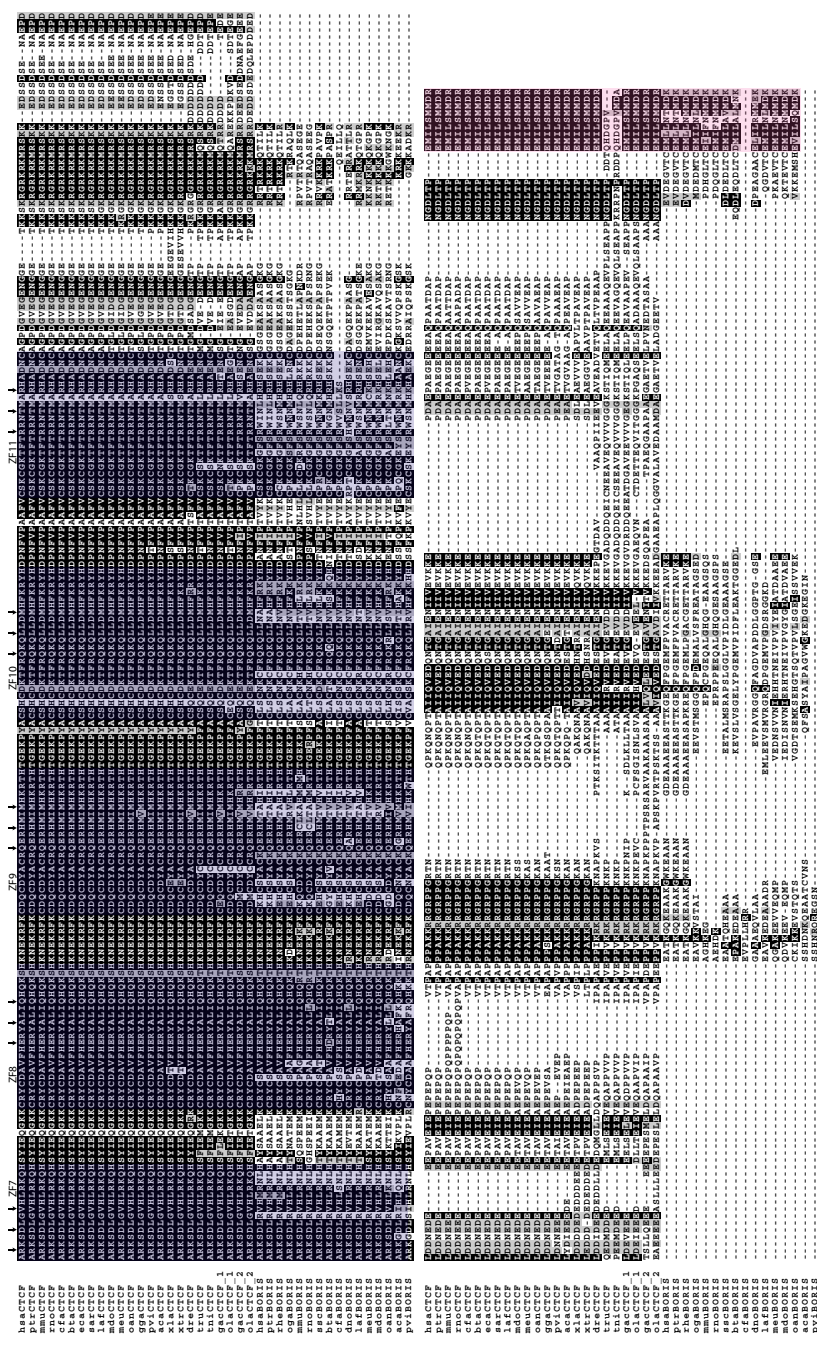




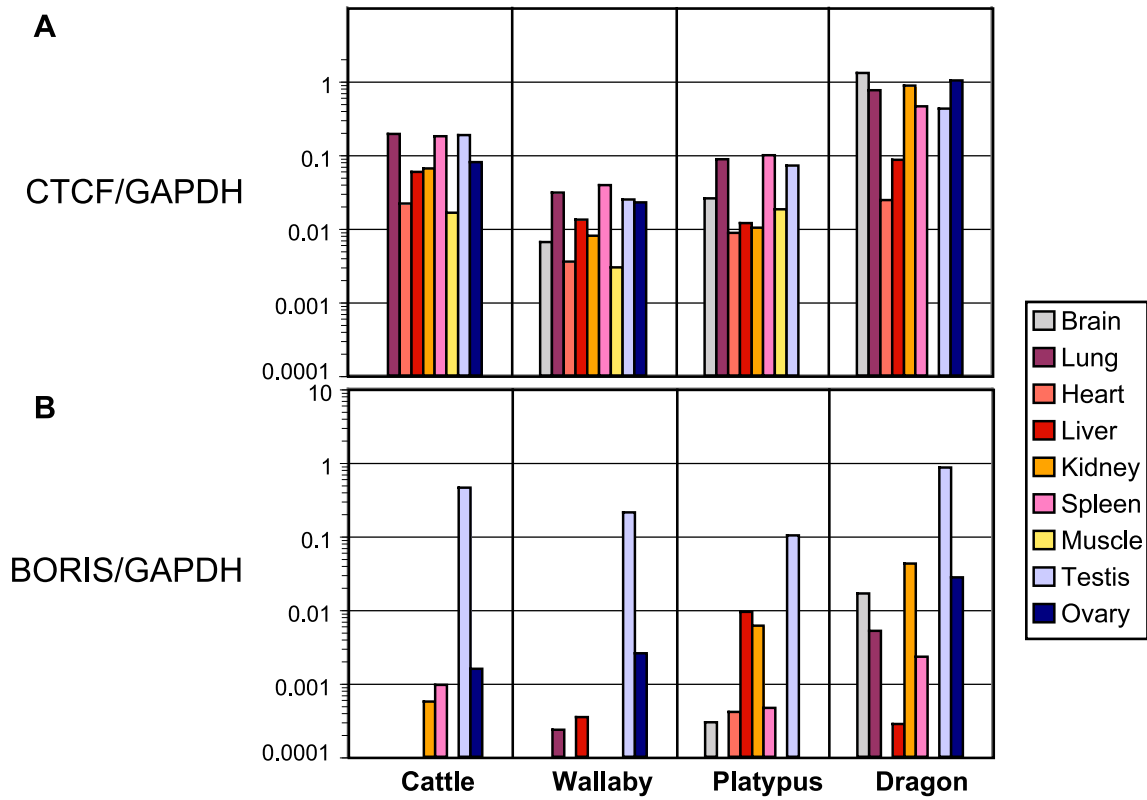
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**Figure S1.** Alignment of predicted CTCF and BORIS sequence in a range of vertebrates. Amino acids from the 11 zinc fingers (blue), likely to interact with DNA are shown indicated with arrows, and regions of similarity at the start and end of CTCF and BORIS are highlighted in red. Full species names can be obtained from Table S2.



**Figure S2.** Quantification of (A) *CTCF* and (B) *BORIS* transcripts in various tissues and species relative to *GAPDH* as determined by real-time PCR.

**Table S1.** Primers used in this study.

Species	Gene	Primer Name	Sequence	Product size	Location	Description	
Cattle	CTCF	btCTCF_F1	TACAGATGGTGATGATGGAACAGC	737 bp	Exons 1-2	RT-PCR primers	
		btCTCF_R1	GAATGCCCTGCCACAGAGATG				
		btCTCF_qF1	GCATCGTCGTTACAAACACACC	390 bp	Exons 3-6		qPCR primers*
		btCTCF_qR1	CATTTCTTGCCCTGCTCAAT				
	BORIS	btBORIS_F8	CACGCTTCTGGGTGAGGAC	208 bp	5'UTR-exon 1	Used to amplify EU527855	
		btBORIS_R8	CTCTGGTTCTTTGATTTGGGTG				
		btBORIS_F4	CATAATGGCAGGGGCTGAG	1069 bp	Exons 1-6		
		btBORIS_R4	GCAGAGGGAACACTGAAAGG				
		btBORIS_F5	GCCACATCCGCTCACACAC	892 bp	Exons 6-11		
		btBORIS_R5	CCTGCCTCCTGTCACTTATTCATC				
		btBORIS_F6	AAAAGAAAGAGCAACGAAGAGGAA	1509 bp	Exons 11-3'UTR		
		btBORIS_R6	TATGGGAGGGGAAGAGTTCACAG				
		btBORIS_F2	GCACTCCAAGAAACAGCAATA	242 bp	Exons 8-10		RT-PCR and qPCR primers
		btBORIS_R2	GCTCTCCACTCACCAGGGATAC				
	GAPDH	btGAPDH_qF1	GTGATGCTGGTCTGAGTATGTG	299 bp	Exons 4-7	qPCR control	
btGAPDH_qR1		AGTCTTCTGGTGGCAGTGATG					

Species	Gene	Primer Name	Sequence	Product size	Location	Description	
Wallaby	CTCF	meCTCF_F1	ATGGAAGGTGAGGCAGTTGAAG	2214 bp	N-term to C-term	Used to amplify EU527852	
		meCTCF_R1	CCAGTTTGGTGGCAGAGC				
		meCTCF_F3	CCAGCCAGCCCAAGCAAG	1524 bp	C-term to 3'UTR		
		meCTCF_R5	TCCTGAACACTTTGCTGCTTCTT				
		meCTCF_R_F1	TTTATTCCGAGCACATGGATACTGG	99 bp	3'UTR		Used as nested forward primers for 3'RACE
		meCTCF_R_F2	GGTTTGGAAAGCTGGGAAGGTGAA				
		oCTCF_R1	GCAAGGCAAGAAATGTCGTTATTG	-	Exon 3		Sequencing primer
		meCTCF_F12	GGGGCTTACGAGAATGAGGT	547 bp	Exons 1-2		RT-PCR primers
		meCTCF_R11	CTGTGTGAGTATTTAGGTGGTTCC				
		meCTCF_F11	CGTTTCCGTGTATGACTTTGAG	287 bp	Exons 1-2		qPCR primers
	meCTCF_R11	CTGTGTGAGTATTTAGGTGGTTCC					
	BORIS	meBORIS_F19	GGAATGGGGACGGAGGC	1428 bp	N-term to C-term	Used to amplify EU527856	
		meBORIS_R9	GCAAGAAAGGCACGTAATGGT				
		meBORIS_F4	ATGCGTTCACATACTGGAGA	2097 bp	ZF to 3'UTR		
		meBORIS_R5	AGGCATTTCAATTTGAGTTATTAGA				
		meBORIS_F8	ACGAGATGGAGCTGGTGGAGA	-	N-term		Sequencing primer
		meBORIS_R8	CATGGCAGTGTGGCTT	-	3' UTR		Sequencing primer
		meBORIS_F17	GAAACCATTACGTGCCTTTCTT	284 bp	Exons 8-10		RT-PCR and qPCR primers
meBORIS_R17		CTGGCATCTGCTCAACAACCTTCT					
	oBORIS_F1	TTATGAATGCTACGTCTGCCATGC	355 bp	Exons 6-8	Extra RT-PCR		

		oBORIS_R1	GGTGAATGGTTTCTCTCCTGTGTG			primers
	GAPDH	meuGAPDH_qF1	AAGTTCAAGGCACTGTCAAGG	131 bp	Exons 2-3	qPCR control
		meuGAPDH_qR1	GACTCTACAACATACTCGGCTCCA			

Species	Gene	Primer Name	Sequence	Product size	Location	Description	
Platypus	CTCF	meCTCF_F1	ATGGAAGGTGAGGCAGTTGAAG	1143 bp	N-term to ZF	Used to amplify EU527853	
		oaCTCF_R_R2	GCACTGGAATGGACGCTCT				
		oaCTCF_F3	AAACGTCATATTGCTCTCACAC	686 bp	ZF		
		oaCTCF_R3	TTACCCTTCTGGTTTCTCCTC				
		oaCTCF_F4	TGGTGTAGAAGGAGAGAATGGAGGA	1454 bp	ZF to 3'UTR		
		oaCTCF_R4	CAGAGCAAAGAAAGTGTAGGTGTGA A				
		oaCTCF_F7	CTTCTACGTCATCCTCCAAG	1007 bp	3'UTR		
		oaCTCF_R7	TGTTAATCCGTTATTATTATTAGC TG				
		oaCTCF_R_F1	ATGACTTCTATGTTGAAAGCAAAGT GG	93 bp	3'UTR		Used as nested forward primers for 3'RACE
		oaCTCF_R_F2	GGGTTTGAAAGCTAGGAAGGAGAAT A				
		oaCTCF_F12	TCAGGAAGCAGAGGCAACC	638 bp	N-term to ZF		RT-PCR primers
		oaCTCF_R12	ATTTGTGTGGTCTTTCATCAGTGT				
	oaCTCF_F11	CAATGGCGAGGTGGAGAC	375 bp	N-term to ZF	qPCR primers		
	oaCTCF_R12	ATTTGTGTGGTCTTTCATCAGTGT					
	BORIS	oaBORIS_F1	GATCCCGCAGAAGGAAAC	381 bp	Exons 1-3	Used to amplify EU527857	
		oaBORIS_R7	GTGGCGATTGAGACTTGACTG				
		oaBORIS_F2	CCACAGGATATGTCTCCGTCAGT	1179 bp	Exons 2-10		
		oaBORIS_R2	TTGGCTTGTACCCTGCTCTGATTT				
		oaBORIS_R_F3	CGGTTGGAGATACGTCGAGATGAA A	333 bp	3' UTR		
		oaBORIS_R_F4	CACATGAAGTGATACTCAGCCAGAT GGA				
oaBORIS_F10		ACCCTTTTCTGCCTTCACTGC	291 bp	Exons 8-10	RT-PCR and qPCR primers		
oaBORIS_R10		GTATCTCCAACCGATGCTGCGTA					
oaBORIS_F7		TATGGCAGAAGAAGGAAAGCAC	276 bp	Exons 2-3	Extra RT-PCR primers		
oaBORIS_R7	GTGGCGATTGAGACTTGACTG						
GAPDH	oanGAPDH_qF1	GTATGATTCCACCACGGCA	210 bp	Exons 3-5	qPCR control		
	oanGAPDH_qR1	CGCTTGGCTCCTCCCTTC					

Species	Gene	Primer Name	Sequence	Product size	Location	Description
Bearded Dragon	CTCF	acaCTCF_F1	GTGACATGGAGGCGAAGTAGTT	958 bp	N-term to ZF	Used to amplify EU527854
		acaCTCF_R5	CCTGTATGAGTGTGAGATGGTTTC			
		acaCTCF_F2	AAAGACATTCCAGTGTGAACTGTG	2762 bp	ZF to 3'UTR	
		acaCTCF_R1	CAGCAGTATATTCTCCTTCCCAG			
	pvCTCF_F1	CTGCCCTTGTCTGCTCCAAGTGT	-	ZF	Sequencing primer	
	pvCTCF_R1	GCAAAGTATCAGGGAAGAAAGACAC C	-	3' UTR	Sequencing primer	
	pviCTCF_F1	ATGGCGAAGTGGAGACATTAGA	438 bp	Exons 1-2	RT-PCR primers	
	pviCTCF_R1	CTGTGTGAGTGTGAGGTGATTT				
	pviCTCF_qF1	GGTGAGTTGGTTCGGCATC	187 bp	Exons 3-4	qPCR primers	
	pviCTCF_qR1	GCCTTTCAGTTTGTAAAGTGTCTCT				

BORIS	acaBORIS_F1	AGGCTTTGGGAGAAGGAGAGAAAC	859 bp	N-term to ZF	Used to amplify EU527858
	pvBORIS_R3	CCTGTATGGGAGCGAATGTGA			
	acaBORIS_F5	GAATGTGATATGGCCTTTGTGAC	787 bp	ZF to C-term	
	pviBORIS_R3	CTTTGCTGGGCTGAATCGCT			
	pviBORIS_R_F1	CAAACAGGAACGCCACATGGTGATA	624 bp	ZF	Forward primer for 3' RACE
	pviBORIS_F3	CAGGAACGCCACATGGTGATA	247 bp	Last two exons	RT-PCR and qPCR primers
	pviBORIS_R3	CTTTGCTGGGCTGAATCGCT			
	pviBORIS_F1	CGTCACATTGCTCCCATAC	299 bp	ZF	Extra RT-PCR primers (ZF)
	pviBORIS_R1	GCACCTCAACGGCACTTCT			
GAPDH	pviGAPDH_qF1	GTGGAGGGATGGCAGAGGT	130 bp	Exons 6-7	qPCR control
	pviGAPDH_qR1	TGGAGTTGGGACACGGAAAG			

\*qPCR primers are those primers used in 'quantitative' or real-time PCR.

**Table S2.** Vertebrate homologues of *CTCF* and *BORIS*.

Common Name	Species Name	Classification	Gene	Accession number	Experimental evidence?	Reference
Human	Homo sapiens	Eutherian mammal	hsaBORIS	NM_080618.2	Yes	Loukinov <i>et al.</i> , (2002)
Human	Homo sapiens	Eutherian mammal	hsaCTCF	NM_006565.2	Yes	Filippova <i>et al.</i> , (1996)
Chimpanzee	Pan troglodytes	Eutherian mammal	ptrBORIS	ENSPTRP00000023498**	No	
Chimpanzee	Pan troglodytes	Eutherian mammal	ptrCTCF	XM_511035.2	No	
Rhesus macaque	Macaca mulatta	Eutherian mammal	rheBORIS	ENSMMUP00000022088**	No	
Small-eared galago	Otolemur garnettii	Eutherian mammal	ogaBORIS	ENSOGAP00000013137**	No	
Mouse	Mus musculus	Eutherian mammal	mmuBORIS	NM_001081387.2	Yes	Loukinov <i>et al.</i> , (2002)
Mouse	Mus musculus	Eutherian mammal	mmuCTCF	NM_007794.1	Yes	Loukinov <i>et al.</i> , (2002)
Rat	Rattus norvegicus	Eutherian mammal	rnoBORIS	ENSRNOP00000034635**	No	
Rat	Rattus norvegicus	Eutherian mammal	rnoCTCF	NM_031824.1	Yes	
Cattle	Bos taurus	Eutherian mammal	btaBORIS	EU527855	Yes	This Study
Cattle	Bos taurus	Eutherian mammal	btaCTCF	NM_001075748.1	Yes	
Dog	Canis familiaris	Eutherian mammal	cfaBORIS	XM_534463 with edition	No	
Dog	Canis familiaris	Eutherian mammal	cfaCTCF	XM_859339.1	No	
Western European hedgehog	Erinaceus europaeus	Eutherian mammal	eeuBORIS	ENSEEUP00000010716**	No	
European shrew	Sorex araneus	Eutherian mammal	sarCTCF	ENSSARP00000005634**	No	
Horse	Equus caballus	Eutherian mammal	ecaBORIS	GENSCAN000000098721**	No	
Horse	Equus caballus	Eutherian mammal	ecaCTCF	XM_001497850 with edition	No	
Pig	Sus scrofa	Eutherian mammal	sscBORIS	NM_001110174.1	No	
African savanna elephant	Loxodonta africana	Eutherian mammal	lafBORIS	ENSLAFP00000007760**	No	
African savanna elephant	Loxodonta africana	Eutherian mammal	lafCTCF	ENSLAFT00000007101** with trace archive data	No	
Nine-banded armadillo	Dasypus novemcinctus	Eutherian mammal	dnoBORIS	ENSDNOP00000012186**	No	
Gray, short-tailed opossum	Monodelphis domestica	Marsupial mammal	mdoBORIS	ENSMODP00000020611**	No	
Gray, short-tailed opossum	Monodelphis domestica	Marsupial mammal	mdoCTCF	ENSMODP00000007129**	No	

Tammar wallaby	Macropus eugenii	Marsupial mammal	meuBORIS	EU527856	Yes	This Study
Tammar wallaby	Macropus eugenii	Marsupial mammal	meuCTCF	EU527852	Yes	This Study
Duck-billed platypus	Ornithorhynchus anatinus	Monotreme mammal	oanBORIS	EU527857	Yes	This Study
Duck-billed platypus	Ornithorhynchus anatinus	Monotreme mammal	oanCTCF	EU527853	Yes	This Study
Green Anole	Anolis carolinensis	Squamate Reptile	acaBORIS <sup>†</sup>	Scaffold_190**, with genome scan	No	
Green Anole	Anolis carolinensis	Squamate Reptile	acaCTCF	Scaffold_448**, with genome scan	No	
Central bearded dragon	Pogona vitticeps	Squamate Reptile	pviBORIS <sup>†</sup>	EU527858	Yes	This Study
Central bearded dragon	Pogona vitticeps	Squamate Reptile	pviCTCF	EU527854	Yes	This Study
Chicken	Gallus gallus	Neognathae bird	ggaCTCF	NM_205332.4	Yes	Klenova <i>et al.</i> , (1993)
Zebra finch	Taeniopygia guttata	Neognathae bird	tguCTCF	Derived from EST CK317499 and trace data	Partial	
African clawed frog	Xenopus laevis	Amphibian	xlaCTCF	NM_001086461.1	Yes	Burke <i>et al.</i> , (2002)
Western clawed frog	Xenopus tropicalis	Amphibian	xtrCTCF	ENSXETP00000034066**	No	
Zebrafish	Danio rerio	Ray finned fish	dreCTCF	NM_001001844.1	Yes	Pugacheva <i>et al.</i> , (2006)
Green spotted puffer	Tetraodon nigroviridis	Ray finned fish	tniCTCF	GSTENT00017695001**	No	
Tiger puffer	Takifugu rubripes	Ray finned fish	truCTCF	NEWSINFRUT00000156554**	No	
Three-spined stickleback	Gasterosteus aculeatus	Ray finned fish	gacCTCF_2	ENSGACT00000003281**	No	
Japanese medaka	Oryzias latipes	Ray finned fish	olaCTCF_1	ENSORLT00000011018**	No	
Three-spined stickleback	Gasterosteus aculeatus	Ray finned fish	gacCTCF_1	ENSGACT00000020981**	No	
Japanese medaka	Oryzias latipes	Ray finned fish	olaCTCF_2	ENSORLT00000022987**	No	
Sea lamprey	Petromyzon marinus	Jawless fish	pmaCTCF	Derived from EST DW022714 and GENSCAN00000118609**	Partial	

†When 3' RACE was performed on bearded dragon *BORIS* cDNA (EU527858) we could find no sequence homologous to the last coding exon of human *BORIS*, and discovered by genomic PCR that the 3'UTR follows on directly from the region homologous to the second-last exon of human *BORIS* without an interspersed intron (data not shown). Nor could we detect sequence homologous to this region in green anole, so we predict that reptilian *BORIS* is missing this exon entirely.

\*\*Indicates which sequences can be retrieved from Ensembl (<http://www.ensembl.org>). All other sequences can be found at NCBI (<http://www.ncbi.nlm.nih.gov>).