**CAAGGTA
GGGCCG**TCGCGTGC**CAAGTAG
GATCGA**TGTCGTGC**GAATCTT
TCAAGA**TTCCGTGA**GAGCGTG
GACCGG**TTGCGTGA**GAATCTT
AACCGT**TTGCGTGA**GAATCTT
CCACAG**TTGCGTGC**GAATCTT
ATTCAA**TCGCGTGA**CGTGTGG
TCAAGA**TTCCGTGA**CAACTCC
TTCATA**TTGCGTGC**AACGGGC
GATTCA**TCGCGTGC**CGGTCGG
CCACCA**TCGCGTGA**CTAGAAT
TCTCCA**TTGCGTGC**GAATCTT
GGCCCA**TCGCGTGT**GAAATGA
CTATGC**TTGCGTGC**GAATCTT
CAAGAT**TCGCGTGA**CAGAACT
TCAAGA**TTCCGTGC**GAACTCT
TCAAGA**TTCCGTGC**GAACCTT
CAAGAT**TCGCGTGA**CAATAAC
GGCCTT**TAGCGTGC**GAAGGGA
TCAAGA**TTCCGTGA**GAACAGA
CTATGC**TTGCGTGC**GAATCTT

Supplemental Figure S1: Compilation of all DNA sequences isolated in the ligand-dependent DNA selection and amplification studies.
See Materials and Methods for details.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	Wild Type																				
	C	C	G	G	A	G	**T**	T	**G**	**C**	**G**	**T**	**G**	A	G	A	A	G	A	G	C
	TCDD (n = 46)																				
#																					
A	6	14	17	18	12	28	2	4	0	0	0	0	0	14	5	46	32	2	10	7	6
C	14	14	12	10	12	3	0	20	7	46	0	0	0	31	9	0	1	12	20	4	10
G	13	12	8	8	17	5	3	0	39	0	46	0	46	1	32	0	10	12	15	8	12
T	13	6	9	10	5	10	41	22	0	0	0	46	0	0	0	0	3	20	1	27	18
%																					
A	13	30	37	39	26	61	4	9	0	0	0	0	0	30	11	100	70	4	22	15	13
C	30	30	26	22	26	7	0	43	15	100	0	0	0	67	20	0	2	26	43	9	22
G	28	26	17	17	37	11	7	0	85	0	100	0	100	2	70	0	22	26	33	17	26
T	28	13	20	22	11	22	89	48	0	0	0	100	0	0	0	0	7	43	2	59	39
	YH439 (n = 28)																				
#																					
A	1	7	7	10	8	12	1	3	0	0	0	0	0	6	0	26	22	3	8	1	4
C	6	7	8	9	10	4	1	7	3	28	0	0	0	22	7	0	2	6	13	4	7
G	9	11	6	8	7	2	5	1	24	0	28	0	28	0	21	1	1	8	5	7	5
T	12	3	7	1	3	10	21	17	1	0	0	28	0	0	0	1	3	11	2	16	12
%																					
A	4	25	25	36	29	43	4	11	0	0	0	0	0	21	0	93	79	11	29	4	14
C	21	25	29	32	36	14	4	25	11	100	0	0	0	79	25	0	7	21	46	14	25
G	32	39	21	29	25	7	18	4	86	0	100	0	100	0	75	4	4	29	18	25	18
T	43	11	25	4	11	36	75	61	4	0	0	100	0	0	0	4	11	39	7	57	43
	βNF (n = 38)																				
#																					
A	4	6	14	14	9	16	6	7	0	0	0	0	0	13	1	34	29	4	5	6	5
C	12	19	15	9	12	4	0	10	5	38	0	0	0	25	5	0	3	1	19	7	10
G	10	7	5	7	15	11	1	1	33	0	38	0	38	0	31	4	4	12	13	7	4
T	12	6	4	8	2	7	31	20	0	0	0	38	0	0	1	0	2	21	1	18	19
%																					
A	11	16	37	37	24	42	16	18	0	0	0	0	0	34	3	89	76	11	13	16	13
C	32	50	39	24	32	11	0	26	13	100	0	0	0	66	13	0	8	3	50	18	26
G	26	18	13	18	39	29	3	3	87	0	100	0	100	0	82	11	11	32	34	18	11
T	32	16	11	21	5	18	82	53	0	0	0	100	0	0	3	0	5	55	3	47	50

Supplemental Figure S2: Frequency calculation of nucleotide base utilization at each position of the DRE sequences obtained by binding site selection and amplification studies for each ligand.

Continued on next page.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Wild Type																					
	C	C	G	G	A	G	**T**	T	**G**	**C**	**G**	**T**	**G**	A	G	A	A	G	A	G	C
3MC (n= 30)																					
#																					
A	6	5	12	10	4	18	0	3	0	0	0	0	0	7	2	28	22	2	7	3	2
C	6	12	9	9	7	4	0	12	6	30	0	0	0	22	7	0	2	4	11	7	8
G	6	5	5	7	16	2	0	1	24	0	30	0	30	0	21	2	4	14	11	14	10
T	12	8	4	4	3	6	30	14	0	0	0	30	0	1	0	0	2	10	1	6	10
%																					
A	20	17	40	33	13	60	0	10	0	0	0	0	0	23	7	93	73	7	23	10	7
C	20	40	30	30	23	13	0	40	20	100	0	0	0	73	23	0	7	13	37	23	27
G	20	17	17	23	53	7	0	3	80	0	100	0	100	0	70	7	13	47	37	47	33
T	40	27	13	13	10	20	100	47	0	0	0	100	0	3	0	0	7	33	3	20	33
IR (n = 27)																					
#																					
A	3	4	12	10	8	13	4	1	0	0	0	0	0	7	1	26	22	1	1	3	3
C	11	12	11	8	7	3	0	13	2	27	0	0	0	20	4	0	1	2	17	5	6
G	4	7	2	4	9	4	1	1	24	0	27	0	27	0	22	1	4	9	7	4	2
T	9	4	2	5	3	7	22	12	1	0	0	27	0	0	0	0	0	15	2	15	16
%																					
A	11	15	44	37	30	48	15	4	0	0	0	0	0	26	4	96	81	4	4	11	11
C	41	44	41	30	26	11	0	48	7	100	0	0	0	74	15	0	4	7	63	19	22
G	15	26	7	15	33	15	4	4	89	0	100	0	100	0	81	4	15	33	26	15	7
T	33	15	7	19	11	26	81	44	4	0	0	100	0	0	0	0	0	56	7	56	59
LK (n = 27)																					
#																					
A	3	7	15	9	6	15	0	2	0	0	0	0	0	11	2	24	22	2	4	4	5
C	7	13	7	11	5	2	0	8	8	27	0	0	0	15	8	0	1	6	11	4	3
G	7	3	1	4	14	6	0	1	18	0	27	0	27	0	17	2	3	6	7	7	5
T	10	4	4	3	2	4	27	16	1	0	0	27	0	1	0	1	1	13	5	12	14
%																					
A	11	26	56	33	22	56	0	7	0	0	0	0	0	41	7	89	81	7	15	15	19
C	26	48	26	41	19	7	0	30	30	100	0	0	0	56	30	0	4	22	41	15	11
G	26	11	4	15	52	22	0	4	67	0	100	0	100	0	63	7	11	22	26	26	19
T	37	15	15	11	7	15	100	59	4	0	0	100	0	4	0	4	4	48	19	44	52

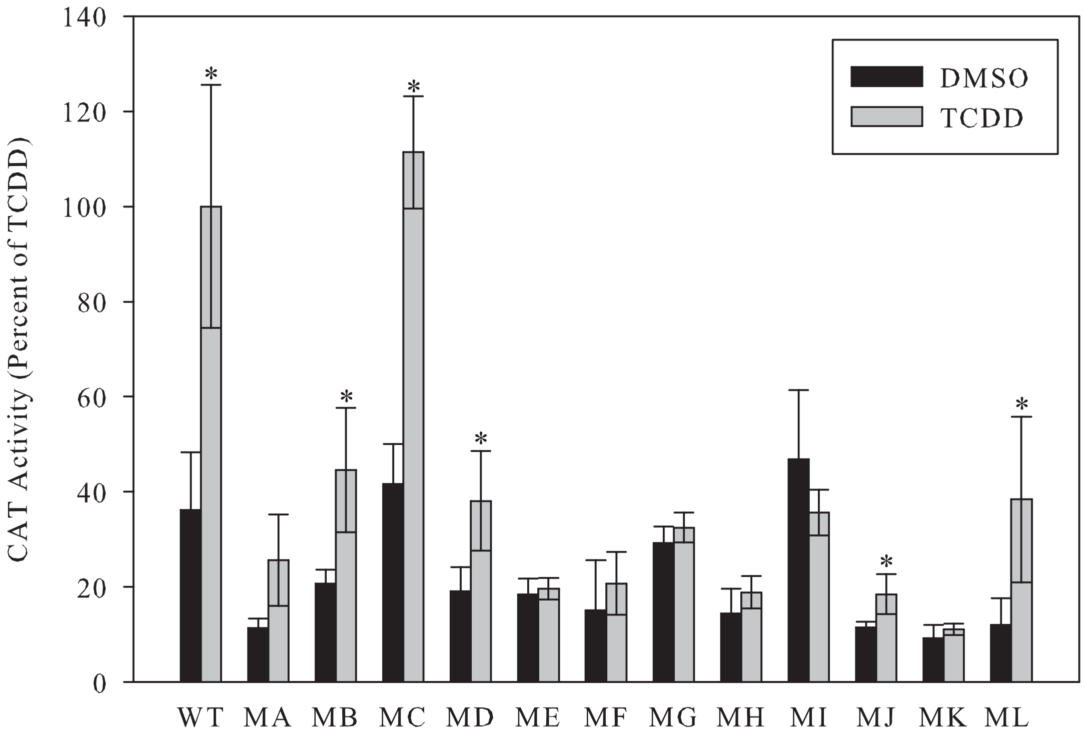
Supplemental Figure S2: Frequency calculation of nucleotide base utilization at each position of the DRE sequences obtained by binding site selection and amplification studies for each ligand.

Continued on next page.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	Wild Type																				
	C	C	G	G	A	G	**T**	T	**G**	**C**	**G**	**T**	**G**	A	G	A	A	G	A	G	C
	All Sequences (n = 196)																				
#																					
A	23	43	77	71	47	102	13	20	0	0	0	0	0	58	11	184	149	14	35	24	25
C	56	77	62	56	53	20	1	70	31	196	0	0	0	135	40	0	10	31	91	31	44
G	49	45	27	38	78	30	10	5	162	0	196	0	196	1	144	10	26	61	58	47	38
T	68	31	30	31	18	44	172	101	3	0	0	196	0	2	1	2	11	90	12	94	89
%																					
A	12	22	39	36	24	52	7	10	0	0	0	0	0	30	6	94	76	7	18	12	13
C	29	39	32	29	27	10	1	36	16	100	0	0	0	69	20	0	5	16	46	16	22
G	25	23	14	19	40	15	5	3	83	0	100	0	100	1	73	5	13	31	30	24	19
T	35	16	15	16	9	22	88	52	2	0	0	100	0	1	1	1	6	46	6	48	45

Supplemental Figure S2: Frequency calculation of nucleotide base utilization at each position of the DRE sequences obtained by binding site selection and amplification studies for each ligand.

The number of times a specific base appears at each position as well as its relative percentage at each position is shown (calculated using the total number of sequences for a particular ligand).



Supplemental Figure S3: Effect of site-directed mutation of the DRE consensus sequence on TCDD-inducible chloramphenicol acetyltransferase (CAT) reporter gene activity.

Hepa1c1c7 cells were transiently transfected with pMcat5 containing a single wild type (WT) or mutant (M) DRE response element, with the lettering indicating a specific mutation described in Table 2. Cells were incubated with DMSO (0.1%) or 1 nM TCDD for 24 hours prior to determination of CAT activity. Values represent the mean ± SD of at least three independent determinations and all activity normalized to that obtained with TCDD-treated cells transfected with pMcat5 containing the WT DRE. The asterisk indicates those values that are significantly greater than the activity of the respective DMSO treated samples (Student's *t*-test; $*p < 0.05$). The CAT activity of all extracts from cells transfected with mutant DRE CAT reporter plasmids and incubated with TCDD (except for that of sample MC), were significantly different than that of TCDD-inducible CAT activity in extracts of cells transfected with a CAT reporter plasmid containing the wild type DRE oligonucleotide (Student's *t*-test; $p < 0.05$).

Oligo		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Binding	Function
		DRE Nucleotide Position																				
		C	G	G	A	G	T	T	G	C	G	T	G	A	G	A	A	G	A	G		
WT		.					.						.							.	+++	+++
MD1	(A)	T					.						.							.	+++	–
MD2		A					.						.							.	+++	+++
JW9		T					.						.							.	+++	+++
JW10		.		T			.						.							.	+++	+++
MD3	(B)	.				A	.						.							.	+++	++
MD4		.				T	.						.							.	+++	+++
JW11		.				A	.						.							.	+++	+++
MD5	(C)	.					G						.							.	+++	+++
JW6		.					C						.							.	+/–?	–
M1		.					A						.							.	+++	++
JW17		.					.	C					.							.	+++	+++
M2		.					.	A					.							.	+++	++
M3		.					.	G					.							.	+++	++
MD6	(D)	.					.		T				.							.	++	++
JW5		.					.		A				.							.	+++	–
M4		.					.		C				.							.	+++	++
MD7	(E)	.					.			A			.							.	–	–
MD8		.					.			T			.							.	–	ND
JW1		.					.			T			.							.	–	ND
JW13		.					.			G			.							.	–	ND
MD9	(F)	.					.				T		.							.	–	–
JW2		.					.				A		.							.	–	–
MD10	(G)	.					.					G	.							.	–	–
JW7		.					.					C	.							.	–	–
JW14		.					.					A	.							.	–	ND
MD11	(H)	.					.						T							.	–	–
JW3		.					.						A							.	–	ND
JW15		.					.						C							.	–	ND
MD12	(I)	.					.						.	G						.	–	–
JW16		.					.						.	G						.	+/–	–
M5		.					.						.	T						.	+++	++
MD13	(J)	.					.						.		T					.	+++	+
JW4		.					.						.		A					.	+/–	–
M6		.					.						.		C					.	+++	+++
MD14	(K)	.					.						.			C				.	++	–
JW12		.					.						.			G				.	+/–	–
M7		.					.						.			G				.	+++	++
MD15		.					.						.			T				.	++	++
M8		.					.						.				G			.	+++	+++
MD16		.					.						.					A		.	++	++
MD17	(L)	.					.						.							T	++	++
MD18		T				A	G						.							.	+++	ND
MD19		.					.						.		T	C				.	++	ND
MD20		.					.						.		T					T	++	ND

Supplemental Table S1: Relative AhR binding and enhancer function of the DRE derived from mutagenesis experiments.

Relative magnitude of binding and function is indicated by a (+) sign, while the lack of activity is indicated by a (-) sign. "MD" mutant data are from Yao and Denison (1992), and a letter in parenthesis indicates the nomenclature of the mutant as used in this paper. "JW" mutant data are from Shen and Whitlock (1992). "M" mutant data are from this paper. ND, not determined.