Supporting Information

Tümpel *et al.* 10.1073/pnas.0806360105

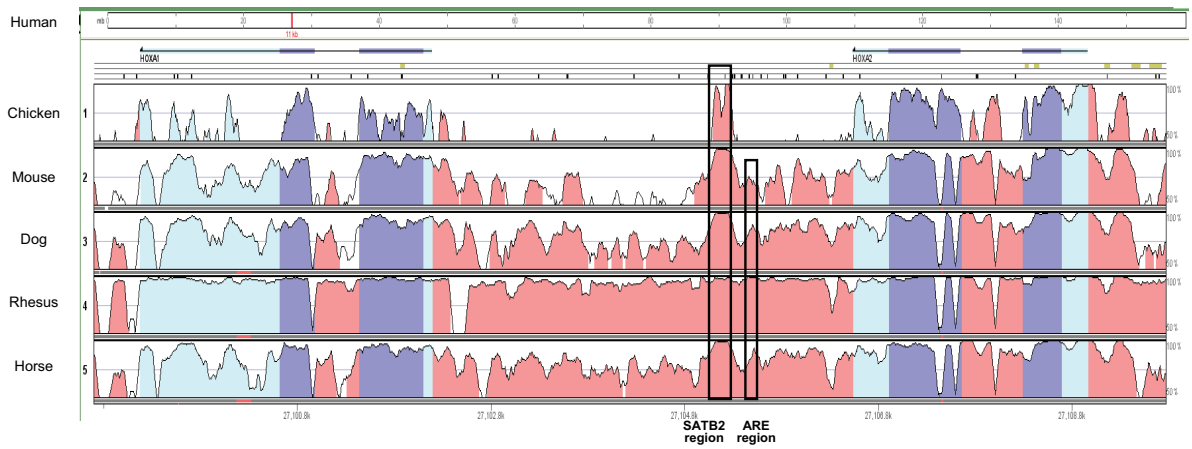

Fig. S1. Vista plot comparing Chicken with Mammalian Hoxa1-Hoxa2 and intergenic regions. The GenomeVista program (http://pipeline.lbl.gov/cgi-bin/GenomeVista) was used. The search was performed starting with Chicken contig AC163712.3, then the viewed region was focused around HoxA1-A2 (base Human March 2006 Chr7:27,098,666–27,109,735). RefSeq Gene annotation was used and the paramters were edited (vs. Chicken, 50, 50, 60, no change to the others). The default chicken view (which is blank in this region) and rat sequences were removed for clarity. The ARE (described in Fig. 1*A*, Fig. S2, and the main text) is located in position 27,098,457–67 in the Human March 2006 release. Global alignments were performed using GenomeVISTA (refs. 1 and 2 and http://genome.lbl.gov/vista/index.shtml).

1. Bray N, Dubchak I, Pachter L (2003) AVID: A global alignment program. *Genome Res* 13:97–102.
2. Couronne O, *et al.* (2003) Strategies and tools for whole-genome alignments. *Genome Res* 13:73–80.

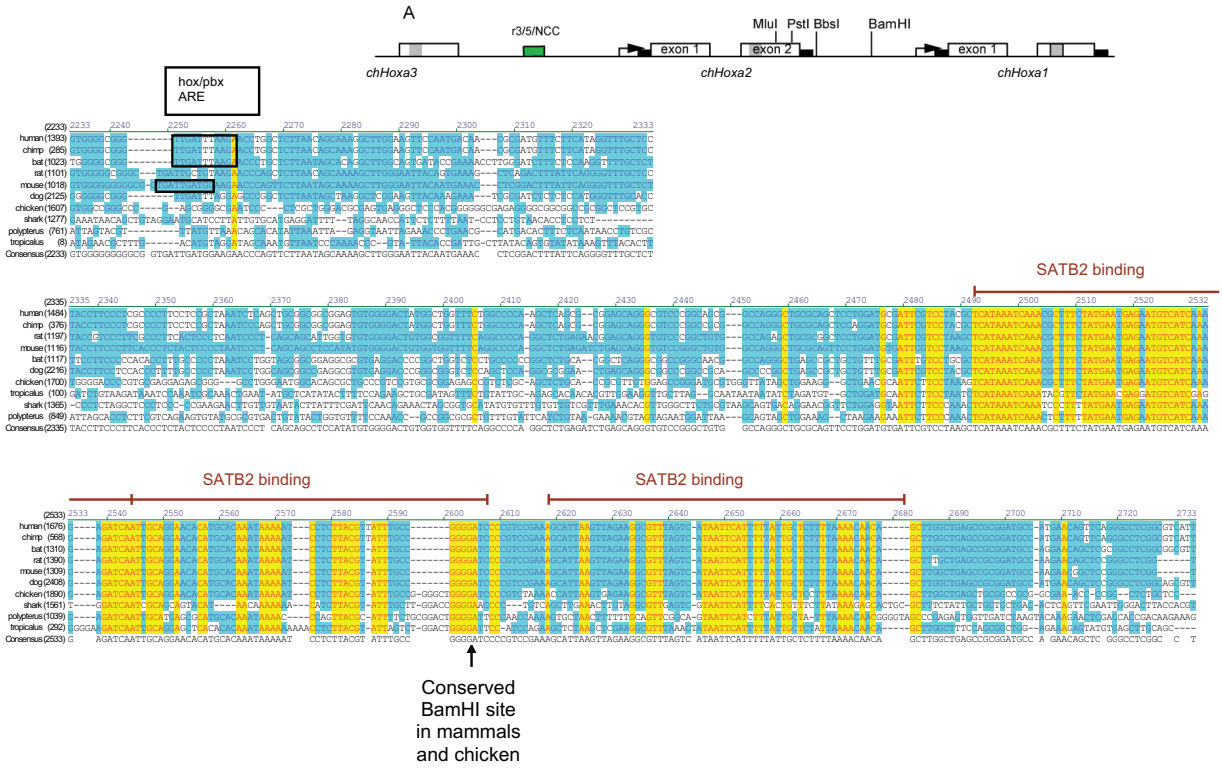

Fig. S2. Alignment of intergenic elements around the BamHI site in mouse, human, chicken, etc. [BamHI marks the 3′ end of the EII ''enhancer'' (1)]. The regions described in SATB2 binding paper (2) are marked. The Hox/pbx site (ARE) proposed by Lampe *et al.* (3) is also marked.

1. Frasch M, Chen X, Lufkin T (1995) Evolutionary-conserved enhancers direct region-specific expression of the murine Hoxa-1 and Hoxa-2 loci in both mice and Drosophila. *Development* 121:957–974.
2. Dobreva G, *et al.* (2006) SATB2 is a multifunctional determinant of craniofacial patterning and osteoblast differentiation. *Cell* 125:971–986.
3. Lampe X, Picard JJ, Rezsohazy R (2004) The Hoxa2 enhancer 2 contains a critical Hoxa2 responsive regulatory element. *Biochem Biophys Res Commun* 316: 898–902.

Table S1. Primers used to construct indicated constructs in Fig. 1

Construct	Sequence	
	Forward	Reverse
3	ACG CGT TTC AGC AGA ATG CG	GCA GGA CCC TGG GAG AGG AC
4	ACG CGT TTC AGC AGA ATG CG	AAA AAG TCA AAG CTG TCA GC
Dog r2 enhancer	GAA ATT TAA AAG CCT CGA AGA CTC	TGC TTT GTT TTG CTT TAA TGT TTT
Mouse r2 enhancer	GCT TCT AGA GAA ATT TAA AAA CCT GGA GGA C	AGC TCT AGA TTG TTT TTC AGG AAA ATC AC
zebrafish r2 enhancer	GAA AGA GAG GGT TAT CCA TT	GTT GGC TAT TTC TTT ATC CG
Xenopus tropicalis r2 enhancer	CTT GAA TAG TGT CTC TGG GG	CAA ATA CTC ATT TAT TCT ACC A
Medaka	ATC GAC TTC CAG CAG GCT GC	CCA AAG CCG AGT CAT TGT TC
7	GCT TCT AGA CAA TGG CGA ATC CCA AAG TT	TCG CCG CGG CTG AAG CTT CTG CAG GCA GGA ATC TGT GG
8	ATA AGA ATG CGG CCG CCC GTT TCG CCT TTA ACG AGC	TCG CCG CGG CTG AAG CTT CTG CAG GCA GGA ATC TGT GG
9	GCT TCT AGA AAA ATC TGA AAC ATT TTC AA	TCG CCG CGG CTG AAG CTT CTG CAG GCA GGA ATC TGT GG
10	ATA AGA ATG CGG CCG CAC ACC AGT CAC CCA CTG TTC A	TCG CCG CGG CTG AAG CTT CTG CAG GCA GGA ATC TGT GG
11	GCT TCT AGA TCA ACA ATG GCC CAG AAC TG	TCG CCG CGG CTG AAG CTT CTG CAG GCA GGA ATC TGT GG
12	GCT TCT AGA GAC AAG CTT ACG CGT TTC AGC AGA ATG CG	TCG CCG CGG CTC AGG ACT GTC ATT GTT GA
13	GCT TCT AGA GAC AAG CTT ACG CGT TTC AGC AGA ATG CG	TCG CCG CGG AGA GGC AGT TTT GAA CAG TG
14	GCT TCT AGA GAC AAG CTT ACG CGT TTC AGC AGA ATG CG	TCG CCG CGG TTC TCA TTG CTC GTT AAA GG

Underlined sequences are linkers added to provide convenient restriction enzyme sites for cloning purposes.

Table S2. Primers used to create deletions in Fig. 2

Construct	Sequence
Δ 1	GCT CTC GCA GCA GCA GGC GCA GAA TGC GCC CCA AAG TTT CCC CGT TTC GCC TTT AAC G
Δ 2	GGC GCA GAA TGC GCA CAA TGG CGA ATC CCC GTT TCG CCT TTA ACG AGC AAT G
Δ 2.1	GGC GCA GAA TGC GCA CAA TGG CGA ATA GTT TCC CCG TTT CGC CTT TAA CGA GCA ATG
Δ 2.2	GGC GCA GAA TGC GCA CAA TGG CGA ATC CCA ACC CCG TTT CGC CTT TAA CGA GCA ATG
Δ 3	GCA GAA TGC GCA CAA TGG CGA ATC CCA AAG TTT CCT TTA ACG AGC AAT GAG AAA AAT CTG AAA
Δ 4	CGA ATC CCA AAG TTT CCC CGT TTC GGC AAT GAG AAA AAT CTG AAA CAT TTT CAA CAC C
Δ 4.1	CGA ATC CCA AAG TTT CCC CGT TTC GCC AAC GAG CAA TGA GAA AAA TCT GAA ACA TTT TCA ACA CC
Δ 4.2	CGA ATC CCA AAG TTT CCC CGT TTC GCC TTT GCA ATG AGA AAA ATC TGA AAC ATT TTC A
Δ 5	CCC AAA GTT TCC CCG TTT CGC CTT TAA CGA AAA TCT GAA ACA TTT TCA ACA CCA GTC ACC
Δ 5.1	CCC AAA GTT TCC CCG TTT CGC CTT TAA CGA GAG AAA AAT CTG AAA CAT TTT CAA CAC CAG TCA CC
Δ 5.2	CCC AAA GTT TCC CCG TTT CGC CTT TAA CGA GCA ATA AAT CTG AAA CAT TTT CAA CAC CAG TCA CC
Δ 6	CCC CGT TTC GCC TTT AAC GAG CAA TGA GAC ACC AGT CAC CCA CTG TTC AAA ACT GCC TCT CAA CAA TGG C
Δ 7	GCA ATG AGA AAA ATC TGA AAC ATT TTC AAA AAC TGC CTC TCA ACA ATG GCC CAG AAC TGC GC
Δ 8	CAT TTT CAA CAC CAG TCA CCC ACT GTT CAC CCA GAA CTG CGC AGC TGG CCT CAA CAA TGA CAG
Δ 8.1	GAA ACA TTT TCA ACA CCA GTC ACC CAC TGT TCA TCA ACA ATG GCC CAG AAC TGC GCA GCT GGC
Δ 8.2	CCA GTC ACC CAC TGT TCA AAA CTG CCT CCC CAG AAC TGC GCA GCT GGC CTC AAC AAT GAC AGT CC
9	CCA CTG TTC AAA ACT GCC TCT CAA CAA TGG CTC AAC AAT GAC AGT CCT GAG GCC CTC GAG G
Δ 10	CCT CTC AAC AAT GGC CCA GAA CTG CGC AGC TGG CGG CCC TCG AGG TCC CCT CTT TAC AGG
Δ 10.1	CCT CTC AAC AAT GGC CCA GAA CTG CGC AGC TGG CAC AGT CCT GAG GCC CTC GAG GTC CCC TCT TTA CAG G
Δ 10.2	GCC CAG AAC TGC GCA GCT GGC CTC AAC AAT GGG CCC TCG AGG TCC CCT CTT TAC AGG ACT TTA ACG
Δ 11	GCG CAG CTG GCC TCA ACA ATG ACA GTC CTG ATA CAG GAC TTT AAC GTT TTC TCC ACA GAT TCC TGC

Table S3. Primers used in Fig. 3 constructs.

Construct	Primer	
	Forward	Annealing
36	GGC CGC AAG CTT AAA CTG CCT CTC AAC AAT GGA AAC TGC CTC TCA ACA ATG GAA ACT GCC TCT CAA CAA TGG AAA CTG CCT CTC AAC AAT GGT CCC CGC	GGG GAC CAT TGT TGA GAG GCA GTT TCC ATT GTT GAG AGG CAG TTT CCA TTG AGA GGC AGT TTC CAT TGT TGA GAG GCA GTT TAA GCT TGC
37	GGC CGC AAG CTT CTC AAC AAT GAC AGT CCT GAC TCA ACA ATG ACA GTC CTG ACT CAA CAA TGA CAG TCC TGA CTC AAC AAT GAC AGT CCT GAT CCC CGC	GGG GAT CAG GAC TGT CAT TGT TGA GTC AGG ACT GTC ATT GTT GAG TCA GGA CTG TCA TTG TTG AGT CAG GAC TGT CAT TGT TGA GAA GCT TGC
38	CTG GAA AGC TTC TCA ACA ATG ACC TCA ACA ATG ACC TCA ACA ATG ACC TCA ACA ATG ACC CGC	GGG TCA TTG TTG AGG TCA TTG TTG AGG TCA TTG TTG AGG TCA TTG TTG AGA AGC TTT
39	CTA GAA AGC TTA CAA TAC AAT ACA ATA CAA TCC GC	GGA TTG TAT TGT ATT GTA TTG TAA GCT TT

The annealed primers were inserted directly into BGZ40 digested with XbaI and SacII.

Table S4. Primers used to construct mutations summarized in Fig. 4*B*

Construct	Primer
Δ 1 TCC to TCG	GCA GCA GGC GCA GAA TGC GCA CAA TGG CGA ATC GCA AAG TTT CCC CGT TTC GCC TTT AAC GAG C
Δ 1 TCC to TCT	GCA GCA GGC GCA GAA TGC GCA CAA TGG CGA ATC TCA AAG TTT CCC CGT TTC GCC TTT AAC GAG C
Δ 1 TCC to TCA	GCA GCA GGC GCA GAA TGC GCA CAA TGG CGA ATC ACA AAG TTT CCC CGT TTC GCC TTT AAC GAG C
Δ 1 CAA to CAG	GCA GCA GGC GCA GAA TGC GCA CAA TGG CGA ATC CCA GAG TTT CCC CGT TTC GCC TTT AAC GAG C
Δ 2 CCT to CCG	CGA ATC CCA AAG TTT CCC CGT TTC GCC GTT AAC GAG CAA TGA GAA AAA TCT GAA ACA TTT TCA ACA CC
Δ 2 CCT to CCA	CGA ATC CCA AAG TTT CCC CGT TTC GCC ATT AAC GAG CAA TGA GAA AAA TCT GAA ACA TTT TCA ACA CC
Δ 2 CCT to CCC	CGA ATC CCA AAG TTT CCC CGT TTC GCC CTT AAC GAG CAA TGA GAA AAA TCT GAA ACA TTT TCA ACA CC
Δ 2 TTA to TTG	CGA ATC CCA AAG TTT CCC CGT TTC GCC TTT GAC GAG CAA TGA GAA AAA TCT GAA ACA TTT TCA ACA CC
Δ 2 ACG to ACA	CGA ATC CCA AAG TTT CCC CGT TTC GCC TTT AAC AAG CAA TGA GAA AAA TCT GAA ACA TTT TCA ACA CC
Δ 2 ACG to ACT	CGA ATC CCA AAG TTT CCC CGT TTC GCC TTT AAC TAG CAA TGA GAA AAA TCT GAA ACA TTT TCA ACA CC
Δ 2 ACG to ACC	CGA ATC CCA AAG TTT CCC CGT TTC GCC TTT AAC CAG CAA TGA GAA AAA TCT GAA ACA TTT TCA ACA CC
Δ 3 GAG to GAA	CCA AAG TTT CCC CGT TTC GCC TTT AAC GAG CAA TGA AAA AAA TCT GAA ACA TTT TCA ACA CCA GTC ACC
Δ 3 AAA to AAG	CCA AAG TTT CCC CGT TTC GCC TTT AAC GAG CAA TGA GAA GAA TCT GAA ACA TTT TCA ACA CCA GTC ACC
Δ 8 TCA to TCC	CCC ACT GTT CAA AAC TGC CTC TCC ACA ATG GCC CAG AAC TGC
Δ 8 TCA to TCT	CCC ACT GTT CAA AAC TGC CTC TCT ACA ATG GCC CAG AAC TGC
Δ 8 TCA to TCG	CCC ACT GTT CAA AAC TGC CTC TCG ACA ATG GCC CAG AAC TGC
Δ 8 ACA to ACC	CCC ACT GTT CAA AAC TGC CTC TCA ACC ATG GCC CAG AAC TGC
Δ 8 ACA to ACG	CCC ACT GTT CAA AAC TGC CTC TCA ACG ATG GCC CAG AAC TGC
Δ 8 ACA to ACT	CCC ACT GTT CAA AAC TGC CTC TCA ACT ATG GCC CAG AAC TGC
Δ 8 ACAAT to AAAAT	CCC ACT GTT CAA AAC TGC CTC TCA AAA ATG GCC CAG AAC TGC
Δ 8 ACAAT to ACAAA	CCC ACT GTT CAA AAC TGC CTC TCA ACA AAG GCC CAG AAC TGC
Δ 8 ACAAT to ACAAG	CCC ACT GTT CAA AAC TGC CTC TCA ACA AGG GCC CAG AAC TGC
Δ 8 ACAAT to ACAGT	CCC ACT GTT CAA AAC TGC CTC TCA ACA GTG GCC CAG AAC TGC
Δ 8 ACAAT to AGAAT	CCC ACT GTT CAA AAC TGC CTC TCA AGA ATG GCC CAG AAC TGC
Δ 8 ACAAT to GCAAT	CCC ACT GTT CAA AAC TGC CTC TCA GCA ATG GCC CAG AAC TGC
Δ 8 CTCAA to CTTAA	CCC ACT GTT CAA AAC TGC CTC TTA ACA ATG GCC CAG AAC TGC
Δ 8 CTCAA to CGCAA	CCC ACT GTT CAA AAC TGC CTC GCA ACA ATG GCC CAG AAC TGC
Δ 8 TGGC to TCGC	CCC ACT GTT CAA AAC TGC CTC TCA ACA ATC GCC CAG AAC TGC
Δ 8 TGGC to TGTC	CCC ACT GTT CAA AAC TGC CTC TCA ACA ATG TC
Δ 10 AAC to AAT	GGC CCA GAA CTG CGC AGC TGG CCT CAA TAA TGA CAG TCC TGA GGC CCT CGA GG,
Δ 10 AAT to AAC	GGC CCA GAA CTG CGC AGC TGG CCT CAA TAA CGA CAG TCC TGA GGC CCT CGA GG

Δ 1, mutations in RTE1; Δ 2, mutations in RTE2. Δ 3, mutations in RTE3; Δ 8, mutations in ACAAT-1; Δ 10, mutations in ACAAT-2.

Table S5. Oligos used in Fig. 4*A*

Oligo name	Forward oligo sequence for EMSA
ACAAT-1 probe	CTG CCT CTC AAC AAT GGC CCA GAA C
1-Mut-1	CTG CCT CTC AAC GAT GGC CCA GAA C
1-Mut-2	CTG CCT CTC AAC AAA GGC CCA GAA C
1-Mut-3	CTG CCT CTC AAT AAT GGC CCA GAA C
1-Mut-4	CTG CCT CTC GAC AAT GGC CCA GAA C
ACAAT-2 probe	GCT GGC CTC AAC AAT GAC AGT CCT G
2-Mut-1	GCT GGC CTC AAC GAT GAC AGT CCT G
2-Mut-2	GCT GGC CTC AAC AAG GAC AGT CCT G
2-Mut-3	GCT GGC CTC AAT AAT GAC AGT CCT G
2-Mut-4	GCT GGC CTC GAC AAT GAC AGT CCT G