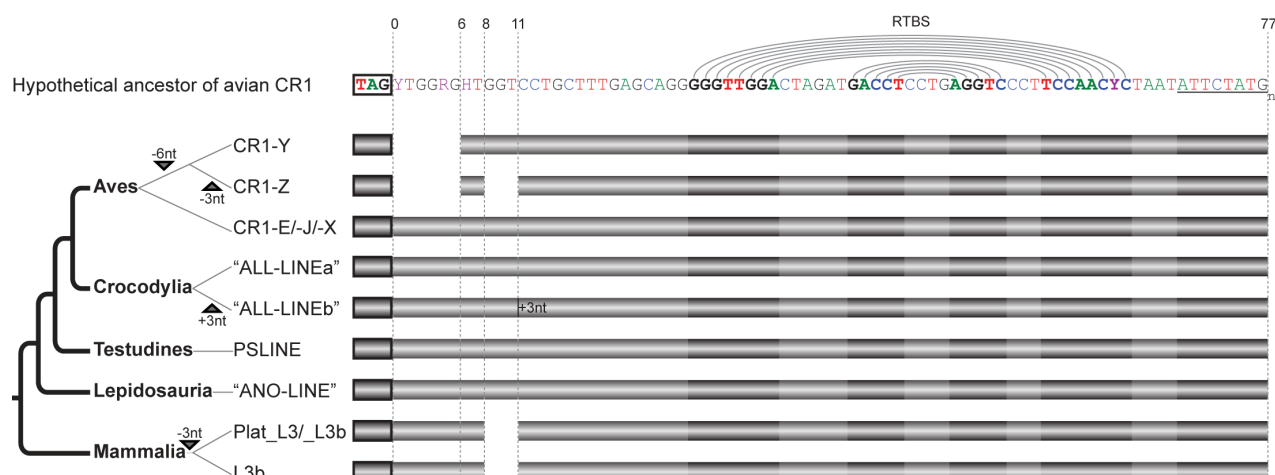
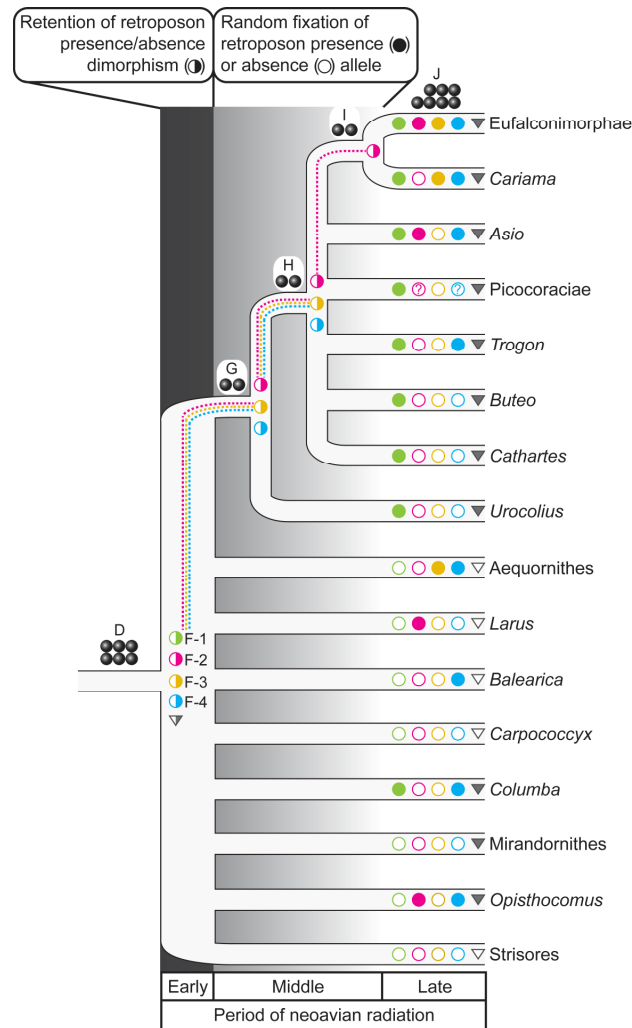
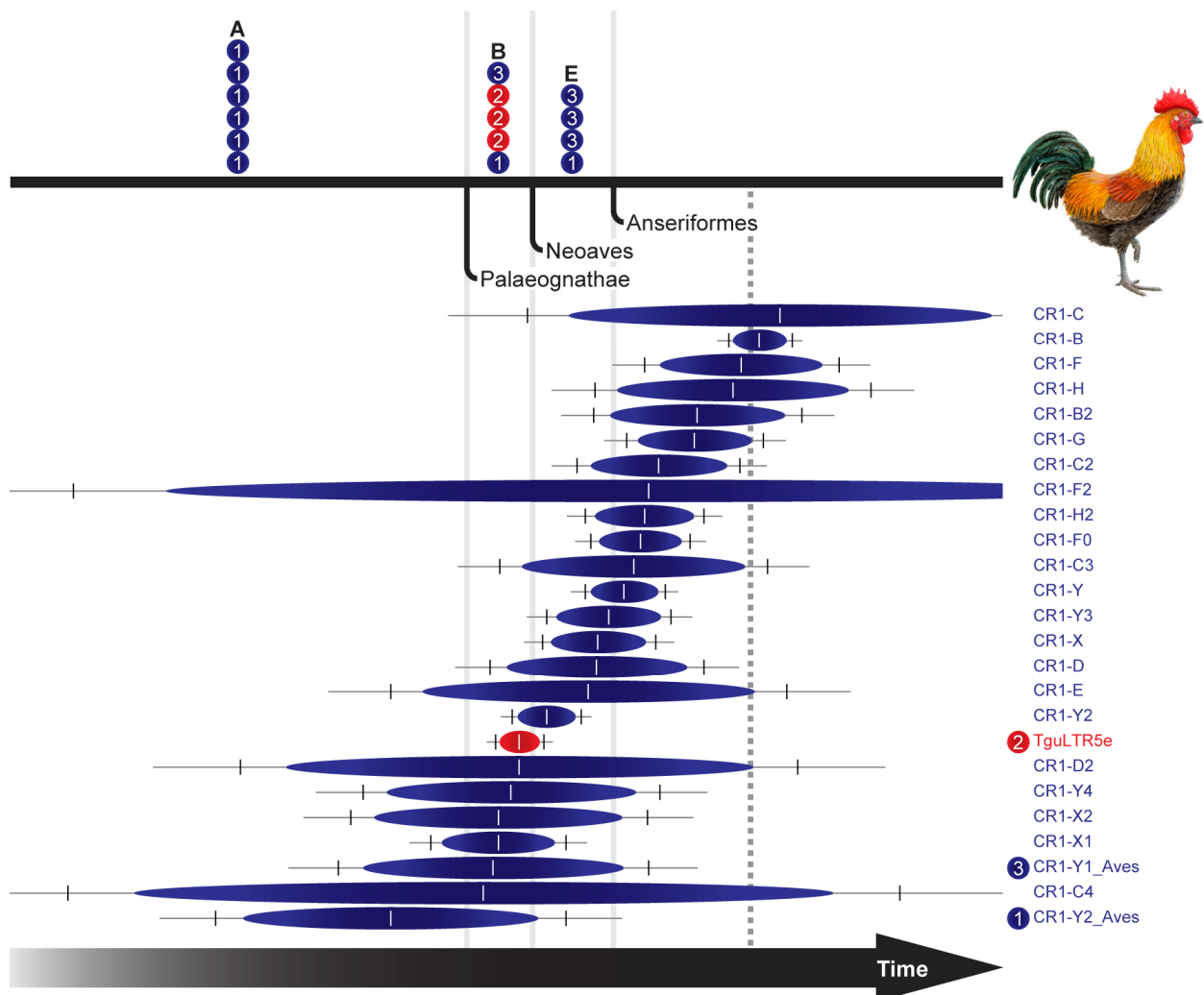




Supplementary Figure S1 | Structural comparison of 3' untranslated regions (3' UTR) in amniote-specific CR1 LINE retroposons. The topology of the amniote species tree (black lines) corresponds to the current understanding of amniote phylogeny⁵¹. Grey straight lines indicate putative relationships among lineage-specific CR1s based on the shown diagnostic random indels (triangles) larger than two nucleotides (nt). To facilitate comprehension, the 3' UTR nucleotide sequence of the putative ancestor of all bird CR1 elements was reconstructed by comparison of bird and reptile CR1s and all extant CR1 sequences were depicted as grey gradient bars. Bold nucleotides or dark grey bars depict highly conserved regions, i.e., the putative binding site for the reverse transcriptase or other CR1-encoded proteins⁵² (RTBS, grey curves indicate a potential hairpin structure in RNA) and the typical octamer microsatellite⁴⁷ (underlined, n = tandem repeats) at the 3' terminus. The first three nucleotides (black box) correspond to the stop codon of the second open reading frame (ORF2), marking the 5' boundary of the 3' UTR. Name suggestions for previously unrecognized CR1 subtypes (in the crocodylian *Alligator mississippiensis* and the lepidosaurian *Anolis carolinensis*) are highlighted by quotation marks.



Supplementary Figure S2 | Incongruent retroposon presence/absence patterns within the neoavian radiation. The topology and the branch labels correspond to those of Fig. 1. Dark grey balls are retroposon markers that (when the four incongruent markers F-1 to F-4 are not considered) yield a topology without conflicts. The presence/absence pattern (filled circle: presence; empty circle: absence; question mark in empty circle: missing data) of each of the four incongruent markers and of an 82-nt random deletion (grey triangle, in the flanking sequences of marker J-5) is shown in corresponding colours on the terminal branches. This complex phenomenon is presumably caused by incomplete lineage sorting²⁶ of retroposon presence/absence dimorphisms (semi-filled circles). Our data suggest that these retroposons inserted during an early period of the neoavian radiation (dark grey shade) and did not have enough time for fixation in the whole population of the ancestral species. In combination with the extensive and accelerated speciation events of the neoavian radiation, these retroposon dimorphisms seemingly persisted (coloured dashed lines) during most of the radiation (light grey gradient) and, subsequently, one of the two alleles was randomly fixed in each of the descendant lineages, leading to incongruence with some of our otherwise congruent markers (branches G, H and I). Notably, the four incongruent markers are not in any conflict with the late period of the neoavian radiation, i.e., the new taxa Eufalconimorphae (branch J) and Psittacopasserae (branch K in Fig. 1). In summary, our results indicate that the neoavian radiation can be divided into an early (occurrence of retroposon presence/absence dimorphisms), a middle (random fixation of dimorphisms leading to incongruent retroposon presence/absence patterns, occurrence of congruent presence/absence patterns) and a late period (occurrence of congruent presence/absence patterns).



Supplementary Figure S3 | Chronology of Mesozoic retroposon activity in the chicken genome.

Computational estimates of activity periods (normal distributions displayed as ovals²⁹) of selected retroposon subtypes were calculated via the TinT model^{25,29} and plotted on a simplified chronogram³⁰ (black lines) by using the experimentally verified retroposon insertion events (numbered blue or red balls, numbers indicate the respective retroposon subtype) of Fig. 1 as temporal landmarks. Single capital letters correspond to the branch labels of Fig. 1 (A, Aves; B, Neognathae; E, Galloanserae). CR1 retroposons are highlighted in blue and LTR retroposons are shown in red. The grey dashed vertical line indicates the estimated end of the Mesozoic Era at the Cretaceous/Tertiary boundary³⁰. Note that during their shared TinT activity range in the ancestor of Aves and Neognathae (see also Fig. 3), several CR1 subfamilies (chicken CR1-C/D/E and zebra finch CR1-E; chicken CR1-F2 and zebra finch CR1-J2_Pass; chicken CR1-X and zebra finch CR1-X; chicken CR1-Y and zebra finch CR1-Y) appear to be identical, which also corresponds to the highly similar nucleotide sequences of their consensus sequences in Repbase (<http://www.girinst.org/repbase/index.html>).

Supplementary Table S1 | Presence/absence table of all retroposon markers including genomic positions, retroposon subtype, and strategies (see Methods for details).

Marker	<i>Taeniopygia</i>	<i>Acanthisitta</i>	<i>Nestor</i>	<i>Falco</i>	<i>Carliama</i>	<i>Asio</i>	<i>Alcedo</i>	<i>Picus</i>	<i>Trogon</i>	<i>Buteo Gyps</i>	<i>Cathartes/Gymnogyps</i>	<i>Urocolius</i>	<i>Ciconia</i>	<i>Larus</i>	<i>Balearica</i>	<i>Carpococcyx/Cuculus</i>	<i>Columba</i>	<i>Tachybaptus/Podiceps</i>	<i>Phoenicopterus</i>	<i>Opisthocomus</i>	<i>Apus</i>	<i>Chrysialmpis</i>	<i>Gallus</i>	<i>Alectura</i>	<i>Anas</i>	<i>Dendrocygna</i>	<i>Dromaius</i>	<i>Eudromia</i>	<i>Pterocnemis</i>	<i>Struthio</i>	
A-1	+										+						+						+				+				
A-2	+																						+				+				
A-3	+										+		+				+						d				+				
A-4	+																						d				+			+	
A-5	+																						+				+				
A-6	+										+		+										+				+				
B-1	+			+							+		+	+			+	+					+		+	+			-		-
B-2	+																+						+		+			-		-	
B-3	+												+										+		+					-	
B-4	+												+										+		+	+		-		-	
B-5	+												+										+		?		-			?	
C-1	-																						-				+	+	+	+	
C-2	-																						-				+	+	+	+	
C-3	-												-										-		-		+	?	?	+	
C-4	-												-										-				+	+	+	+	
D-1	+					+	+		+	+	+	+	+	+	+	+	+	+		+		+	-		-	-					
D-2	+					+		+	+	+	+	+	+	+	+	+	?	+		+		+	-	-	d	-				-	
D-3	+					+	+		+	+	+	+	+	+	+	+	+	+		+		+	-		-						
D-4	+					+	+		+	+	+	+	+	+	+	+	+	+		+	+		-		-						
D-5	+			+		+		?	?	+	+	+	+	+	+	+	+	+	+	+	+		-		?						
D-6	+			+		+	+		+	+	+	d	+	+	+	+	+	+	+	+	+	+	-			-		-		-	
E-1	-																-						+		+			-		-	
E-2	-												-				-						+	?	+	+	-	-			
E-3	-																-					-	+	+	+	+	-	-			
E-4	-												-										+	+	+	?	?				
F-1	+	+	+	+	+	+	?	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-					-			
F-2	+		+	+	-	+		?	-	-	-	-	-	+	-	-	-	-		+		-	-				-	-			
F-3	+		+	+	+	-		-	-	-	-	-	+	-	-	-	-	-			-	-	-								
F-4	+		+	+	+	+		?	+	-	-	-	+	-	+	-	+	-		+		-	-				-				
G-1	+		+	+	+	+		+	+	+	+	+	-	-	d	-	d	-		-	-	-	-				-				
G-2	+		+	+	+	+	+		+	+	+	+	-	-	-	-	-	-		-	-	-	-				-				
H-1	+		+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-		-	-	-	-		-		-				
H-2	+		+	+	+	+	+	d	+	+	+	+	-	-	-	-	-	-		-	-	-	-				-				
I-1	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
I-2	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
J-1	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
J-2	+	+	+	+	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d								
J-3	+	+	+	+	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
J-4	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
J-5	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
J-6	+	+	+	+	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
J-7	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	?	-								
K-1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	?	-								
K-2	+	+	+	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-								
K-3	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
L-1	+	+	-	-			-	-			-		-			-							-								
L-2	+	+	-	-			-	-			-		-			-							-								
L-3	+	+	-	-			-	-		-	-		-			-							-								
L-4	+	+	-	-			-	-			-		-			-							-								
L-5	+	+	-	-			d	-			-		-			-							-								
L-6	+	+	-	-			-	-			-		-			-							-								

Character states are presence (+) or absence (-) of a retroposon insertion at a given genomic locus. Unspecific deletions are marked by 'd' and missing experimental data is indicated by '?'. For each marker, the taxon sampling was optimized to all phylogenetically essential taxa (blank cells: not analysed).

Supplementary Table S1 | (continued).

Marker	RE subtype	RE orientation	Chromosome (taeGut1)	Start and end (taeGut1)	Strategy
A-1	CR1-Y2_Aves	+	2	26059437-26060242	I
A-2	CR1-Y2_Aves	+	2	24760332-24761039	I
A-3	CR1-Y2_Aves	+	2	25303447-25304574	I
A-4	CR1-Y2_Aves	+	2	18685609-18686573	I
A-5	CR1-Y2_Aves	+	1A	20376757-20377989	I
A-6	CR1-Y2_Aves	+	1A	20375110-20375988	I
B-1	CR1-Y2_Aves	+	Z	24736112-24736753	I
B-2	TguLTR5e	+	Z	24741461-24742589	I
B-3	TguLTR5e	-	1	29915747-29916601	II
B-4	TguLTR5e	-	5	14985700-14986976	II
B-5	CR1-Y1_Aves	-	2	25167690-25168178	I
C-1	CR1-Y2_Aves	-	4	64193638-64194132	III
C-2	CR1-Y2_Aves	+	2	54424564-54425428	III
C-3	CR1-Y1_Aves	+	17	8094466-8094952	I
C-4	CR1-Y1_Aves	+	3	108853900-108854584	I
D-1	TguLTR5e	-	1A	13493804-13495145	II
D-2	CR1-E_Pass	+	23	2210820-2211801	I
D-3	TguLTR5d	+	2	43944384-43945448	II
D-4	TguLTR5d	+	3	77815159-77816217	II
D-5	TguLTR5a	+	1A	53818237-53819230	II
D-6	CR1-J2_Pass	-	Z	42095413-42096259	I
E-1	CR1-Y2_Aves	-	Z	24741461-24742589	I
E-2	CR1-Y1_Aves	-	3	108854577-108854902	I
E-3	CR1-Y1_Aves	-	3	108828593-108829533	I
E-4	CR1-Y1_Aves	-	Z	4905862-4906760	II
F-1	CR1-J2_Pass	-	1	38803625-38804919	II
F-2	CR1-J2_Pass	+	3	108756503-108757505	I
F-3	CR1-J2_Pass	-	2	143483216-143484373	II
F-4	CR1-J2_Pass	+	2	25246211-25246841	I
G-1	CR1-X3_Pass	+	3	108701927-108703291	II
G-2	TguLTR5a	+	1A	20294073-20295316	I
H-1	TguLTR5d	+	3	85253635-85255015	II
H-2	TguLTR5a	+	4	58849092-58850972	II
I-1	TguLTR5d	+	5	34776063-34777405	II
I-2	TguLTR5d	-	7	23352566-23354134	II
J-1	TguLTR5d	+	1A	59391304-59392588	II
J-2	TguLTR5d	+	Z	29203733-29205351	II
J-3	TguLTR5d	-	15	8418945-8419797	II
J-4	TguLTR5d	-	5	30767928-30769035	II
J-5	TguLTR5a	+	1A	32879518-32880652	II
J-6	TguLTR5a	+	1	38803625-38804919	II
J-7	TguLTR5a	+	9	21782642-21784130	II
K-1	TguLTR5d	-	1A	43914270-43915689	II
K-2	TguLTR5d	-	4	32753338-32754835	II
K-3	TguLTR5d	-	1	86620295-86621332	II
L-1	TguLTR5d	-	7	10143600-10144882	II
L-2	TguLTR5d	-	3	31049664-31051072	II
L-3	TguLTR5a	+	20	7141389-7142483	II
L-4	TguLTR5a	+	2	108771408-108772227	II
L-5	TguLTR5a	+	1	56216733-56218030	II
L-6	TguLTR5a	+	14	13779122-13780263	II

Supplementary Table S2 | Oligonucleotide primer sequences.

Marker	forward primer (5'-3')	reverse primer (5'-3')
A-1	CATACCTGACACAGTTGGTGAC	GAGAGTGCCTGGGTCAGTAG
A-2	CTTAATCCTGCTGCAAATACAC	GGAAGAGCTACAAGAACGTCTC
A-3	CTACACTGAATGTAATAAGGCTTG	CTCTCCAACCTAAGTCACAGATC
A-4	GAAGGTGTGAAACTGTCTGGAC	GAGAGATCCACCTGAAGAAAGTAG
A-5	TTACCCTGGCATGTGCTG	CTGTTCTTTTGTCTCATTCTCTG
A-6	ATCCAAGCAGAAACAATGTC	CTAGACTTCTTGTGCTGGGAG
B-1	GTCCGTATTTTCTCACAGATGG ²¹	ATGATCCAGTGCTTGTTCC ²¹
B-2	CAGCAGAAATCAATCCAAGAC ²¹	CAGCCCATTTAACTGATAATCTC ²¹
B-3	GGTGAAACTGGACACTTGG	CATGAAATAGACTTGACTGCTTC
B-4	CCTAAAGGAATGTACCTGAACC	GCCTCCCATTAACACAGTAGC
B-5	AGGTCTGATWCCTAAGAAAATAAG	CGCTCCTCTGAGTCCATC
C-1	TAGTTTACAACCAAGACTGAAGG	GGATTAACCCAGAGTAACCTGATTG AACCTGATTGATCTCTGTGTGAG
C-2	AACAGCAAGTCTTCAGTCAGC GAGTAGGTGAGGGTGAGAAGG	GAATCTTTTCACCTTCAGTACAC AAAATCCCAGCCACTTCAG
C-3	TGGAGGTGAAATTCTGCC	TTCTCTTGCTGTTTGGGAC
C-4	CTCAGTTTCCCTTGATGAATG	TAAATCCCACCAACCTCC AACCTCCGCTGCCTGTC
D-1	CTCTGTCAAATGAAACCATAGG TCTTCAAACATGGAGATGACC	AGAGGTCCAAGGATTCTGC GGTCCAAGGATTCTGCTTC
D-2	CGCAGCATGGGAGATTG ACCACTGAAGAACTCATTGG CATTTGGGCCAGTTCTCAG	AAGAGCAACAGCAAGTAAAGAG AAAGCAGACACCTCCTCCTC ACCTCCTCCTCACAGGACAG AACAGAGGGGAAGAGGAAAGC
D-3	GAATGGCTGGGTGCTGTC GCTGGGTGCTGCTTGAATC	GCTCCAATAACTGAATAATCTTCTC
D-4	TCTCTATGGTGGCAGAAAGG ATGGTGGCAGAAAGGTCTG	AGTTCAGCACTTGGCAGTTG GAAGGCTCTGGACAAAACC
D-5	CCAAAGACATGCTTCAAGATTG AAGAAGATCAATGTCTTGTGTC	AGTCCTTCATTTCAGTAACAGTG ACAGTGCTTCTGTAGTAAAGG
D-6	TTGTCAGAGTTGCTGGAGATAC ²¹	AATTGTAGTGGCACATAACTGTAG ²¹
E-1	see B-2	see B-2
E-2	GTAAGGCAGGGATGTAGGAG	AAACKGRGKCTTTCTAACTC
E-3	AGYACAGAATAATGGGTCAAC	AAATCAGGCATCTAGTCTAAACTC
E-4	ATTTCTCTCTATCTTCTCTGC	CGATTATCTGGCTGTTTCATCC
F-1	TTCAATTCAGAACAGTGTGCTTC	GCTTTGTGGAAGAAGCTGATAG CTGGATAGATTACAACCTTTCC
F-2	CATTGCCAGTTTCTYTCCAC CTGAAAAATGGTCTAGGATGAC	TTCTCTGTTCTTTCTTKATTCC CATGCTCCAGAAAGAGTTACAC
F-3	TGGTACTCCAAGTGTCTCAGATG GGTACTCCAGTGTCTCAGATGATAC CAGATGTAGGCACCTGCTGAAG	AGTTGCTGAAACCTCCTGC ACAGAAATTAATCCAGAGAAGC CAAGAATGCACACCACGTAAG
F-4	ATAATGGTGTCTGTCAAAGG GGAGACTTCTGGCTTTAACAG	TGTTGTGCTTGCTAATACTGG AAGCAAAACACAGATCACTGAG GGTGATAGCGATGTGAAATG
G-1	CTCCTCTCTCCAGTTTCAGTG GTGGTTATTCCCCTGCTATG	AACACACAGTGTATCACACATGG CTGTCCAGACTGATAAATTGTTAG
G-2	CATTGAGGASAAGGGGATG TGTAATCCATTTGTGCTAAAGAC TTGTCAGTCTGTCTTTCCCTC	TAMGGTTATTTGATTGCTGWC GGCTGACTGTACTTTAGGTTGC TGTTTAGCCTAGTACCTGAAGC
H-1	GGTTGGTAATCCTGGAGACC ATCCTGGAGACCCTGGTTC	CAAACCTGCTTAATGTGCTG ATCACTTGGTCTTTTCCCTAG
H-2	CAGAAGAATCTGCCAACAC GCTCACCTGGGACTCTCAG CCGCATAGAAGGAGTGGTC CACTGTTTCAACAATCATCTG CACTGCATATTCTGAGAACC	CCAGCATTACAGTCACTTG TCTGAGTTATTGTCTGGTGAGC GACTGCTTTAGAGTTTCCAGC TTTTCCAGCATTACAGTCAC
I-1	GGTCACACGCTGTTAGAGTTAC	GTGTCTCAAGATGATGTGGATG CGCACCAGATACTACAATACAG
I-2	CTGAAAAATAAGAAATGGCACAC CTGGAATTACTTGTAGGTTGATG ATAAAGAATGGCACACCTCC	AGGCAGAATCTGATTGAACAG CAAGCAAGTGACGCTCCTG
J-1	TTGAGCAGAGGAATGAGC	CACAAAGAAACGGACAAAGG

Supplementary Table S2 | (continued).

Marker	forward primer (5'-3')	reverse primer (5'-3')
J-2	CATCCATTGACCCATAAACTG TATCAATGCTGATGACCTTCC	ACTAACACTCCACTGATATGCTTC TTTAATAAGCCGTGTCTGTCC
J-3	ATCTGAGGATTGGCTGTGC CAGTTCCATCACCGACACAG	TCAAAGGAGATGAGGGAGC TGCCTCTCAACACAGAATCC
J-4	GCGTAAATTCAACAGAAACCAG	CGAAAAATCAGCATGATAACAGAG CAGCATGATAACAGAGTATGAGTTG
J-5	TCTGTACTGGCATCCTGGTC CACTGTATGGGCTGTCACTG	CTAAACAAAATCATTAGGCTGG GCTGGAGATGACTATTGGTAAG
J-6	see F-1	see F-1
J-7	CCCTCTAGTTTACTGCTGTG ATGTCATTAGCATCTGTCTTGG	GGCTATGAAAATGGTAACAAGG CTGAGCAGTCTCATCCCTTC
K-1	AAGTGAGCAACATCTCCAGG CAACATCTCCAGGGAAAGC	CATGGGCTTTCTGTAAGTCTG GTGCTGACAAAATAGACTGCTG
K-2	CATTCATGTTAAGTGGTTTCG	ATTCTTACCAGGTTCAAGTTGC
K-3	CAAGTCCAGACCATGATATGTG	TCTCTAGGCAAACTGTGTAAAC
L-1	AACTGTGCTGCCTGATGC GCGGATGTATTTGGATGC	GCCTATTAGCTTCCTTCTGTG TTAGCTTCCTTCTGTGTGTC
L-2	GGAAGTTGGTGATTGAAGATG TGGTGATTGAAGATGAAACAGAG	ATAGGGGTTAGACCTTGTTTCG GCATTTCTTCTCCATCCAGC
L-3	TGTGAGCCTTGTGTAAGTTGAG	CCTTTGAGTTTAGCCAGAC
L-4	ATGTACTACCACCAAGTTATCCAC	GCTTTACGTTCTCTATTGAGG
L-5	TCCAGCATCAACATCTCCTG	TGGTTACAATGCCTTTCCTG
L-6	CTCCAATGACAGTTGTTACAGC	ATGGAAAGTCTGTGGATGATG
L5216 + H6313	GGCCCATACCCCGRAAATG	ACTCTTRTTTAAGGCTTTGAAGGC