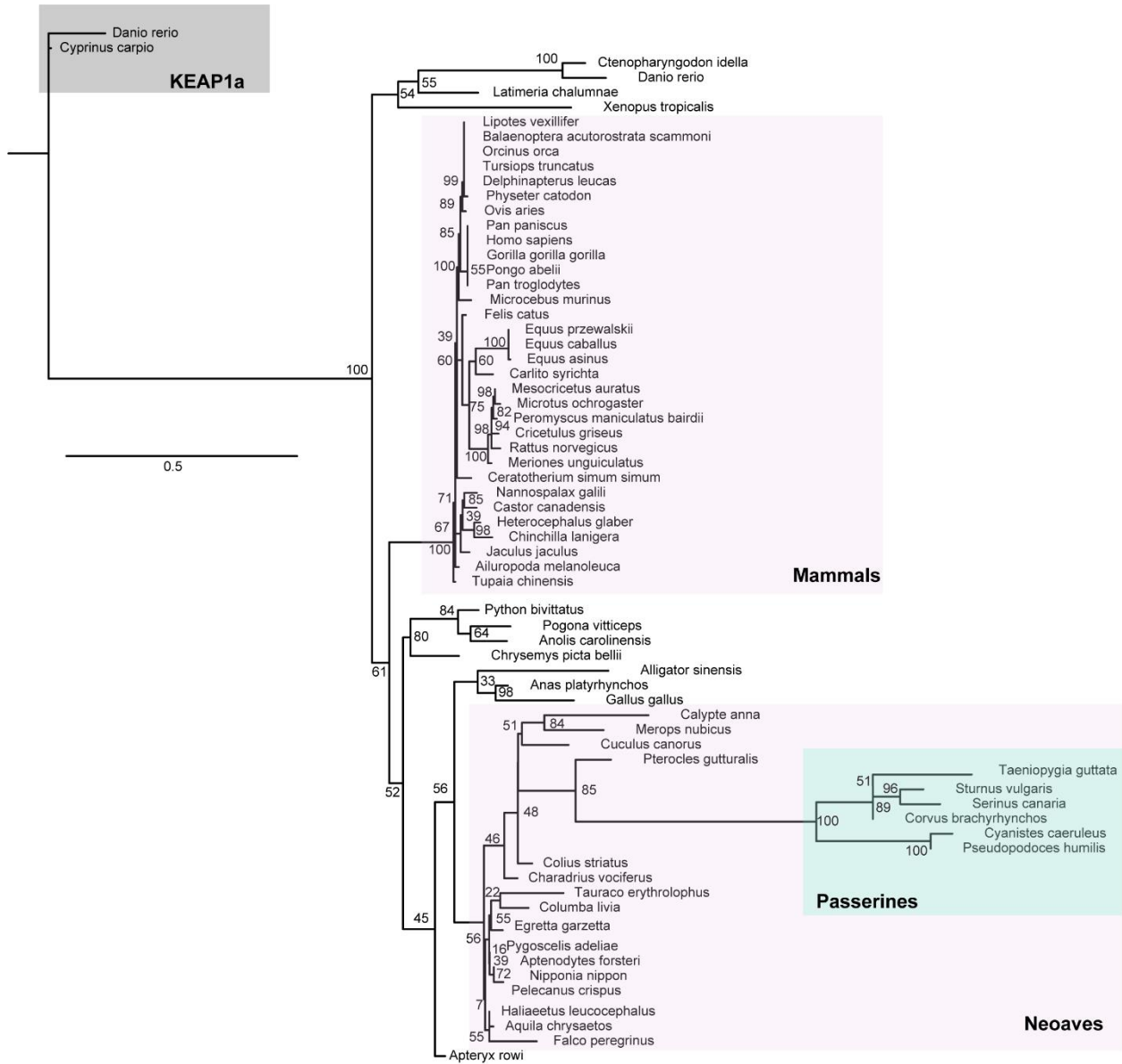


Supplementary Information

Adaptation of the master antioxidant response connects metabolism, lifespan and feather development pathways in birds

Castiglione et al.

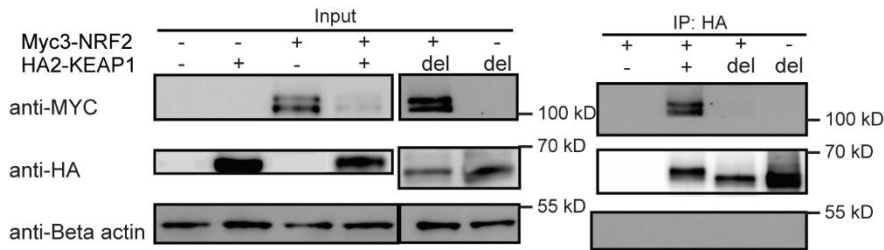
Supplementary Figure 1



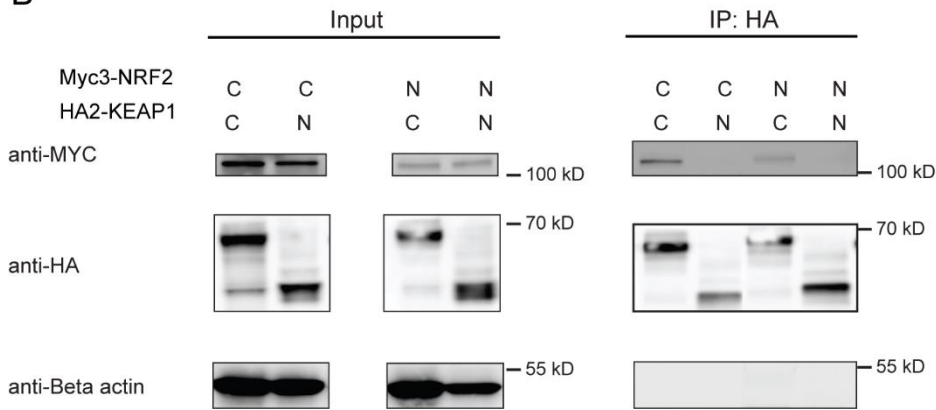
KEAP1 gene tree generated to confirm identity of KEAP1 coding-sequences.

Supplementary Figure 2.

A

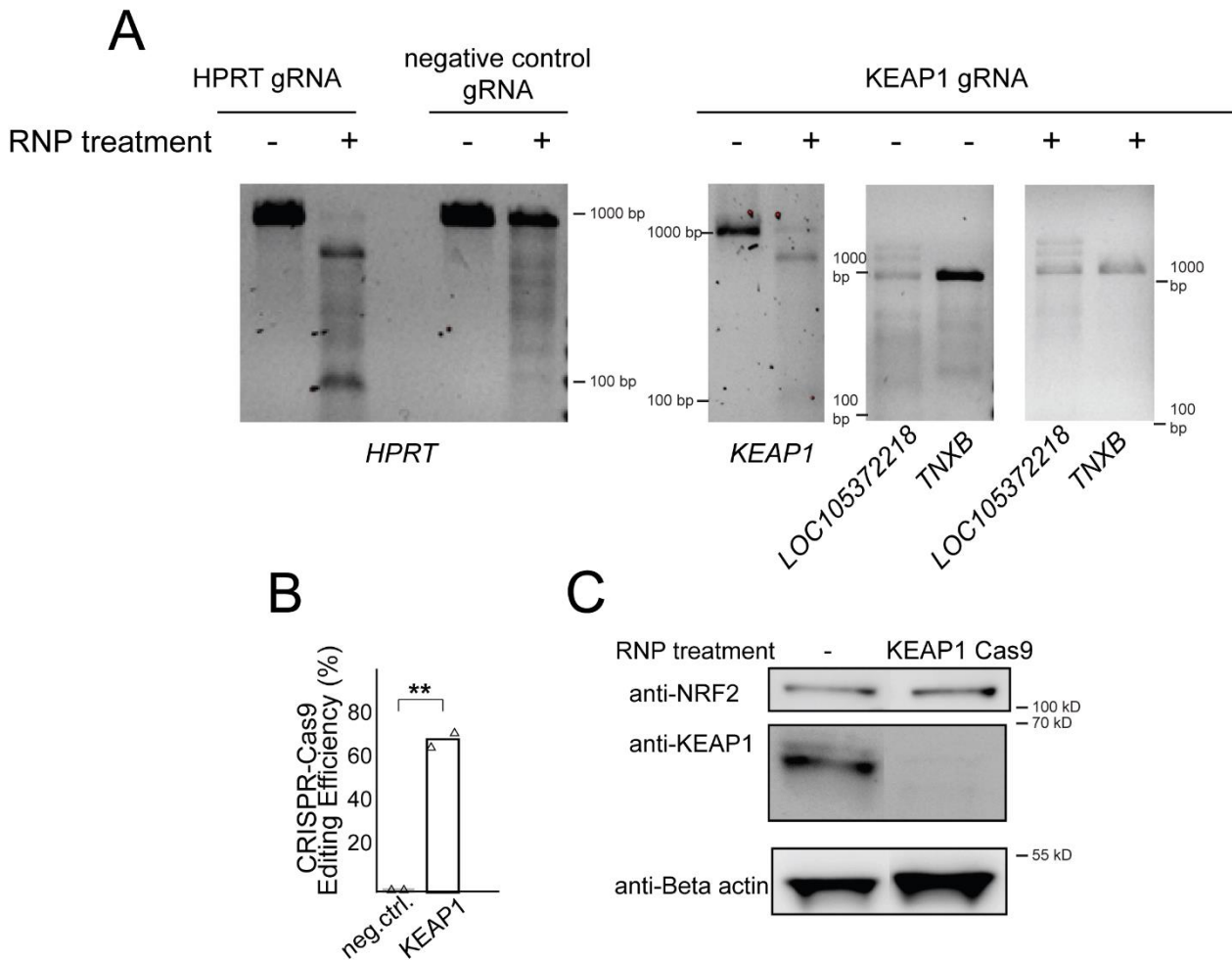


B



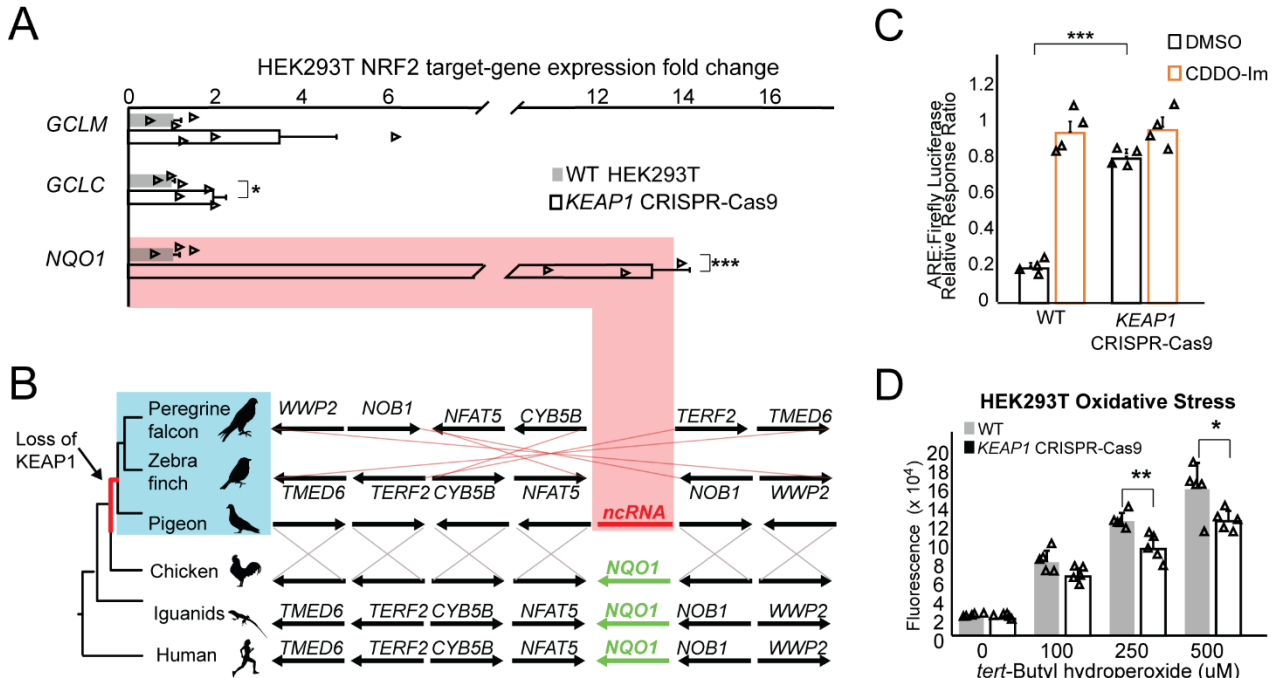
Co-immunoprecipitation of HA2-KEAP1 and Myc3-NRF2 from HEK293T cells transfected with various NRF2 and KEAP1 constructs. **(A)** Human wild-type NRF2 (+) was co-transfected with either wild-type KEAP1 (+) or Δ 442-488 KEAP1 (del). **(B)** Chicken (C) or Neoaves (N; *Zonotrichia albicollis*) NRF2 was co-transfected with Chicken (C) or Neoaves (N) (*Taeniopygia guttata*) KEAP1. Source data are provided as a Source Data file. All of these immunoblot experiments were repeated twice each, with each independent replicate showing similar results.

Supplementary Figure 3.



CRISPR-Cas9 genome editing detection. **(A)** Amplicons of control (HPRT), KEAP1, and off-target loci were digested by T7 endonuclease I (T7EI) to evaluate mismatch between annealed DNA strands from genomic DNA isolated from RNP treated HEK293T cells. HPRT gRNA RNP served as positive and negative control for Cas9 editing. KEAP1 gRNA RNP editing efficiency was assessed at both KEAP1 and off-target loci (LOC105372218; TNXB) and results were analyzed by gel electrophoresis. **(B)** Quantification of band intensity from T7EI-digested KEAP1 gRNA RNP-treated HEK293T cell amplicons was performed using the Image J program (version 1.52d) [N=2; p=0.001, one-sided t-test]. **(C)** Western blot of cell lysates from HEK293T cells treated with KEAP1 gRNA Cas9 RNP demonstrates loss of KEAP1 expression. Source data are provided as a Source Data file. All data are presented as mean values. All error bars represent standard error. This immunoblot experiment was repeated twice, with each independent replicate showing similar results.

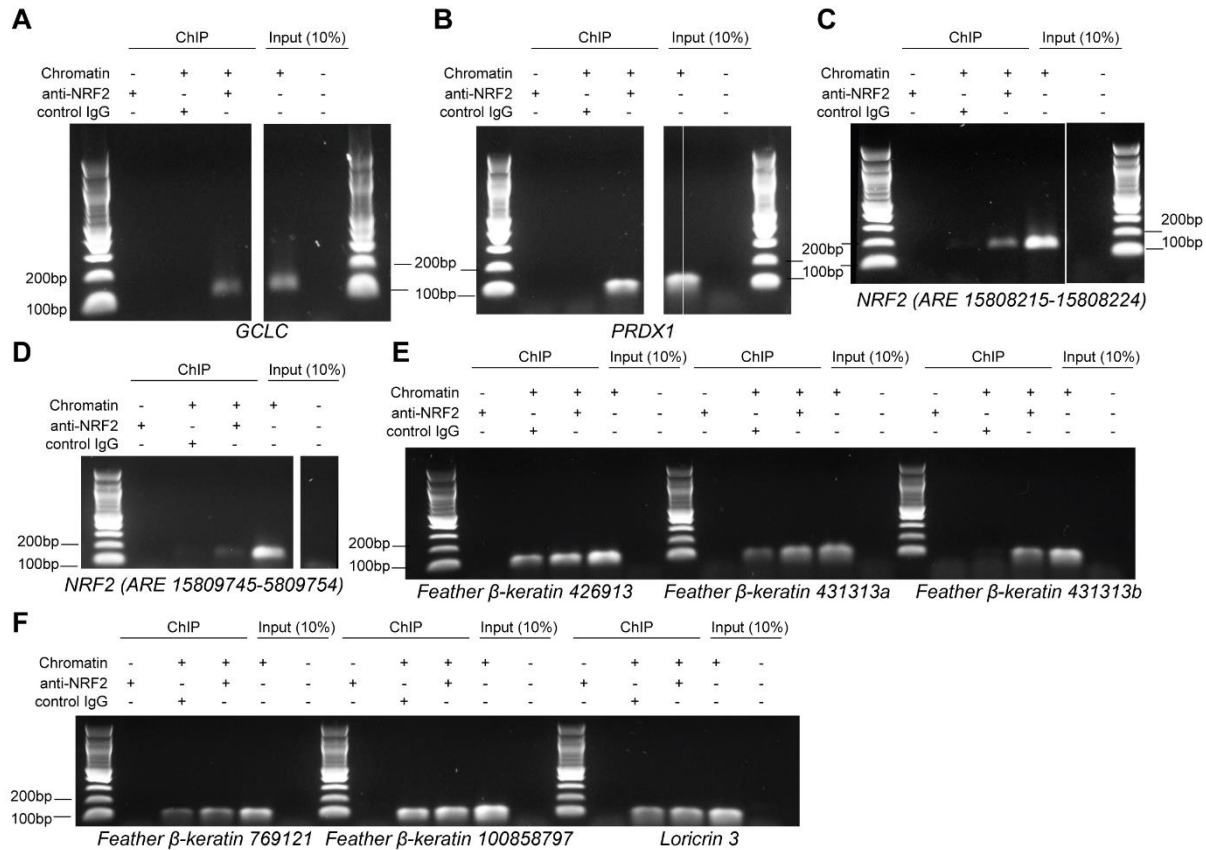
Supplementary Figure 4.



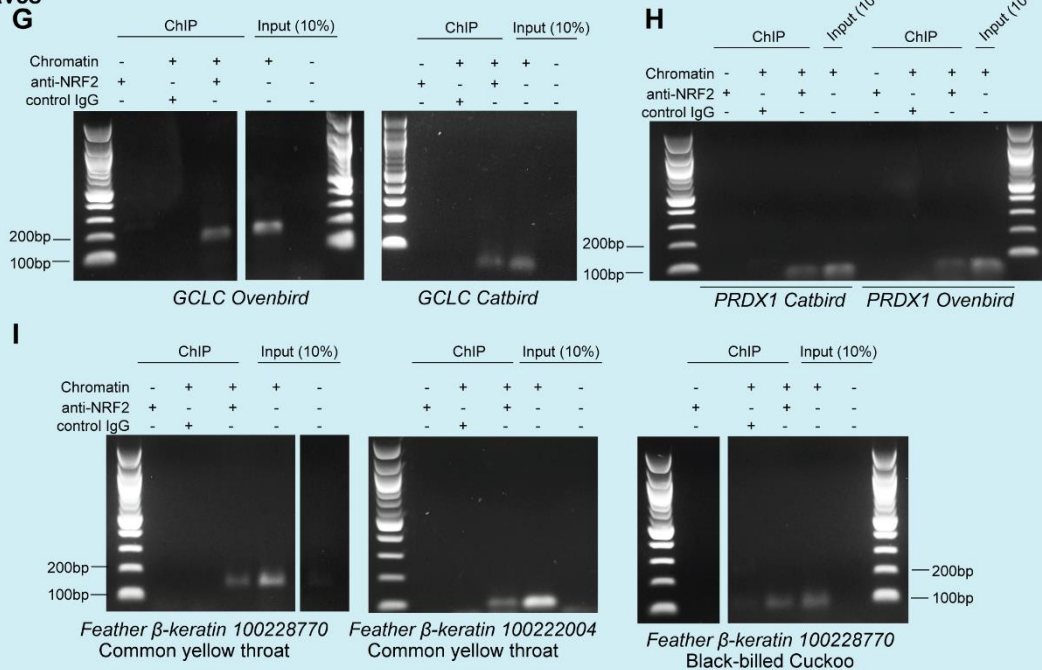
Increased NRF2 activity and decreased oxidative stress in KEAP1-gRNA-Cas9 treated HEK293T cells. **(A)** Disruption of KEAP1 function through CRISPR-Cas9 genome editing in HEK293T cells leads to a significant overexpression of *NQO1*—an important NRF2-regulated detoxification enzyme – and *GCLC*³² [$N=3$; one-sided t-test]. **(B)** *NQO1* is deleted in Neoaves genomes despite high syntenic conservation of neighboring genes across Human, Anolis, and Chicken. **(C)** When treated with 50nM of the KEAP1-mediated NRF2-activator CDDO-Im⁶⁶, the induced NRF2(ARE)-driven Firefly luciferase expression in Cas9-RNP-treated HEK293T cells is significantly muted relative to that of WT [$N=4$ biologically independent cells; $p=5.6 \times 10^{-7}$, one-sided t-test]. **(D)** Decreased cellular oxidative stress results from KEAP1 loss-of-function in HEK293T cells treated with *tert*-Butyl hydroperoxide [$N=5$ biologically independent cells; 250uM tBh, $p=0.002$; 500uM tBh, $p=0.017$; one-sided t-test]. Source data are provided as a Source Data file. All data are presented as mean values. All error bars represent standard error.

Supplementary Figure 5.

Chicken

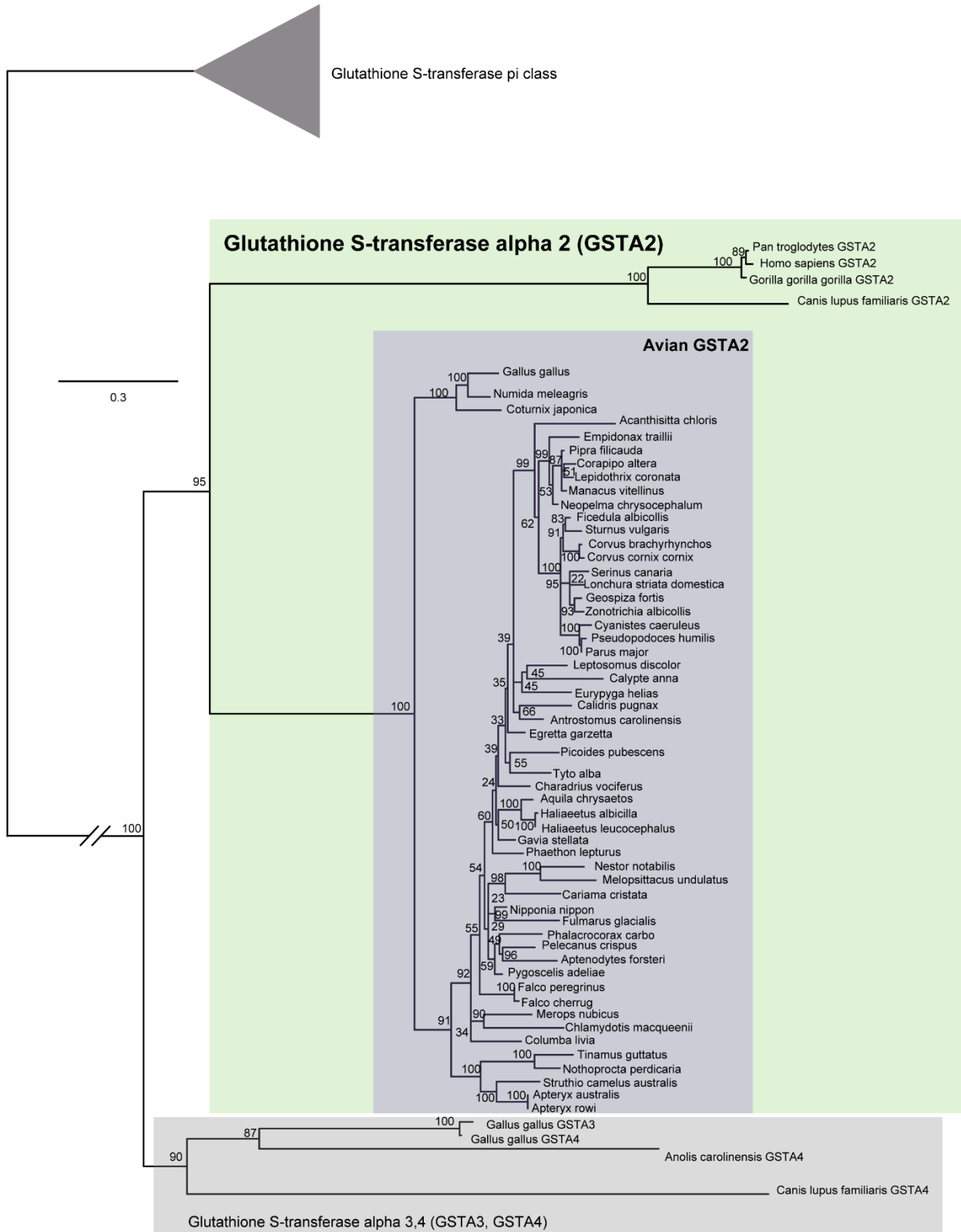


Neoaves



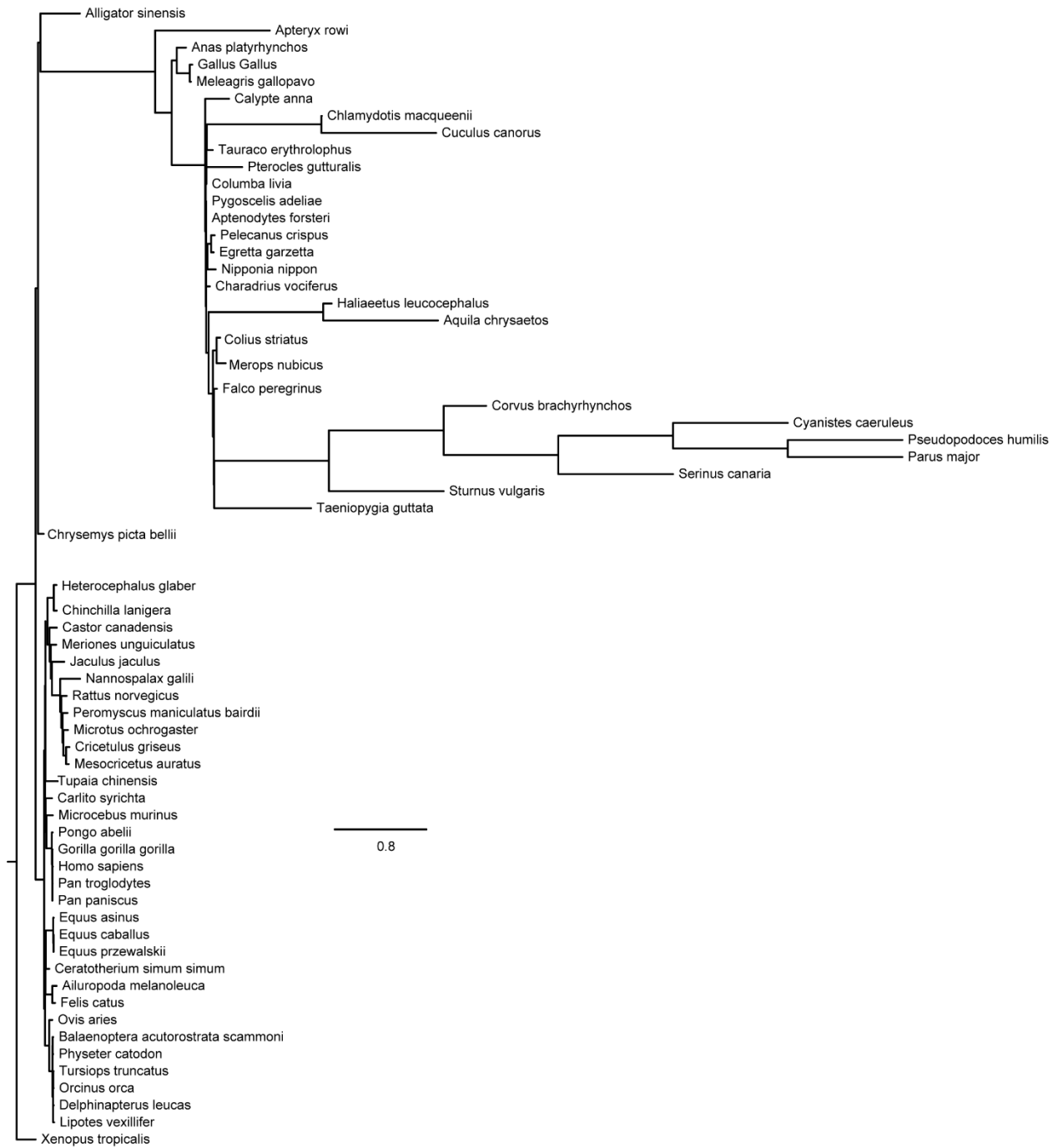
Validation of PCR amplicons quantified in qPCR analysis of Avian Chromatin. These experiments were repeated twice each, with each independent replicate showing similar results. Source data are provided as a Source Data file.

Supplementary Figure 6.



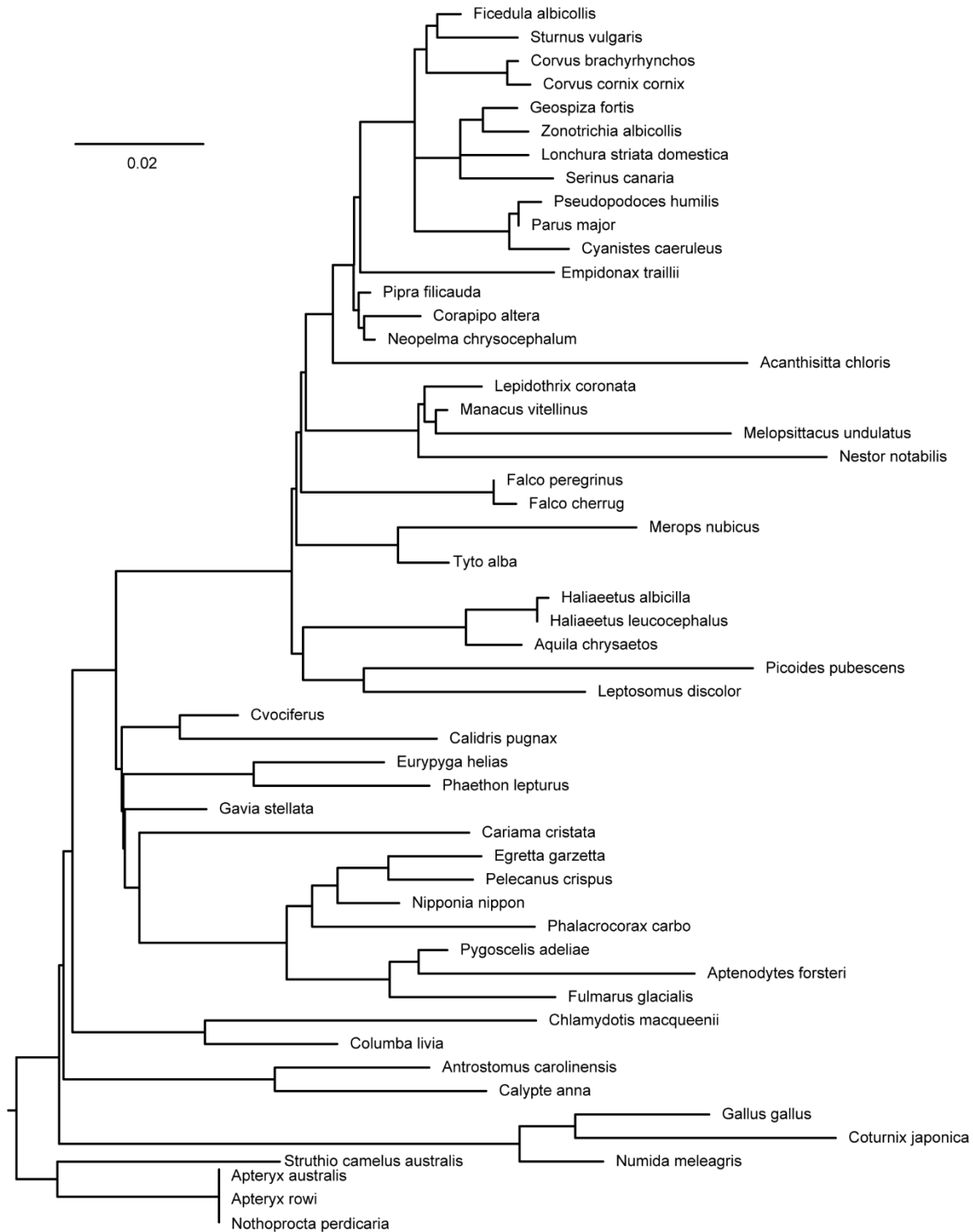
GSTA2 gene tree generated to confirm identity of GSTA2 coding-sequences

Supplementary Figure 7.



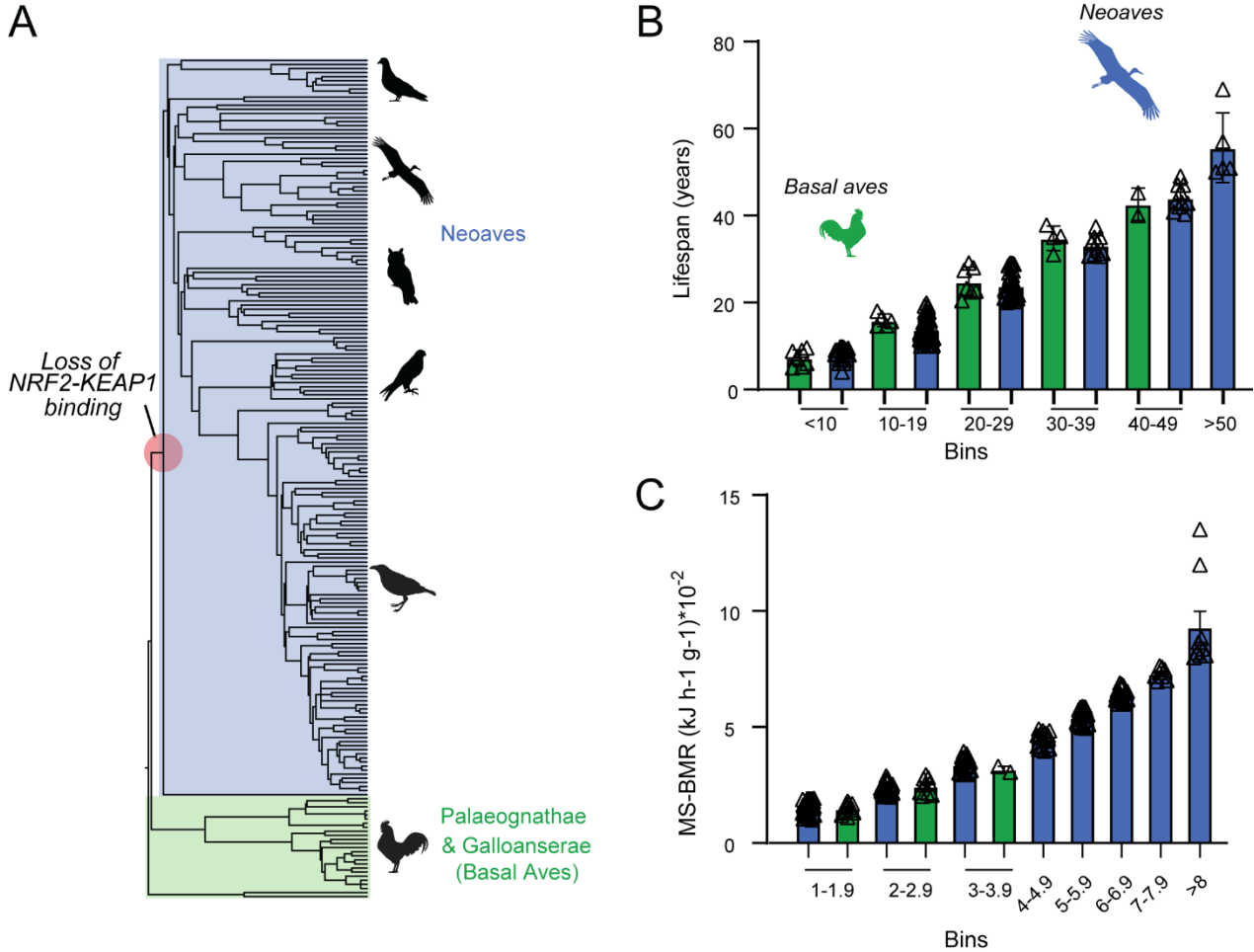
Species tree used in statistical analyses of *KEAPI* evolutionary rates, with branch lengths estimated by PAML.

Supplementary Figure 8.



Species tree used in statistical analyses of *GSTA2* evolutionary rates, with branch lengths estimated by PAML.

Supplementary Figure 9



(A) Phylogenetic relationships of avian taxa for which both MS-BMR and lifespan data were available (Methods). Red highlight denotes the Neoavian ancestor where KEAP1 binding of NRF2 was lost. Neoaves and basal Aves species stratified into (B) lifespan groups [Neoaves: $n=180$ biologically independent animals; Basal Aves: $n=25$ biologically independent animals] and (C) mass-specific basal metabolic rate (MS-BMR) groups [Neoaves: $n=175$ biologically independent animals; Basal Aves: $n=23$ biologically independent animals]. Within each group the mean lifespans and MS-BMR of basal Aves and Neoaves were statistically indistinguishable (Mann-Whitney; Supplementary Table 11). All error bars represent standard error. Source data are provided as a Source Data file. All data are presented as mean values.

Supplementary Table 1.

Non-avian genome accession numbers

scientific name	accession
<i>Drosophila melanogaster</i>	NP_788685.1
<i>Danio rerio</i>	XM_021476739
<i>Latimeria chalumnae</i>	XM_005994311
<i>Xenopus tropicalis</i>	NM_001008023
<i>Lipotes vexillifer</i>	XM_007460670
<i>Delphinapterus leucas</i>	XM_022557191.1
<i>Orcinus orca</i>	XM_012535762
<i>Tursiops truncatus</i>	XM_019933070
<i>Physeter catodon</i>	XM_024134256
<i>Balaenoptera acutorostrata scammoni</i>	XM_007168981
<i>Ovis aries</i>	XM_027969637
<i>Felis catus</i>	XM_023245303
<i>Ailuropoda melanoleuca</i>	XM_002921371
<i>Equus przewalskii</i>	XM_008537450.1
<i>Equus caballus</i>	XM_023645423
<i>Equus asinus</i>	XM_014865526
<i>Ceratotherium simum simum</i>	XM_004442630
<i>Pan paniscus</i>	XM_008961848
<i>Pan troglodytes</i>	XM_009434584
<i>Homo sapiens</i>	XM_011528452.1
<i>Gorilla gorilla gorilla</i>	XM_019015776.1
<i>Pongo abelii</i>	XM_009252776
<i>Microcebus murinus</i>	XM_012790647
<i>Carlito syrichta</i>	XM_008050920
<i>Mesocricetus auratus</i>	XM_021232069
<i>Cricetulus griseus</i>	XM_027411505
<i>Microtus ochrogaster</i>	XM_013346291
<i>Peromyscus maniculatus bairdii</i>	XM_006987015
<i>Rattus norvegicus</i>	XM_006242591
<i>Nannospalax galili</i>	XM_008850040
<i>Jaculus jaculus</i>	XM_004667452
<i>Meriones unguiculatus</i>	XM_021649200
<i>Castor canadensis</i>	XM_020174298
<i>Heterocephalus glaber</i>	XM_004865503
<i>Chinchilla lanigera</i>	XM_013505131
<i>Tupaia chinensis</i>	XM_027765540
<i>Python bivittatus</i>	XM_007426568
<i>Pogona vitticeps</i>	XM_020798692
<i>Anolis carolinensis</i>	XM_003216399
<i>Chrysemys picta bellii</i>	XM_008175416

<i>Alligator sinensis</i>	XM_006023442.3
<i>Ctenopharyngodon idella (Keap1b)</i>	KJ729125
<i>Cyprinus carpio (Keap1a)</i>	JX470752
<i>Danio rerio (Keap1a)</i>	NM_182864

Supplementary Table 2.

Analyses of selection on Mammalian KEAP1 using PAML random sites models.

Model	<i>lnL</i>	Parameters ¹			Null	<i>p</i> [df] ²
		ω_0/p	ω_1/q	ω_2/ω_p		
M0	-10727.53	0.01	-	-	N/A	-
M1a	-10699.67	0.01 (98%)	1.00 (2%)	-	M0	1 [1]
M2a	-10699.67	0.01 (98%)	1.00 (1%)	1.00 (1%)	M1a	1 [2]
M3	-10565.06	0.00 (68%)	0.03 (26%)	0.18 (6%)	M0	0.000 [4]
M7	-10569.21	0.10	4.24	-	N/A	-
M8	-10569.21	0.10	4.24	1.00	M7	1 [2]

¹ ω values of each site class are shown for model M0-M3 (ω_0 – ω_2) with the proportion of each site class in parentheses. For M7 and M8, the shape parameters, p and q, which describe the beta distribution are listed instead. In addition, the ω value for the positively selected site class (ω_p , with the proportion of sites in parentheses) is shown for M8.

²Significant *p*-values ($\alpha \leq 0.05$) are bolded. Degrees of freedom are given in square brackets after the *p*-values. Significance was determined through a likelihood-ratio test of null and alternative models, with reference to a χ^2 distribution.

Abbreviations—*lnL*, ln Likelihood; *p*, *p*-value; N/A, not applicable.

Supplementary Table 3.

Analyses of recombination (GARD HYPHY) in Avian KEAP1 coding-sequences.

Breakpoints	AICc	Delta AICc	Segments (human nucleotide numbering)	Significant topological incongruence breakpoints (LHS; RHS p-values)¹
0	53827.95	----	166-1896	----
1	50358.33	3469.62	166-1430; 1431-1896	----
2	48310.18	2048.15	166-630; 631-1411; 1412-1896	631 (0.0004;0.0004); 1412 (0.0004;0.0004)

¹ Shimodaira and Hasegawa (1999) test (SH test) as described in Ref 73.

Supplementary Table 4.

Accession numbers for sequences generated in this study

Definition	Accession
Gallus gallus NF-E2-related factor 2 (NRF2) mRNA complete cds	MN416129
Zonotrichia albicollis Kelch-like ECH-associated protein 1 (KEAP1) mRNA partial cds	MN416130
Zonotrichia albicollis NF-E2-related factor 2 (NRF2) mRNA complete cds	MN416131
Gallus gallus Kelch-like ECH-associated protein 1 (KEAP1) mRNA complete cds	MN416132
Zonotrichia albicollis Kelch-like ECH-associated protein 1 (KEAP1) gene, partial cds	MN416133

Supplementary Table 5.

Results of Clade Model D (CmD) analyses of Tetrapod *KEAPI* under various partitions.

Model & Foreground ¹	ΔAIC	$\ln L$	Parameters ³			Null	p [df] ⁴
			ω_0	ω_1	ω_2/ω_d		
M3	10.25	-11019.64	0.001(38%)	0.02(48%)	0.13 (14%)	N/A	-
CmD_ <i>Passerines</i>	7.44	-11017.24	0.002 (41%)	0.12 (16%)	0.01 (44%) Passerines:0 .04	M3	0.02 [1]
CmD_ <i>Birds vs. Mammals</i>	0.00*	-11012.51	0.001 (41%)	0.02 (46%)	0.25 (13%) Mammals: 0.08 Birds: 0.17	M3	0.000 [2]

¹The foreground partition is listed after the underscore for the clade models and consists of either: the clade of 'Passerines', 'Mammals', or the clade 'Birds'. In any partitioning scheme, the entire clade was tested, and all non-foreground data are present in the background partition.

²All ΔAIC values are calculated from the lowest AIC model. The best fits are bolded with an asterisk (*).

³ ω_d is the divergent site class, which has a separate value for the foreground and background partitions.

⁴Significant p -values ($\alpha \leq 0.05$) are bolded. Degrees of freedom are given in square brackets after the p -values. Significance was determined through a likelihood-ratio test of null and alternative models, with reference to a χ^2 distribution.

Abbreviations— $\ln L$, ln Likelihood; p , p -value; AIC , Akaike information criterion

Supplementary Table 6.

Avian specimens collected and analyzed in this study

Experiment	Common name (biological replicates)	Species	Collection date
qPCR	American Woodcock (n=2)	<i>Scolopax minor</i>	October 2018
	White-throated sparrow (n=3)	<i>Zonotrichia albicollis</i>	
ChIP, IB	Ovenbird (n=4)	<i>Seiurus aurocapilla</i>	May 2019
	Black-billed cuckoo (n=1)	<i>Coccyzus erythrophthalmus</i>	
ChIP	Common yellowthroat (n=2)	<i>Geothlypis trichas</i>	
ChIP, IF	Gray catbird (n=1)	<i>Dumetella carolinensis</i>	
ChIP, IB, IF qPCR	Chicken (n=5)	<i>Gallus gallus</i>	
			Dec 2018/May 2019

ChIP= chromatin immunoprecipitation; IB= immunoblot; qPCR= quantitative PCR; IF= immunofluorescence

Supplementary Table 7.

Antioxidant response elements (ARE) detected in avian genomes

Reference Organism	Genome (Chromosome accession)	Gene	ARE	Coordinates		
Chicken	Chicken GRCg6a (Chr 3 NC_006090.5)	GCLC	TGACACAGCT	88173981	88173990	
	Chicken GRCg6a (Chr 8 NC_006095.5)	PRDX1	TGAGAAAGCC	21508092	21508101	
	Chicken GRCg6a (Chr7 NC_006094.5)	NFE2L2	TGAGACAGCA	15809745	15809754	
			TGAGAAAGCG	15808215	15808224	
	Chicken GRCg6a (Chr25 NC_006112.4)	Feather keratin 1-like	TGAGTAAGCA	2178857	2178866	
			feather beta keratin-like	TGACAAATCT	2270450	2270459
				TGAGACATCT	2270135	2270144
			feather keratin 1-like	TGAGAAAGCA	3122498	3122507
			feather keratin 1-like	TGAGTCATCG	3125460	3125469
			Loricrin 3	TGACACAGCC	3224257	3224266
Chicken GRCg6a (Chr 3 NC_006090.5)	GSTA2	TGACAAAGCT	88412260	88412269		
Zebra finch	Taeniopygia_guttata 3.2.4 (Chr 3 NC_011466.1)	GCLC	TGACACAGCT	90519291	90519300	
	Taeniopygia_guttata 3.2.4 (GCF_000151805.1) NC_011471.1	NFE2L2	None	N/A	N/A	
Common Cuckoo	Cuculus canorus (GCF_000709325.1) NW_009243908.1	NFE2L2	none	N/A	N/A	
Manakin	Manacus vitellinus (GCF_001715985.3) NW_021939471.1	NFE2L2	None	N/A	N/A	
Common Canary	Serinus canaria SCA1 (GCF_000534875.1) NW_007931131.1	PRDX1	TGAGTCATCT	17942995	17943004	
Starling	Sturnus_vulgaris-1.0(GCF_001447265.1) NW_014650503.1		TGAGTCATCT	1002016	1002025	
Sparrow	GeoFor_1.0(GCF_000277835.1) NW_005054347.1		TGAGTCATCT	3368547	3368556	
Zebra finch	Zebrafinch 3.2.4 (Chr 25 NC_011489.1)	feather keratin 1-like	TGACAAATCA	789704	789714	

		feather keratin 4-like	TGACAAATCA	795758	795767
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Supplementary Table 8.

GSTA2 accession numbers (presence of ARE investigated)

Name	accession
<i>Apteryx australis</i>	XM_013953262
<i>Acanthisitta chloris</i>	XM_009084876
<i>Aquila chrysaetos</i>	XM_030037898
<i>Aptenodytes forsteri</i>	XM_009288570
<i>Apteryx rowi</i>	XM_026086143
<i>Corapipo altera</i>	XM_027641169
<i>Calypte anna</i>	XM_008495629
<i>Corvus brachyrhynchos</i>	XM_008637649
<i>Cyanistes caeruleus</i>	XM_023922506
<i>Antrostomus carolinensis</i>	XM_010170651
<i>Corvus cornix cornix</i>	XM_010406318
<i>Cariama cristata</i>	XM_009709554
<i>Coturnix japonica</i>	XM_015859291
<i>Columba livia</i>	XM_005506790
<i>Chlamydotis macqueenii</i>	XM_010116437
<i>Calidris pugnax</i>	XM_014948928
<i>Charadrius vociferus</i>	XM_009882144
<i>Egretta garzetta</i>	XM_009638768
<i>Eurypyga helias</i>	XM_010160429
<i>Empidonax traillii</i>	XM_027898226
<i>Ficedula albicollis</i>	XM_005044193
<i>Falco cherrug</i>	XM_027809110
<i>Fulmarus glacialis</i>	XM_009585672
<i>Falco peregrinus</i>	XM_027787880
<i>Gallus gallus</i>	XM_015284825
<i>Geospiza fortis</i>	XM_005426381
<i>Gavia stellata</i>	XM_009812395
<i>Haliaeetus albicilla</i>	XM_009922637
<i>Haliaeetus leucocephalus</i>	XM_010561073
<i>Lepidothrix coronata</i>	XM_017814538
<i>Leptosomus discolor</i>	XM_009956935.1
<i>Lonchura striata domestica</i>	XM_021551287
<i>Merops nubicus</i>	XM_008940286
<i>Melopsittacus undulatus</i>	XM_005153205
<i>Manacus vitellinus</i>	XM_018088908
<i>Neopelma chrysocephalum</i>	XM_027690338
<i>Numida meleagris</i>	XM_021389848
<i>Nipponia nippon</i>	XM_009461358
<i>Nestor notabilis</i>	XM_010022308
<i>Nothoprocta perdicaria</i>	XM_026034283
<i>Pygoscelis adeliae</i>	XM_009333197
<i>Phalacrocorax carbo</i>	XM_009508114

<i>Pelecanus crispus</i>	XM_009478080
<i>Pipra filicauda</i>	XM_027715482
<i>Pseudopodoces humilis</i>	XM_005521908
<i>Phaethon lepturus</i>	XM_010296442
<i>Parus major</i>	XM_015623193
<i>Picoides pubescens</i>	XM_009904173
<i>Struthio camelus australis</i>	XM_009677028
<i>Serinus canaria</i>	XM_018920082
<i>Sturnus vulgaris</i>	XM_014869515
<i>Tyto alba</i>	XM_009962792
<i>Zonotrichia albicollis</i>	XM_005495970

Supplementary Table 9.

Analyses of selection on Avian *GSTA2* using PAML random sites models.

Model	<i>lnL</i>	Parameters ¹			Null	<i>p</i> [df] ²	Δ AIC ³
		ω_0/p	ω_1/q	ω_2/ω_p			
M0	-6143.41	0.24	-	-	N/A	-	699.9
M1a	-5844.16	0.05 (79%)	1.00 (21%)	-	M0	0.000 [1]	103.38
M2a	-5802.86	0.05 (78%)	1.00 (19%)	3.87 (3%)	M1a	1 [2]	24.78
M3	-5796.21	0.03 (73%)	0.68 (24%)	3.51 (3%)	M0	0.000 [4]	13.49
M8a	-5827.47	0.27	2.58	1.00 (13%)	N/A	-	72
M7	-5835.17	0.14	0.49	-	N/A	-	85.4
M8	-5790.47	0.17	0.71	3.49 (2%)	M7	0.000 [2]	0*
					M8a	0.000 [1]	

¹ ω values of each site class are shown for model M0-M3 (ω_0 – ω_2) with the proportion of each site class in parentheses. For M7 and M8, the shape parameters, *p* and *q*, which describe the beta distribution are listed instead. In addition, the ω value for the positively selected site class (ω_p , with the proportion of sites in parentheses) is shown for M8.

²Significant *p*-values ($\alpha \leq 0.05$) are bolded. Degrees of freedom are given in square brackets after the *p*-values. Significance was determined through a likelihood-ratio test of null and alternative models, with reference to a χ^2 distribution.

³Model fits were assessed by Akaike information criterion differences to the best fitting model (bolded asterisk). Abbreviations—*lnL*, ln Likelihood; *p*, *p*-value; **N/A**, not applicable.

Supplementary Table 10.

Conventional and phylogenetically-independent non-parametric Kruskal-Wallis tests comparing mass-specific basal metabolic rate (MS-BMR) or lifespan of various groups using non-functional (0) vs functional (1) KEAP1-based categories

Group ¹	Category	Response Variables	N	Median	Mean Rank	Z-value	DF	H-value	Conventional P value	Phylogenetic P value*																										
<10 Lifespan	0	MS-BMR	7	0.021	4.7	-3.81	1	14.53	0.000	0.03																										
	1		27	0.061	20.8	3.81					10-19.9 Lifespan	0	5	0.017	14.0	-2.80	1	7.83	0.005	0.02	1	87	0.049	48.4	2.80	Neoaves >0.08, basal Aves 0.02-0.029, MS-BMR	0	Lifespan	9	10.1	8.1	-1.10	1	1.22	0.270	---
10-19.9 Lifespan	0		5	0.017	14.0	-2.80	1	7.83	0.005	0.02																										
	1		87	0.049	48.4	2.80					Neoaves >0.08, basal Aves 0.02-0.029, MS-BMR	0	Lifespan	9	10.1	8.1	-1.10	1	1.22	0.270	---	1	9	14.7	10.9	1.10										
Neoaves >0.08, basal Aves 0.02-0.029, MS-BMR	0	Lifespan	9	10.1	8.1	-1.10	1	1.22	0.270	---																										
	1		9	14.7	10.9	1.10																														

Degrees of freedom (DF)

¹Data was grouped according to either lifespan (years) or MS-BMR (kJ/h/g; see figures 5C-D, respectively).

*Only significant conventional P values (<0.05) were further investigated for phylogenetic significance. Phylogenetic significance was determined by empirical null distribution generated by phylogenetic simulations. Bold P values are significant.

Supplementary Table 11.

Conventional Mann-Whitney testing equivalency between basal Aves vs Neoaves MS-BMR and lifespan stratifications

Group¹	Sample	N	Median	W-value	P value
<10 Lifespan	Basal Aves	7	7.4	99.0	0.33
	Neoaves	27	8.6		
10-19 Lifespan	Basal Aves	5	15.7	344.0	0.06
	Neoaves	87	13.1		
20-29 Lifespan	Basal Aves	7	23.2	199.5	0.42
	Neoaves	41	22.9		
30-39 Lifespan	Basal Aves	4	35.1	42.0	0.21
	Neoaves	11	33.7		
40-49 Lifespan	Basal Aves	2	42.6	9.0	0.45
	Neoaves	10	43.2		
0.01-0.019 MS-BMR	Basal Aves	12	0.015	256	0.44
	Neoaves	35	0.016		
0.02-0.029 MS-BMR	Basal Aves	9	0.025	203	0.453
	Neoaves	30	0.023		
0.03-0.039 MS-BMR	Basal Aves	2	0.032	18	0.55
	Neoaves	21	0.034		

¹Data was grouped according to either lifespan (years) or MS-BMR (kJ/h/g ; see figures S9B-C, respectively).

Supplementary Table 12.

Primer sequences used in this study

	Template Species	Gene	Forward	Reverse	
Avian NRF2 KEAP1 cloning	Chicken	KEAP1	ATGTACGGCCCCGAATGCCA	ACAGGGGCACTCGTGGCG	
		NRF2	ATGAACTTGATTGACATCCTT TGGAGGC	CAGTTTAGTCTCTGCCTTTCT GCTTTTA	
	White-throated sparrow	NRF2	ATGAACCTGATTGACATCCTT TGGAGG	AAGTTTAGTCCCCGACTGCC TG	
		KEAP1	CCAGCCCCGTCTTCAGGGCCA TGTTACAGCGAGGCTGCG	CCCGGTTTGCACGGTCCAT GGTGACGGCCACGCTCCAG	
ChIP qPCR	Chicken	GCLC	CTCATGTGGTAACGCCACCAA AG	TGTGTCACAAGGGAAGAGTT CTGC	
		PRDX1	TCGGCATTACCCGGGCTTTA	CTTTCTCATGGCGCAGTGCTT A	
			CATCTGACTCATCCTGCCGTG A	CTCAGCGCTTGGATGGTGG	
		NFE2L2	GTAGTGGTAGTGGCACAGGT ATGAAA	GCCACAAGACAGCAACCTGT GA	
			GGGACAATTTGGCCCCTGGA AAT	CTACCTCAGCAATGCAGATC CCTTT	
		Feather keratin 1-like	GTGCCATGACTGGAAAGGTG ATTTT	CAGGCAAGGAGAGCCCACA TTAA	
		feather beta keratin-like	CTCGTGTCTGAGCTGACAAAT CTGG	GAGAGTGAGGAGTGATGTGC AACA	
			GCACCCAAGTGGATGAGACA TCTC	CTCCTCCTTGGCAAAGACTC CTT	
		feather keratin 1-like	CCTTAGCAGGAAGTCTGAGA AAGCA	TCAGATCCCCATGCTGAGGG TATA	
		feather keratin 1-like	CAACAGTCATGAGTCATCGTG AGCT	AAGTCACCTTCCCTCCTCTG GTT	
	Loricrin 3	CATTGCTTTGACACAGCCACT GT	AGCAGCACCGCTCACACT		
	Neoaves species	GCLC	CTCACTGCCCAAAGCTAGA AAG	TGATAGCCAACAGCTGTGTC AG	
		PRDX1	CATCTGGTCCCAAATGAGTC ATCTCT	GTGTAGTGTTGCTGGGTGAC CATAAC	
			TGAGTCATCTCTGGGTGAACC		
		feather keratin 1-like	GGAGGGCCAGATGGTCAGT	GCAGGACGTGCCCAGTCA	
		feather keratin 4-like	GGAACAGGAGGGGTTTAGAG GTT	AATTTGAGGCACAGCTGGAA GAAC	
	HEK Cell qPCR	Human	GCLM	TGTGTGATGCCACCAGATTT	TTCACAATGACCGAATACCG
			GCLC	ACCATCATCAATGGGAAGGA	GCGATAAACTCCCTCATCCA
			HO1	AACTTTCAGAAGGGCCAGGT	GTAGACAGGGGCGAAGACT G
			GSTM1	TTCCAATCTGCCCTACTTG	TCTCCAAAATGTCCACACGA
NQO1			CAGCTCACCGAGAGCCTAGT	ACCACCTCCCATCCTTTCTT	
Avian qPCR	Chicken and	NRF2	GCAGAACATAGAGCAAGTTT GGGAAG	GTGCTCACCTCAGCCAGGTT	

	Neoaves species	GAPDH	GGAGGGTCTCATGACCACTGTC	CAGGAGACAACCTGGTCCTCT
		GCLC	CTAGAGCCACTGAAGAACAAC	GTGTGCAATATGCTGTGCCAA
		GCLM	CARDAAGATTGTTGCCATAG	CATCACRCAACAKGAAGCTAG
		PRDX1	TGCATACAGRGGTCTSTTCA	CTTCCAGCCAGCTGGGCACA
		TXNRD1	GRTTTCMCATTGCCACTGGTG	TARGAAGCTCCAACCACCAG
		PTGR1	GGAGAGTTGCTGCTTGAATCA	GCAACCTGCGTGCCAATCAT
			GTGTTTC	TA
	GAGAGCTGCTGCTTGAATCA	GCAACCTGCGTGCCTATCAT		
	GTGTTT	TATG		
T7EI assay	Human	KEAP1	GGCTAATTGCGTAGGGCTTTG	TCAATGAGGCGCTCCATGAC
		TNXB	CCACTCTTCCTAGTGCCTCAG	CCAAGTCCCGCTCACAGGAT
		LOC105372 218	TGCTGGAGGGAGAGGCAC	ACAGCGTGCACAGACCCA

Supplementary Table 13.

crRNA (Hs.Cas9.KEAP1.1.AD) used in CRISPR-Cas9 genome editing of *KEAP1* in HEK293T cells (information provided by Integrated DNA Technologies)

Position	Strand	Sequence	PAM	On-Target Score	Off-Target Score (Genes and loci)
10499768	-	GGTCAAGTACCAGGATGCAC	CGG	98	71 (TNXB chr6:-32081514;LOC105372218 chr18:-77983723)