Genomic Analyses Reveal Genetic Adaptations to Tropical Climates in Chickens

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HIGHLIGHTS
Gene flow was detected from Tibetan chickens to Sri Lankan and Saudi Arabian chickens

Twelve genes showed chicken adaptations to both tropical desert and monsoon climates

Advantageous alleles of TLR7 and ZC3HAV1 showed immune adaptation in chickens

Convergent adaptation to tropical desert climate was found between birds and mammals
Genomic Analyses Reveal Genetic Adaptations to Tropical Climates in Chickens

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SUMMARY
The genetic footprints of adaptations to naturally occurring tropical stress along with domestication are poorly reported in chickens. Here, by conducting population genomic analyses of 67 chickens inhabiting distinct climates, we found signals of gene flow from Tibetan chickens to Sri Lankan and Saudi Arabian breeds and identified 12 positively selected genes that are likely involved in genetic adaptations to both tropical desert and tropical monsoon island climates. Notably, in tropical desert climate, advantageous alleles of TLR7 and ZC3HAV1, which could inhibit replication of viruses in cells, suggest immune adaptation to the defense against zoonotic diseases in chickens. Furthermore, comparative genomic analysis showed that four genes (OC90, PLA2G12B, GPR17 and TNFRSF11A) involved in arachidonic acid metabolism have undergone convergent adaptation to tropical desert climate between birds and mammals. Our study offers insights into the genetic mechanisms of adaptations to tropical climates in birds and other animals and provides practical value for breeding design and medical research on avian viruses.

INTRODUCTION
Chickens were domesticated from red jungle fowl (RJF) at least 4,000–4,500 years ago and have multiple origins within the geographic range of their wild ancestors in South and Southeast Asia (Fumihito et al., 1996; Larson and Fuller, 2014; Liu et al., 2006). Domestication of chickens is closely related to human activities. For instance, the complex and ancient interactions resulting from the Silk Route have facilitated maritime and terrestrial intercontinental translocation of several domestic chicken breeds among the Middle East and South, Southeast, and East Asia (Boivin and Fuller, 2009; Boulos, 2004; Fuller and Boivin, 2009). After a long period of artificial and natural selection by humans, chicken breeds have evolved genetic adaptations to their local environmental conditions, such as hot and arid climates and high-altitude environments (Lawal et al., 2018; Wang et al., 2015). It is well known that the detection of genomic differences can shed light on the genetic basis of adaptation to diverse environments and provide insights into functionally important genetic variants (Andersson and Georges, 2004). The completion of the chicken genome sequence and genetic variation maps has promoted the exploration and research of chicken genetic mechanisms using whole-genome-based strategies (Hillier et al., 2014; Johnsson et al., 2012; Li et al., 2017a; Qanbari et al., 2019; Rubin et al., 2010; Wong et al., 2004). Conversely, considering their short reproductive and growth periods and wide distribution, chickens can be used as ideal models to study genetic adaptations to environments.

Environmental pressure is an important driver shaping the animal genome, which is also affected by pathogens (Fumagalli et al., 2011), disease (Saberi et al., 2007), diet (Jiao et al., 2019), altitude (Huerta-Sanchez et al., 2013), and climate (Ai et al., 2015; Frichot et al., 2013; Lv et al., 2014). High altitudes are among the most challenging extreme environments, and high-altitude adaption has been widely studied in humans (Simonson et al., 2010), Tibetan chickens (TCs) (Wang et al., 2015; Zhang et al., 2016), yaks (Qiu et al., 2012), pigs (Li et al., 2013), ground tit (Qu et al., 2013), and sheep (Yang et al., 2016). Recently, genomic studies have also reported the genomic responses to acclimation to hot and arid environments in camels (Jirimutu et al., 2012; Wu et al., 2014), sheep (Yang et al., 2016), and goats (Kim et al., 2016). Although the acute heat response has been studied in commercial chickens (Lara and Rostagno, 2013), to the best of our knowledge, genetic adaptations to naturally occurring hot and arid environments in domestic chickens...
remain poorly known. In view of the current and future global effects of climate change, the genetic basis of adaptation to hot or arid climates in chickens is of practical value for breeding design and medical research (Diamond, 2002).

In this study, we performed a large-scale genomic analysis of chicken populations, including 36 high-altitude TCs from our work (Li et al., 2017a), 5 RJFs (Wang et al., 2015), 10 TCs (Wang et al., 2015), 11 Sri Lankan indigenous chickens (SL) (Lawal et al., 2018), and 5 Saudi Arabian indigenous chickens (SA) (Lawal et al., 2018), from habitats in harsh environments (high-altitude, tropical desert, and tropical monsoon island climates) (Figure 1A and Table S1). Genome-wide analysis for selective sweeps was performed to elucidate the genetic mechanisms of cutaneous melanin formation (thermotolerance), hormonal regulation, angiogenesis, vasodilation, and mitochondrial respiration, which may work together to enable chickens to adapt to tropical desert and tropic monsoon island climates. We also revealed the immune adaptation to the defense against zoonotic diseases in chickens. Additionally, we revealed convergent adaptation to tropical climates.
desert climate between birds and mammals. Our study adds to knowledge on genetic mechanisms of adaptations to tropical climates in vertebrates and is of fundamental importance for the potential application in functional genomics, breeding design, and the development of medical models for avian viruses.

RESULTS AND DISCUSSION
Characterization of Genomic Variants
After performing strict data quality filtering, a total of 1.56 Tb (terabases) of high-quality genome sequences from 67 chickens were mapped to the chicken reference genome (GRCg6a, GCF_000002315.6), resulting in an average of 94.74% non-gap genome coverage and 20.25-fold average depth (Table S2).

We identified 12.78 million biallelic high-quality population genomic variants (PGVs) that contained 10.69 million SNPs and 2.09 million InDels with lengths ranging from 1 to 306 bp (Tables S3 and S4). Overall, more than half of the PGVs (52.63% and 52.65% for total SNPs and InDels, respectively) were located in intronic regions, followed by intergenic regions, with 39.28% and 40.00% for SNPs and InDels, respectively. The densities of the PGVs also showed a higher ratio in the promoter region than in the coding region (Figures S2 and S3). We annotated 180,729 SNPs (1.67% of the total) and 10,901 InDels (0.52% of the total) located within 15,370 protein-coding genes (89.31% of the total 17,209 genes). We identified a total of 55,480 missense, 6,798 frameshift, 629 stop-gain, and 92 stop-loss PGVs that caused amino acid changes, transcript elongation, or premature stopping, leading to structural changes in 11,407 genes (66.29% of total genes). We also observed an enrichment of \( \leq 30 \text{ bp} \) in coding sequences (95.13% of the total) that are multiples of 3 bp, which is expected to preserve the open reading frame (Figure S3).

The ratio of nonsynonymous to synonymous changes was estimated to be lower in chickens (0.45) than in other agricultural economic animals (Table 1), such as pigs (0.68) (Li et al., 2013), sheep (0.66) (Yang et al., 2016), and cattle (1.21) (Daetwyler et al., 2014), possibly indicating a weaker artificial selection accumulation effect in chickens than in other animals due to the short domestication time (Larson and Fuller, 2014). We also identified a greater proportion of unique PGVs (13.73% and 1.84% for SNPs and InDels, respectively) in RJF than in other chicken groups (Table 1), which is consistent with the loss of genetic diversity during domestication (Larson and Fuller, 2014).

Phylogeny and Introgression in Chickens
On the basis of the maximum likelihood tree and principal-components analysis (PCA), there were five distinct major clusters (i.e., TC1, TC2, SL, SA, and RJF) (Figures 1B and 1C). We further used an admixture model (FRAPPE) to explore the level of shared ancestry among individuals within each breed (Tang et al., 2005). As the ancestry component (K value) increased, we observed a simultaneous increase in the composition of ancestors in the RJF (Figure S4). This result is expected because the genetic materials of other
chicken breeds were from components of RJF ancestry (Figure 1C). The subcluster ancestry composition showed considerable genetic difference between the TC1 and TC2 clusters, which is consistent with a previous report showing that multiple origins of TCs can be traced to two independent sources (Wang et al., 2015). We also observed that some SL individuals always maintained a single ancestry component and others showed a simultaneous increase in the composition of ancestors with increasing K value. We speculated that this single ancestor might be the result of a continent-island genetic model in Sri Lanka. We further identified four admixture category clusters (TC1_Sub1, TC1_Sub2, TC2_Sub1 and TC2_Sub2) on the basis of the two distinct TC clusters that were previously observed (Wang et al., 2015), according to phylogenetic analysis (Figure 1B). We found that the TC2_Sub2 cluster had more shared ancestry components with the SA and SL breeds than the other clusters (Figure 1E). Based on TreeMix (Pickrell and Pritchard, 2012), we observed signals of genetic flow from TCs to SA and SL breeds (Figures 1D and S5). Subsequently, the D-statistic (Durand et al., 2011; Green et al., 2010; Patterson et al., 2012) also indicated that the SA and SL breeds have a significant level of introgression with TC2_Sub2 chickens (Figure S6, Tables S6 and S7). Previous studies showed that Southwest China was one of the origin regions of chickens (Liu et al., 2006; Wang et al., 2020) and that trade activities have facilitated maritime and terrestrial intercontinental translocation of several domestic chicken breeds among the Middle East and South, Southeast, and East Asia (Figure S7) (Boivin and Fuller, 2009; Boulnois, 2004; Fuller and Boivin, 2009). Our admixture analysis supported the hypothesis that Chinese indigenous chicken breeds may have been transported to Sri Lanka and Saudi Arabia via the land and maritime Silk Route voyages (Fuller and Boivin, 2009).

**Genomic Sweep Footprints in Chickens**

The SA indigenous breed is well adapted to hot and arid environments because those breeding centers are located in the Eastern Province of Saudi Arabia and belong to a tropical desert climate zone with high solar radiation, very large diurnal ambient temperature differences, high daytime temperatures (ranging from 21.2°C to 50.8°C), frequent floating dust weather, and extremely low rainfall (74 mm per year) (Figures 1A and Table S1). The SL indigenous breed is from the Puttalam district of Sri Lanka, which is a tropical monsoon island country with an average annual rainfall of ~1,000 mm and a temperature of 27°C, whereas TCs live in a temperate humid plateau climate in China. To identify genomic sweep footprints in chickens, we applied a modified population-branch statistic (PBS) method, which was very powerful in detecting incomplete selective sweeps over short divergence times (Simonson et al., 2010; Zhan et al., 2014). This approach was designed to take advantage of three outgroups (TC2_Sub2, TC2_Sub1 and RJFs), aiming to identify genomic regions under selection in SA/SL breeds. We identified 24.18 Mb (2.30% of the genome) and 15.58 Mb (1.48% of the genome), encompassing 723 and 464 positively selected genes (PSGs), in SA and SL breeds, respectively. Next, we employed three methods: log2 (θπ ratio) (Nei and Li, 1979), cross-population extended haplotype homozygosity (XP-EHH) (Sabeti et al., 2007), and cross-population composite likelihood ratio (XP-CLR) (Chen et al., 2010), although these methods could not remove noise signals from domestication if compared with RJFs (the ancestral population of domestic chickens). Ideally, for our purpose of detecting tropical adaptation, the best control group would be those breeds with a similar level of domestication and a distinct level of environmental temperature compared with study groups. We selected TC2_Sub2 as the control group, because our analyses of phylogeny and introgression showed that there is significant genetic mixing between SA/SL breeds and TC2_Sub2 cluster (Figures 1B and 1D). Thus, using the three methods, we performed a genome-wide selection sweep scanning with two comparisons, i.e., SA and SL breeds were compared with TC2_Sub2 cluster, respectively. Specifically, 376 and 396 PSGs were identified by XP-CLR, 233 and 305 from log2 (θπ ratio), and 383 and 267 from XP-EHH in SA and SL breeds, respectively (Figure 2). Combined with the PBS method, only 87 and 45 PSGs were detected by all four methods in SA and SL breeds, respectively (Figures S8 and S9). The low number of overlapped PSGs could have resulted from different signatures of population variations in the four methods (Akey, 2009; Sabeti et al., 2006; Wang et al., 2016). Finally, we obtained 942 PSGs in SA breed and 923 PSGs in SL breed after integrating all candidate selection regions from the four methods, and 150 of them were found in both SA and SL breeds (Figure S10).

**Genetic Adaptations to Tropical Climates in Chickens**

Among the PSGs in the SA and SL breeds, we found significant enrichment of functional pathways (p < 0.05) that directly or indirectly influence several traits that are critical for survival under heat stress in tropical climates, e.g., 18 PSGs involved in the vascular smooth muscle contraction pathway (VSMC, 12 PSGs in SA breed and 9 PSGs in SL breed), 19 PSGs located in the melanogenesis pathway (13 in SA and 9 in SL), 27 PSGs located in the adrenergic signaling in cardiomyocytes pathway (12 in SA and 18 in SL), and 26...
PSGs involved in the calcium signaling pathway (13 in SA and 17 in SL) (Tables S8 and S10). We found five PSGs (ADCY1, ADCY2, ADCY7, ADCY8, and ADCY9) associated with adenylyl cyclase (AC), affecting the formation of cAMP; these genes also play roles in the above four pathways (Figure 3). Adrenergic signaling in cardiomyocytes plays a pivotal role in regulating cardiac function in response to ever-changing environments. Systolic and diastolic function and heart rate are primarily controlled by the adrenergic and muscarinic systems in response to varying physiological demands (Schaub et al., 2006). The calcium signaling pathway acts as an intracellular messenger for signal transduction that is primarily modulated by adrenergic control of phosphorylation involved in intracellular Ca\textsuperscript{2+} handling (Schaub et al., 2006). We identified five PSGs (CACNA1B, CACNA1C, CACNA1G, CACNA1I, and CACNA1S) that are critical for calcium channel function, especially CACNA1C, which encodes the L-type voltage-gated calcium channel Cav1.2 and is under positive selection in both SA and SL breeds. This protein activates the VSMC pathway (Figure 3). The CAMK2D gene is under selection in chickens living in different tropical climates. It encodes calmodulin-dependent protein kinase-II (CaMKII), which is activated in response to adrenergic stimulation (Grimm and Brown, 2010), affects a variety of Ca\textsuperscript{2+}-handling proteins (Schaub et al., 2006) and regulates melanin synthesis (Figure 3). In addition, we found that 68 PSGs are involved in central nervous system developmental processes, such as regulation of nervous system development, neurogenesis, neuron remodeling, cholinergic synapse, synaptic transmission regulation, cerebellar development, and axonal transport (Tables S9 and S11). The central nervous system of avian organisms exhibits high thermosensitivity in the control of thermoregulatory responses (Boulant and Dean, 1986). A deficit of thermal stimulation causes alterations, retardation, and deleterious effects in the central nervous system (Ahmed, 2005). Therefore, PSGs involved in these relevant functional ontologies suggested that nerve development, cutaneous melanin formation, hormonal regulation, angiogenesis, and vasodilation may have worked together to enable chickens to adapt to heat stress in tropical climates, reflecting common and local adaptations.

**PSGs Associated with Thermotolerance**

The cutaneous melanin pigment plays a critical role in protecting animals against the harmful effects of solar radiation, and Wnt signaling plays a crucial role in orchestrating epidermal stratification. Of the 19 PSGs located in the melanogenesis pathway, three PSGs (WNT7B, WNT8B, and GNAO1) in SA breed and two (WNT7B and GSK3β) in SL breed are involved in Wnt signaling, which induces epidermal stratification and regeneration.
Figure 3. Genetic Adaptations of Chickens to Tropical Climates

Dotted arrows indicate an indirect effect. Dotted lines indicate indirect inhibition. Solid lines indicate association. The PSGs simultaneously present in the SA and SL breeds are shown in red. The PSGs in SA breed are shown in brown. The PSGs in SL breed are shown in green. The names of the KEGG pathways are shown in purple.

(Zhu et al., 2014); three PSGs (KIT, RAF1, and MAP2K1) located only in SA breed are involved in the MAPK signaling pathway, which is an upstream regulator of melanogenesis and melanoma angiogenesis (Figure 3). In particular, the interaction of KIT with MITF has been reported to confer resistance to melanosis and the ability to cope with solar radiation (Satzger et al., 2008). One PSG (UG3) in SA breed was involved in "negative regulation of mitochondrial DNA replication" and "base-excision repair, DNA ligation" (Table S9); two PSGs (SIRT1 and MCT1) of SL were significantly enriched in "UV-damage excision repair," of which SIRT1 was selected in SA breed based on the XP-EHH value (Table S11). We found significantly higher (p < 10^{-16}, Mann–Whitney U test) selective pressure in the SA breed than in the SL breed for SIRT1, indicating that the genome characteristics were sensitive to solar radiation (Figure S11).

PSGs Associated with the Circulatory and Respiratory Systems

The living climates of the SA and SL breeds are characterized by very large diurnal ambient temperature differences and high daytime temperatures caused by intense solar radiation (Table S1). Indigenous chickens use a variety of solutions to maintain core body temperature and internal homeostasis, such as convective and evaporative heat loss through diastolic blood vessels (Lara and Rostagno, 2013; Mutaf et al., 2009). Therefore, the balance between vascular contraction and relaxation plays a pivotal role in dissipating excessive heat through regulation and maintenance of blood pressure to maintain core body temperature and internal homeostasis in chickens. Eighteen PSGs involved in the VSMC pathway encode seven vasoconstrictors, eight vasodilators, two voltage-dependent calcium channel L-type alpha proteins, and one calcium-activated potassium channel subunit alpha protein. Notably, three genes (PRKCH, ADCY1, and CACNA1C) were simultaneously selected by SA and SL breeds and separately activated vasoconstrictors, vasodilators, and voltage-dependent calcium channels (Figure 4A). Furthermore, we performed quantitative real-time PCR (qRT-PCR) to investigate changes in gene expression level between heat stress and normal temperature using the C2C12 cell line. We found significantly higher expression levels of CACNA1C under heat treatments (Figure 4B). Subsequently, we analyzed the population genotype frequency in a large dataset of 845 Gallus gallus accessions (Wang et al., 2020). We first divided 845 accessions into four groups, of which 109 domesticated chickens live in tropical climates (TCs) (Table S12), 112 are TCs, 475 are other domesticated chickens (non-tropical chickens, or NTRCs), and 149 are RJFs. Based on the recently released population SNP data of chickens (http://bigd.big.ac.cn/chickensd/), we screened out 50,582 SNPs within CACNA1C gene and up-/downstream 500-kb regions based on 845 accessions. We discovered directional increased or reduced genotype frequency through a priority order with TRCs → NTRCs → TCs, as demonstrated by a linear regression model (Transparent Methods); especially 147 mutations (29.05%) within top 1% outlier of absolute K value belonged to the region of CACNA1C gene (Figure 4C). Indeed, 11 PSGs in SA breed and 14 PSGs in SL breed were significantly enriched (p < 0.05) in GO terms “regulation of blood circulation” (Table S9). More specifically, the significantly enriched GO terms...
Figure 4. Genomic Regions with Strong Selective Sweep Signals in SA/SL Breeds

(A) Example of genes with strong selective signals in SA and SL breeds. Genomic regions located above the upper horizontal red, green, and yellow dashed lines and under the horizontal blue dashed line were termed regions with strong selective sweep signals (gray regions). Genome annotations are shown in the middle (gray bar, coding sequence; purple bar, gene body). The boundary of CACNA1C is marked in red.

(B) Quantitative real-time PCR (qRT-PCR) for the CACNA1C gene in C2C12 cells under 42°C for heat stress (HS) and 37°C for the normal control (NC) groups (n = 3 replicates per group; two-sided Student’s t test (***: p < 0.001, **: 0.001 < p < 0.01, ns: non-significant); data are represented as mean ± SEM).

(C) Genotype frequency analysis detects selection signals upstream and downstream 500 Kb around CACNA1C gene based on 845 chickens. Below left showed directional reduced frequency of 52 mutations and below right showed directional reduced frequency of 95 mutations across five chicken populations in CACNA1C gene.

(D) Selection signals in the SA breed for the VPS13C gene. The upper half is similar to (A).

(E) qRT-PCR for the VPS13C gene in C2C12 cells under HS and NC groups (n = 3 replicates per group; two-sided Student’s t test (***: p < 0.001, **: 0.001 < p < 0.01, *: 0.01 < p < 0.05, ns: non-significant); data are represented as mean ± SEM).
were “regulation of systemic arterial blood pressure by hormone” (RASL10B, DRD3 and RPS6KA2) and “blood vessel maturation” (MMP2 and ANKRD17) in SA breed, whereas GO terms included “regulation of blood vessel diameter” (ADRA2C, FGG, FGA, SMNL2, DOCKS, GRIP2, UTSS2R, and SCPEP1) in SL breed.

Heat stress causes excessive oxygen demand in animals (Portner, 2001), leading to increased blood flow in circulation and optimized oxygen delivery. Consistent with this phenomenon in SA breed, we discovered that three PSGs (EPAS1, CREBBP, and HIF1AN) are significantly involved in the “cellular response to hypoxia,” whereas five PSGs (SIRT1, PPARD, HP1BP3, SDHD, and HIF1AN) were involved in the “cellular response to decreased oxygen levels” (Table S9). Among those genes, EPAS1 was detected by PBS (PBS value = 0.096; p = 0.007), and was known as hypoxia-inducible factor 2α (HIF-2α), and HIF1AN was involved in transcriptional repression through interaction with HIF1α, VHL, and histone deacetylases. Also, a unique set of regulatory targets was acted by each of HIF-1α and EPAS1 (Hu et al., 2003). It has been suggested that there is a close relationship between EPAS1 and the regulation of red blood cell production (Percy et al., 2008; Yi et al., 2010). Indeed, we found one PSG (KIT) that is involved in “cellular response to erythropoietin” (Table S9). One SNP of EPAS1 with the greatest genotype frequency difference between SA and TCs is located in the sixth intron, with a derived allele at 0% frequency in TCs and 70% in the SA breed, as well as ≤10% in SL breed and RJFs (Table S13). This is consistent with a previous finding that the intron 5 of EPAS1 harbored a highly differentiated SNP associated with erythrocyte abundance to positively influence adaptation to hypoxia (Yi et al., 2010). Similarly, a previous study also found that a non-synonymous SNP in the exon 7 of EPAS1 may contribute to the hypoxia adaptation of TCs (Li et al., 2017b).

To test whether positive selection has acted on different regions of EPAS1 for coping with heat stress and high altitude, we analyzed genotype frequency of EPAS1 from 845 G. gallus accessions (Wang et al., 2020). In addition to one SNP in the sixth intron of EPAS1 identified in our dataset, two more SNPs located in the sixth intron with a significant frequency preference were found in tropical chickens using the large dataset of 845 accessions (Table S14). By contrast, the corresponding genotypes in TCs and RJFs were fixed, indicating that this trait was retained before the ancient divergence of chickens. However, after comparing these genotypes in 109 TRCs and 475 NTRCs with the background of RJFs and all 845 chickens, we observed significant deviations of p values using chi-square tests, indicating that the two SNPs in tropical chickens are not random and lineage-specific positive selection should have favored the tropical chickens (Table S15). Indeed, our PBS method was considered to be robust for identifying recent natural selection. Therefore, although the regulation mechanism of EPAS1 by the intronic mutations remains to be discovered, these findings supported that the naturally occurring tropical stress may have led to the alleles targeted by natural selection, which may confer a functionally relevant adaptation to excessive oxygen demand. This result also indicated that different mutations of same genes may have occurred to meet similar physiological needs to cope with different environment stresses.

Additionally, chickens can expel internal heat by evaporation of moisture by increasing the frequency of panting (Collier and Gebremedhin, 2015). Correspondingly, we found that one PSG (EDA) was involved in “trachea submucosa development” in SA breed (Table S9). These functions play an important role in the regeneration and repair of airway epithelial cells (Tata et al., 2013). Therefore, the synergistic action of these biological functions may have enabled chickens to cope with heat stress in the heart, a trachea burden, and vascular pressure.

**PSGs Associated with Mitochondrial Respiration**

We also found that 15 PSGs and 5 PSGs are associated with the cellular component term “mitochondrial membrane” in SA and SL breeds, respectively (Tables S9 and S11). Under heat stress, oxygen levels in chicken body fluids may decrease, reflecting excessive oxygen demand (Portner, 2001). The adjustment of mitochondrial densities in addition to molecular or membrane adjustments appears crucial for maintaining aerobic scope and for shifting thermal tolerance. Of the eight PSGs (NDUF53, NDUF44, NDUF65, NDUF99, SDHD, VPS13C, PARK2, and PACRG) involved in the response to the mitochondrial respirasome based on the annotations in the PANTHER and UniProt databases, four (SDHD, VPS13C, PARK2, and PACRG) were under selection in both SA and SL breeds. VPS13C and PARK2 play a role in mitochondrial maintenance, such as in the regulation of...
mitochondrial respiration rates, suggesting compensatory adaptation aimed at preserving mitochondrial transmembrane potential levels (Lesage et al., 2016; Mortiboys et al., 2008). In our study, SDHD was involved in complex II of the mitochondrial electron transport chain, and this gene is responsible for transferring electrons from succinate to ubiquinone (Tables S9 and S11). We hypothesize that VPS13C regulates parkin-mediated stimulation of mitophagy in response to mitochondrial depolarization, thereby inhibiting the generation of reactive oxygen species and reducing irreversible mitochondrial damage (Figure 4D) (Youle and van der Bliek, 2012). The higher expression level of VPS13C in the C2C12 cell line under heat treatment may also support this hypothesis (Figure 4E). In addition, genotype frequency analysis of 845 accessions showed that 59 mutations (16.95%) within top 1% outlier of absolute K value are located within the region of VPS13C gene (Figure 4F; Transparent Methods) (Wang et al., 2020).

**PSGs Associated with Defense against Zoonotic Diseases**

Avian influenza and salmonellosis are caused by pathogens capable of infecting humans and animals and cause significant morbidity and mortality worldwide. Chickens are an important medical model for studying low-pathogenicity avian influenza viruses due to partial immunity owing to previous exposure (Seo et al., 2002). The Saudi Arabian climate, as a high-temperature climate, is conducive to the propagation of pathogenic bacteria in chicken coops, resulting in poultry that are vulnerable to infection. Cytokines are key players in the regulation of the immune response, particularly during infection, joint inflammation, and endocrinological autoimmune diseases (Evans, 1993). Among 942 PSGs of SA breed, we found that 60 were significantly (< 0.05) involved in several functional categories related to infection and defense against zoonotic diseases, including the influenza A pathway (14 PSGs), the Salmonella infection pathway (11 PSGs), cytokine–cytokine receptor interaction (13 PSGs), immune system development (26 PSGs), and immune response (25 PSGs) (Tables S8 and S9). These functional terms indicate that the thermal climate can indirectly affect autoimmune regulation in animals through its effects on the environments that chickens inhabit.

Of these PSGs, TLR7 is a key component of innate and adaptive immunity and is involved in RNA virus recognition, especially in the recognition of highly pathogenic avian influenza viruses. Small antiviral compounds activate immune cells via the TLR7-MyD88-dependent signaling pathway. TLR7 may activate IRF7 through activation of MyD88, BTK, and TRAF6, thus inducing antiviral responses via the production of IFN-α. IFNLR2 interacts with IFNLR1 to form a receptor for the cytokine IFN-γ (Soh et al., 1994). A previous study reported that loxoribine induces antiviral gene expression, such as the expression of type I IFNs (IFN-α and IFN-β) and IFN-γ in primary chicken splenocytes, and can inhibit influenza A replication in vitro and in vivo in a dose-dependent manner (Stewart et al., 2012). The PSG IL12B is a cytokine that can act as a growth factor for T and NK cell activation, which can enhance the lytic activity of natural killer (NK)/lymphokine-activated killer cells, and stimulate the production of IFN-γ by resting peripheral blood mononuclear cells (Oppmann et al., 2000). Another PSG IL18 is a proinflammatory cytokine primarily involved in polarized T-helper 1 cell and NK cell immune responses (Tominaga et al., 2000). Another study found that members of the Toll-like receptor (TLR) family are critical for the recognition and clearance of Salmonella (Talbot et al., 2009; Vazquez-Torres et al., 2004). One consequence of Salmonella-induced TLR activation is the production of inflammatory cytokines and antimicrobial compounds, including pro–IL-1β, pro–IL-18, IFN-γ, TNF-α, and reactive oxygen species, which are critical mediators for the control of bacterial growth in host tissues (Eckmann and Kagnoff, 2001).

Remarkably, within the TLR7 gene, we found that two nearly fixed missense mutations in SA breed, i.e., Val121-Ile (G361-A) and Thr135-Ser (A403-T), have significantly higher frequencies than those observed in the other breeds (Figure 5A), possibly due to strong selective pressure. In addition, a similar pattern was identified in the gene ZC3HAV1, which has high breed genotype spectrum frequencies and harbors three missense mutations (Figure 5B). ZC3HAV1 encodes an antiviral protein that inhibits the replication of viruses by recruiting cellular RNA degradation machinery to degrade viral mRNAs (Hayakawa et al., 2011). In addition, our genotype frequency analysis of 845 accessions showed that 12 mutations within top 1% outlier of absolute K value are located within the region of these two genes (TLR7 and ZC3HAV1) (Wang et al., 2020) (Figures 5C–5F; Transparent Methods).

Thus, the immune adaptation of the chickens was shaped by the hot environment for resistance to invasion by pathogens. Our findings suggest the importance of specific advantageous alleles for the defensive response to pathogens in chickens.
Convergent Evolution to Tropical Desert Climate between Birds and Mammals

Intriguingly, we identified seven PSGs (CYP2J19, CYP2J21, CYP2J22, CYP2J23, PLA2G12B, TBXAS1, and HPGDS) that are significantly enriched in the pathway of “Arachidonic acid metabolism” and three PSGs (OC90, DRD3, and PLA2G12B) related to the GO term “arachidonic acid secretion (GO: 0050482, p < 0.05)” specific in SA breed (Tables S8 and S9). The arachidonic acid metabolism pathway plays a key role in converting arachidonic acid to hydroxyeicosatetraenoic acids (HETE) and exhibits strong expression in the kidney (Rouault et al., 2003). Members of the CYP2J family could help convert arachidonic acid into 19(S)-HETE, which has been demonstrated to be a potent vasodilator of renal preglomerular vessels for stimulating water reabsorption (Carroll et al., 1996; Croft et al., 2000; Escalante et al., 1991). In addition, CYP2J2 is regulated by high-salt diet and its suppression can lead to high blood pressure (Carroll et al., 1996). We also observed specific genotype blocks of the two genes (OC90 and PLA2G12B) in SA breed, which have significantly higher or lower frequencies than those observed in the other breeds (Figures 6A and 6B). Indeed, PLA2G12B catalyzes the conversion of arachidonic acid to 20-HETE eicosanoid, which is a potent vasoconstrictor produced in vascular smooth muscle cells that depolarizes and stimulates angiogenic responses in vivo (Miyata and Roman, 2005). Consistently, we also identified in the SA breed two PSGs (KIF26B and AR) involved in urogenital system development and one PSG (CRH) harboring positive regulation of defecation (Table S9), which may be involved in the response to renal...
stress. In conclusion, those genes are important for the adaptation of chickens to tropical desert climates.

Additionally, the arachidonic acid metabolism pathway was reported to be important for desert adaptation in the Bactrian camel (Jirimutu et al., 2012) and sheep (Yang et al., 2016), indicating possible convergent evolution between chickens and mammals for inhabiting similar tropical desert climates.

Molecular convergence is viewed as evidence for common adaptations in divergent lineages. We used 11 species, including six birds and five mammals (Figure 6D), to further identify convergent amino acid substitutions for tropical climate adaptation by applying an established method for detecting convergent and parallel evolution (Zhang and Kumar, 1997). Of these species, three also experience desert stress, such as the southern ostrich (Struthio camelus australis) (Zhang et al., 2015), which is abundant in dry areas with low rainfall in Africa; the yellow-throated sandgrouse (Pterocles gutturalis), which was collected from the Sharjah Breeding Center in the United Arab Emirates (Zhang et al., 2014); and Bactrian camels (Jirimutu et al., 2012), which live in desert regions.
We first replaced the bases of gene sequences from the reference genome with the corresponding fixed genotypes in SA chickens, where the fixed genotype was defined if the allele frequency was more than 0.8. We identified 4,844 single-copy orthologs using OrthoMCL (Li et al., 2003) and then compared the observed number of convergent sites with the random expected number for each gene under the JTT- f amino acid substitution models (Figures 6D and 6E) (Zou and Zhang, 2015). As expected, the ratio of molecular convergence between the numbers of observed and expected values declined with the increase in genetic distance between the two branches compared (Figure 6F). Only genes with a significantly higher number of observed convergent sites were considered to be truly under convergent adaptation (p < 0.05, Poisson test) (Thomas and Hahn, 2015).

Subsequently, we screened convergent genes harboring nonsynonymous amino acids on the basis of PGVs in SA breed. We observed 11, 32, and 37 adaptively convergent genes (ACGs) between SA chickens and Bactrian camels, the southern ostrich, and the yellow-throated sandgrouse, respectively. Gene function enrichment analyses revealed several significant terms involved in the regulation of the response to stimulus and stress (Table S16), especially terms related to “immune system development,” “cardiac muscle adaptation,” “glomerular development,” and “blood vessel development.”

Among the 11 ACGs detected between SA chickens and Bactrian camels, GPR17 has been identified as a dual uracil nucleotide and cysteinyl-leukotriene (CysLT) receptor and is involved in the Reactome pathway named “UDP/CysLT receptor can bind cysteinyl leukotrienes” (R-HSA-391937) (Ciana et al., 2006). CysLTs are derived from the ubiquitous membrane constituent arachidonic acid and are members of a large family of molecules known as eicosanoids. Another convergently evolved gene, TNFRSF11A, functions upstream of the biological process “positive regulation of eicosanoid secretion” (GO: 0032305) (Figure 6C). These findings confirmed that arachidonic acid metabolism was an important molecular adaptive convergence mechanism for the tropical desert climate (Table S16). The kidney is a key tissue for regulating water retention and reabsorption. Interestingly, we found several genes associated with kidney size and function, such as ASXL1, which are involved in “regulation of kidney size” (GO: 0035564) in the southern ostrich, and KLF15, which is involved in “glomerular epithelium development” (GO: 0070210) in the yellow-throated sandgrouse. Additionally, GPR17 and TNFRSF11A were also involved in the biological process “regulation of inflammatory response” (GO:0050727), and similar convergence mechanisms were also found in the southern ostrich and the yellow-throated sandgrouse, which indicated that heat stress could increase inflammatory signaling (Table S16) (Ganesan et al., 2016; Pearce et al., 2013).

Conclusions

In sum, selective sweep analyses of the chicken genomes revealed a variety of important genes, pathways, and GO categories associated with genetic adaptations of chicken to tropical desert/monsoon island climates. Specifically, 12 PSGs (ADCY1, CACNA1C, CAMK2D, PACC, PARK2, PKRCH, SDHD, SIRT1, WNT7B, TXAS1, IL18, and VPS13C) were detected to play roles in chicken adaptations to both tropical desert and tropical monsoon island climates. Our study revealed that nerve development, cutaneous melanin formation, hormonal regulation, angiogenesis, and vasodilation may have worked together to enable chickens to adapt to heat stress. We also discovered different mutations of the same EPAS1 gene to cope with different environmental stresses. Furthermore, a number of PSGs are functionally related to the immune adaptation to the defense against zoonotic diseases and water reabsorption in the tropical desert climate; some of these findings were confirmed by analysis of population genotype frequency in a large dataset of 845 chicken accessions just released. In addition, we provided insights into the genetic mechanism of water reabsorption that have resulted in adaptive convergence between birds and mammals inhabiting tropical desert climate. In view of the current and future global effects of climate change, our study adds to knowledge on genetic mechanisms of adaptations to tropical climates in vertebrates and also has inestimable value for breeding heat-, drought-, and stress-tolerant chicken lines and/or breeds, as well as for medical research related to zoonotic diseases.

Limitations of the Study

To make up for the adverse effect caused by the small sample size, we combined four classic methods to independently identifying genome-wide selection signals for two chicken breeds inhabiting both types of tropical climates; this could reduce the false-positive rate caused by the deviation of a single method. We subsequently revealed genetic adaptations to tropical climates in chickens of two populations inhabiting two similar tropical climates and confirmed some of these results by analysis of the population genotype frequency in a large dataset of 845 chicken accessions just released. Furthermore, we employed comparative genomic analysis between six birds and five mammals, combining with population genomics analysis,
to explain shared genetic mechanisms associated with adaptations to the tropical desert climate from divergent lineages. Due to the protection of local chicken breeds abroad, it is difficult to obtain samples with similar genetic backgrounds. In the future, more samples from diverse breeds are needed to further understand the genetic mechanisms of adaptations to tropical climates in chickens or other vertebrates.

**Resource Availability**

**Lead Contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Huabin Zhao (huabinzhao@whu.edu.cn).

**Data Availability**

The sequence data of 67 chicken genomes in this study are available under the NCBI accession numbers of SRP067615, PRJNA241474, and PRJNA453469.

**Materials Availability**

This study did not generate new reagents or other materials.

**METHODS**

All methods can be found in the accompanying Transparent Methods supplemental file.

**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101644.

**ACKNOWLEDGMENTS**

The work was supported by the National Natural Science Foundation of China (31722051 and 31672272), Natural Science Foundation of the Hubei Province (2019CFA075), Sichuan Provincial Department of Science & Technology Program (2019JTD0009, 20GJHZ0069 and 20SYSX0249), and Fok Ying-Tong Education Foundation for Young Teachers in the Higher Education Institutions of China (161026).

**AUTHOR CONTRIBUTIONS**

H.Z., D.L., and S.T. designed the study. S.T. and D.Z. performed the bioinformatics analysis. C.N. performed the qRT-PCR experiment. T.P. and N.Z. made an effort to sample Tibetan chickens. S.T. and H.Z. wrote the manuscript. X.Z. and D.L. revised the paper.

**DECLARATION OF INTERESTS**

The authors declare no conflicts of interest.

Received: May 26, 2020
Revised: August 19, 2020
Accepted: September 30, 2020
Published: November 20, 2020

**REFERENCES**

Ahmed, R.G. (2005). Heat stress induced histopathology and pathophysiology of the central nervous system. Int. J. Dev. Neurosci. 23, 549–557.

Ai, H., Fang, X., Yang, B., Huang, Z., Chen, H., Mao, L., Zhang, F., Zhang, L., Cu, L., He, W., et al. (2015). Adaptation and possible ancient interspecies introgression in pigs identified by whole-genome sequencing. Nat. Genet. 47, 217–225.

Alkey, J.M. (2009). Constructing genomic maps of positive selection in humans: where do we go from here? Genome Res. 19, 711–722.

Andersson, L., and Georges, M. (2004). Domestic-animal genomics: deciphering the genetics of complex traits. Nat. Rev. Genet. 5, 202–212.

Boivin, N., and Fuller, D.Q. (2009). Shell middens, ships and seeds: exploring coastal subsistence, maritime trade and the dispersal of domesticates in and around the ancient Arabian Peninsula. J. World Prehistory 22, 113–180.

Boulant, J.A., and Dean, J.B. (1986). Temperature receptors in the central nervous system. Annu. Rev. Physiol. 48, 639–654.

Boulnois, L. (2004). Silk Road: Monks, Warriors & Merchants on the Silk Road (WW Norton & Co Inc).

Carroll, M.A., Balazy, M., Margiotta, P., Huang, D.D., Falck, J.R., and McGiff, J.C. (1996). Cytochrome P-450-dependent HETEs: profile of biological activity and stimulation by vasoactive peptides. Am. J. Phys. 271, R863–R869.

Chen, H., Patterson, N., and Reich, D. (2010). Population differentiation as a test for selective sweeps. Genome Res. 20, 393–402.
The orphan receptor GPR17 identified as a new 20-HETE from rat renal microvessels. Am. J. Physiol. 48, 322–330.

Hayakawa, S., Shiratori, S., Yamato, H., Kameyama, T., Kitatsui, C., Kashihi, F., Goto, S., Kameoka, S., Fujikura, D., and Yamada, T. (2011). ZAP7 is a potent stimulator of signaling mediated by the RNA helicase RIG-I during antiviral responses. Nat. Immunol. 12, 37.

Hillier, L.W., Miller, W., Birney, E., Warren, W., Hardison, R.C., Ponting, C.P., Bork, P., Burt, D.W., Groenen, M.A., and Delany, M.E. (2014). Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 423, 695–777.

Hu, C.J., Wang, L.Y., Chodosh, A.A.L., Keith, B., and Simon, M.C. (2003). Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in human small airway epithelial cells. J. Biol. Chem. 278, 19006–19011.

Jiao, H., Zhang, L., Xie, H., Simmons, N., Liu, H., Sun, Z., Zhang, H., Wang, L., Haisi, S., Zhang, Y., et al. (2012). Genome sequences of wild and domesticated pigs. PLoS Genet. 8, e1002914.

Kim, E.S., Elbeltagy, A.R., Aboul-Naga, A.M., Rischkowsky, B., Sayre, B., Mwacharo, J.M., and Rothschild, M.F. (2016). Multiple genomic signatures of selection in goats and sheep indigenous to a hot and arid environment. Heredity (Edinb) 116, 255–264.

Lara, L.J., and Rostagno, M.H. (2013). Impact of heat stress on poultry production. Animals (Basel) 50.

Miyata, N., and Roman, R.J. (2005). Role of 20-hydroxyeicosatetraenoic acid (20-HETE) in vascular system. J. Smooth Muscle Res. 39, 175–193.

Morfitoys, H., Thomas, K.J., Koopman, W.J., Klafke, S., Abou-Sleiman, P., Olfen, S., Willems, P.H., Steinitz, J.A., Cookson, M.R., et al. (2008). Mitochondrial function and morphology are impaired in parkinsonian fibroblasts. Ann. Neurol. 64, 555–565.

Mutaf, S., Kahraman, N.S., and Firat, M.Z. (2009). Intermittent partial surface wetting and its effect on body-surface temperatures and egg production of white and brown domestic laying hens in Antalya (Turkey). Br. Poult. Sci. 50, 33–38.

Nei, M., and Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. U S A 76, 5269–5273.

Oppmann, B., Lesley, R., Blom, B., Timans, J.C., Xu, Y., Hunte, B., Vega, F., Yu, N., Wang, J., and Singh, K. (2000). Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity 13, 715–725.

Patterson, N., Moorjani, P., Lawal, R.A., Al-Atiyat, R.M., Silva, A., Molina, J., Faucher, S., et al. (2018). Genome-wide association mapping identifies distinct patterns of selection in domesticated pigs and Tibetan wild boars. Nat. Genet. 49, 1431–1438.

Tarekegn, A., Ekong, R., Antao, T., Cardona, A., et al. (2013). Genetic signatures reveal high-altitude adaptation in a set of ethiopian domestic chickens. Mol. Genet. Genom. 38, 1–19.

Zhang, Y.P., and Koval, A.M. (2011). Testing for ancient admixture between human domestication and Neandertal genomes. Science 332, 710–722.

Mitochondrial dysfunction and increased PINK1/Parkin-dependent mitophagy. Am. J. Hum. Genet. 98, 500–513.

Li, D., Che, T., Chen, B., Tian, S., Zhou, X., Zhang, G., Li, M., Gaur, U., Li, Y., Luo, M., et al. (2017a). Genomic data for 78 chickens from 14 populations. Gigascience 6, 1–5.

Li, L., Stocekert, C.J., and Roos, D.S. (2003). OrthoMCL-identification of ortholog groups for eukaryotic genomes. Genome Res. 13, 2178–2189.

Li, M., Tian, S., Jin, L., Zhou, G., Li, Y., Zhang, Y., Wang, T., Yeung, C.K., Chen, L., Ma, J., et al. (2013). Genomic analyses identify distinctive patterns of selection in domesticated pigs and Tibetan wild boars. Nat. Genet. 45, 1431–1438.

Li, S., Li, D., Zhao, X., Wang, Y., Yin, H., Zhou, L., Zhong, C., and Zhu, Q. (2017b). A non-synonymous SNP with the allele frequency correlated with the altitude may contribute to the hypoxia adaptation of Tibetan chicken. PLoS One 12, e0172211.

Liu, Y.P., Wu, G.S., Yao, Y.G., Yao, M.W., Lung, G., Bia, M., Beja-Pereira, A., Ding, Z.L., Palanichamy, M.G., and Zhang, Y.P. (2006). Multiple maternal origins of chickens: out of the Asian jungles. Mol. Phylogenet. Evol. 38, 12–19.

Li, F.H., Agha, S., Kantanen, J., Colli, L., Stucki, S., Kijas, J.W., Joost, S., Li, M.H., and Aymone Maras, P. (2014). Adaptations to climate-mediated selective pressures in sheep. Mol. Biol. Evol. 31, 3324–3343.
favors intestinal glucose transport in growing pigs. PLoS One 8, e70215.

Percy, M.J., Furlow, P.W., Lucas, G.S., Li, X., Lappin, T.R., McMullin, M.F., and Lee, F.S. (2008). A gain-of-function mutation in the HIF2A gene in familial erythrocytosis. N. Engl. J. Med. 358, 162–168.

Pickrell, J.K., and Pritchard, J.K. (2012). Inference of population splits and mixtures from genome-wide allele frequency data. PLoS Genet. 8, e1002967.

Portner, H.O. (2001). Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88, 137–146.

Qanbari, S., Rubin, C.J., Maqbool, K., Weigend, S., Weigend, A., Geibel, J., Kerje, S., Wurmser, C., Peterek, A.T., Briaghi, I., and Guttmann, G. (2019). Genetics of adaptation in modern chicken. PLoS Genet. 15, e1007989.

Qi, Z., Zhang, G., Ma, T., Qian, W., Wang, J., Ye, Z., Cao, C., Hu, Q., Kim, J., and Larkin, D.M. (2012). The yak genome and adaptation to life at high altitude. Nat. Genet. 44, 946–949.

Qu, Y.H., Zhao, H.W., Han, N.J., Zhou, G.Y., Song, G., Bao, B., Tian, S.L., Zhang, J.B., Zhang, R.Y., Meng, X.H., et al. (2013). Ground tit genome reveals avian adaptation to living at high altitudes in the Tibetan plateau. Nat. Commun. 4, 2071.

Rouault, M., Bollinger, J.G., Ladzinski, M., Geib, M.H., and Lambeau, G. (2003). Novel mammalian group XII secreted phospholipase A2 lacking enzymatic activity. Biochemistry 42, 11494–11503.

Rubin, C.J., Zody, M.C., Erksson, J., Meadows, J.R., Sherwood, E., Webster, M.T., Jiang, L., Ingrman, M., Sharpe, T., Ka, S., et al. (2010). Whole-genome resequencing reveals loci under selection during chicken domestication. Nature 464, 587–591.

Sabetti, P.C., Schaffner, S.F., Fry, B., Lohmueller, J., Varilly, P., Shamovsky, O., Palma, A., Mikkelsen, T.S., Alshuler, D., and Lander, E.S. (2006). Positive natural selection in the human lineage. Science 312, 1614–1620.

Sabetti, P.C., Varilly, P., Fry, B., Lohmueller, J., Hostetter, E., Cotsapas, C., Xie, X., Byrne, E.H., McCarroll, S.A., Gaudet, R., et al. (2007). Genome-wide detection and characterization of positive selection in human populations. Nature 449, 913–918.

Satzger, J., Schaefer, T., Kuettler, U., Broecker, V., Voelker, B., Otstergaard, H., and Guttmann, R. (2008). Analysis of c-KIT expression and KIT gene mutation in human mucosal melanomas. Br. J. Cancer 99, 2065–2069.

Schaub, M.C., Helti, M.A., and Zaugg, M. (2006). Integration of calcium with the signaling network in cardiac myocytes. J. Mol. Cell Cardiol. 41, 183–214.

Seo, S.H., Peiris, M., and Webster, R.G. (2002). Protective cross-reactive cellular immunity to lethal A/Goose/Guangdong/1996-like H5N1 influenza virus is correlated with the proportion of pulmonary CD8+ T cells expressing gamma interferon. J. Virol. 76, 4886–4890.

Simonsen, T.S., Yang, Y., Huff, C.D., Yun, H., Qin, G., Witterspon, D.J., Bai, Z., Lorenzo, F.R., Xing, J., Jorde, L.B., et al. (2010). Genetic evidence for high-altitude adaptation in Tibet. Science 329, 72–75.

Soh, J., Donnelly, R.J., Kotenko, S., Mariano, T.M., Cook, J.R., Wang, N., Emanuel, S., Schwartz, B., Mile, T., and Peskka, S. (1994). Identification and sequence of an accessory factor required for activation of the human interferon gamma receptor. Cell 76, 793–802.

Stewart, C.R., Bagnaud-Baule, A., Karpala, A.J., Lowther, S., Mohr, P.G., Wise, T.G., Lowenthal, J.W., and Bean, A.G. (2012). Toll-like receptor 7 ligands inhibit influenza A infection in chickens. J. Interferon Cytokine Res. 32, 46–51.

Talbot, S., Tomterey, S., Yamamoto, M., Akira, S., Hughes, K., Gray, D., Barr, T., Mastroeni, P., Maskell, D.J., and Bryant, C.E. (2009). Toll-like receptor 4 signalling through MyD88 is essential to control Salmonella enterica serovar Typhimurium infection, but not for the initiation of bacterial clearance. Immunology 128, 472–483.

Tang, H., Peng, J., Wang, P., and Risch, N.J. (2005). Estimation of individual admixture: analytical and statistical study and design considerations. Genet. Epidemiol. 28, 289–301.

Tata, P.R., Mou, H., Pardo-Saganta, A., Zhao, R., Prabhu, M., Law, B.M., Vinarsky, V., Cho, J.L., Breton, S., and Sahay, A. (2013). De-differentiation of committed epithelial cells into stem cells in vivo. Nature 503, 218–223.

Thomas, G.W., and Hahn, M.W. (2015). Determining the null model for detecting adaptive convergence from genomic data: a case study using echolocating mammals. Mol. Biol. Evol. 32, 1232–1236.

Tominaka, K., Yoshimoto, T., Torigoe, K., Kurimoto, M., Matsui, K., Hada, T., Okamura, H., and Nakanishi, K. (2000). IL-12 synergizes with IL-18 or IL-1beta for IFN-gamma production from human T cells. Int. Immunol. 12, 151–160.

Vazquez-Torres, A., Vallance, B.A., Bergman, M.A., Finlay, B.B., Cookson, B.T., Jones-Carson, J., and Fang, F.C. (2004). Toll-like receptor 4 dependence of innate and adaptive immunity to Salmonella: importance of the Kupffer cell lineage. J. Immunol. 172, 6202–6208.

Wang, M.S., Su, L.Y., Peng, M.S., Zhang, R.W., Lu, Y., Zhang, W., Li, Q.Y., Tu, X.L., Dong, Y., Zhu, C.L., Wang, L., et al. (2015). Genomic analyses reveal potential independent adaptation to high altitude in Tibetan chickens. Mol. Biol. Evol. 32, 1880–1889.

Wang, M.S., Thakur, M., Peng, M.S., Jiang, Y., Frantz, L.A.F., Li, M., Zhang, J.J., Wang, S., Peters, J., Otecko, N.O., et al. (2020). 863 genomes reveal the origin and domestication of chicken. Cell Res. 693–701.
Supplemental Information

Genomic Analyses Reveal Genetic Adaptations to Tropical Climates in Chickens

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Supplemental Information for

Genomic analyses reveal genetic adaptations to tropical climates in chickens

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This supplementary file contains:
Figures S1-S11
Tables S1-S16 (Tables S9, S11 and S16 are provided as separate Excel files)
Transparent Methods
Figure. S1. Nonredundant SNPs (left) and InDels (right) increase as individuals are added. Related to Table 1. The number of genomic variants was plotted on the y axis, whereas the number of individuals sequentially added was shown on the x axis.
Figure. S2. Occurrence possibility of SNPs and InDels in different genomic elements. Related to Table 1.
Figure. S3. Length distribution of small indels (1-30bp) in the whole genome (a) and coding sequence (CDS) regions (b). Related to Table 1.
Figure. S4. Genetic structure of chickens. Related to Figure 1. Each colored segment represents the ancestral component in an individual from $K = 2$ to $K = 9$. The names of each breed/cluster are shown at the bottom of the figure, and the subcluster names are provided on the top.
Figure. S5. Residual fit. Related to Figure 1. Plotted is the residual fit from the maximum likelihood tree in Figure 1D. Residual values greater than zero represent populations that are more closely related to each other in the data than in the best-fit tree, which are thus considered candidates for admixture events.
Figure S6. Four-taxon ABBA/BABA test of introgression. Related to Figure 1.
Figure. S7. The Maritime Silk Route. Related to Figure 1.
Figure S8. Venn map of PSGs for hot adaptation in the SA breed detected by the four methods. Related to Figure 2.
Figure. S9. Venn map of PSGs for hot adaptation in the SL breed detected by the four methods. Related to Figure 2.
Figure. S10. Venn map of PSGs for hot adaptation in SA and SL breeds compared with TC2_Sub2. Related to Figure 2.
Figure. S11. XP-EHH values for the SIRT1 gene between SA and SL breeds. Related to Figure 2.
Table S1. Sample information for chickens in this study. Related to Table 1 and Figure 1.

| Sample ID | NCBI BioSample/RUN ID | Sampling location | Average annual rainfall | Average annual temperature | Altitude |
|-----------|-----------------------|-------------------|-------------------------|---------------------------|----------|
| SL1       | SAMN08979083          |                   |                         |                           |          |
| SL2       | SAMN08979089          |                   |                         |                           |          |
| SL3       | SAMN08979090          |                   |                         |                           |          |
| SL4       | SAMN08979091          |                   |                         |                           |          |
| SL5       | SAMN08979092          |                   |                         |                           |          |
| SL6       | SAMN08979093          | Sri Lankan        | ~1,000mm                | 27°C                      | 60m      |
| SL7       | SAMN08979084          |                   |                         |                           |          |
| SL8       | SAMN08979085          |                   |                         |                           |          |
| SL9       | SAMN08979086          |                   |                         |                           |          |
| SL10      | SAMN08979087          |                   |                         |                           |          |
| SL11      | SAMN08979088          |                   |                         |                           |          |
| SA1       | SAMN08979081          |                   |                         |                           |          |
| SA2       | SAMN08979082          |                   |                         |                           |          |
| SA3       | SAMN08979078          | Saudi Arabian     | 74mm                    | 26°C (ranging from 21.2 to 50.8°C) | 110-130m |
| SA4       | SAMN08979079          |                   |                         |                           |          |
| SA5       | SAMN08979080          |                   |                         |                           |          |
| TC3       | SAMN02712048          | Tibet             | 50-5000m                | 8°C (ranging from -16 to 16°C) | 3,000-6,000m |
| TC4       | SAMN02712049          | Tibet             | decreases from east to west |                           |          |
| TC5       | SAMN02712050          | Tibet             | 200-430mm               | 6.3 - 14°C                | 4000m+   |
| TC6       | SAMN02712051          | Tibet             |                         |                           |          |
| TC7       | SAMN02712052          | Shigatse, Tibet   | 200-430mm               | 6.3 - 14°C                | 4000m+   |
| TC1       | SAMN02712045          | Tibet             | 650mm                   | 8.7°C                     | 3100m    |
| TC2       | SAMN02712047          | Tibet             |                         |                           |          |
| TC8       | SAMN02712053          | Tibet             |                         |                           |          |
| TC9       | SAMN02712054          | Tibet             |                         |                           |          |
| TC10      | SAMN02712046          | Nyingchi, Tibet   |                         |                           |          |
| TCLZ1     | SRR3041450            | Tibet             |                         |                           |          |
| TCLZ2     | SRR3041451            | Tibet             |                         |                           |          |
| TCLZ3     | SRR3041452            | Tibet             |                         |                           |          |
| TCLZ4     | SRR3041453            | Tibet             |                         |                           |          |
| TCLZ5     | SRR3041454            | Tibet             |                         |                           |          |
| TCAB1     | SRR3041433            | Tibet             |                         |                           |          |
| TCAB2     | SRR3041434            | Tibet             |                         |                           |          |
| TCAB5     | SRR3041435            | Aba, Sichuan      | 705mm                   | 9.3°C                     | 3300m    |
| TCAB6     | SRR3041436            | Tibet             |                         |                           |          |
| TCAB7     | SRR3041437            | Tibet             |                         |                           |          |
| Code   | District                | Province          | Precipitation | Temperature | Elevation |
|--------|-------------------------|-------------------|---------------|-------------|-----------|
| TCDQ1  | Diqing, Yunnan          | SRR3041440        | 2261.2mm      | 4.7 - 16.5°C | 3280m     |
| TCDQ2  |                         | SRR3041439        |               |             |           |
| TCDQ3  |                         | SRR3041440        |               |             |           |
| TCDQ4  |                         | SRR3041441        |               |             |           |
| TCDQ5  |                         | SRR3041442        |               |             |           |
| TCDQ6  |                         | SRR3041443        |               |             |           |
| TCGZ1  |                         | SRR3041444        |               |             |           |
| TCGZ2  |                         | SRR3041446        |               |             |           |
| TCGZ3  |                         | SRR3041447        |               |             |           |
| TCGZ4  |                         | SRR3041448        |               |             |           |
| TCGZ5  |                         | SRR3041449        |               |             |           |
| TCGZ6  |                         | SRR3041450        |               |             |           |
| TCQH1  |                         | SRR3041451        |               |             |           |
| TCQH2  |                         | SRR3041452        |               |             |           |
| TCQH3  |                         | SRR3041453        |               |             |           |
| TCQH4  |                         | SRR3041454        |               |             |           |
| TCQH5  |                         | SRR3041455        |               |             |           |
| TCQH6  |                         | SRR3041456        |               |             |           |
| TCQH7  |                         | SRR3041457        |               |             |           |
| TCQH8  |                         | SRR3041458        |               |             |           |
| TCQH9  |                         | SRR3041459        |               |             |           |
| TCQH10 |                         | SRR3041460        |               |             |           |
| TCQH11 |                         | SRR3041461        |               |             |           |
| TCSN1  |                         | SRR3041462        |               |             |           |
| TCSN2  |                         | SRR3041463        |               |             |           |
| TCSN3  |                         | SRR3041464        |               |             |           |
| TCSN4  |                         | SRR3041465        |               |             |           |
| TCSN5  |                         | SRR3041466        |               |             |           |
| TCSN6  |                         | SRR3041467        |               |             |           |
| TCSN7  |                         | SRR3041468        |               |             |           |
| TCSN8  |                         | SRR3041469        |               |             |           |
| TCSN9  |                         | SRR3041470        |               |             |           |
| RJF1   |                         | SAMN02712039      |               |             |           |
| RJF2   |                         | SAMN02712040      |               |             |           |
| RJF3   |                         | SAMN02712041      |               |             |           |
| RJF4   |                         | SAMN02712042      |               |             |           |
| RJF5   |                         | SAMN02712043      |               |             |           |

**Note:** Precipitation and temperature ranges are approximate and may vary by location and season.
Table S2. Summary of sequence quality control and alignment. Related to Table 1 and Figure 1.

| Sample ID | Raw bases (Gb) | High-quality ratio (%) | Q20 (%) | Q30 (%) | Mapping rate (%) | Average sequencing depth (X) | Coverage |
|-----------|----------------|------------------------|---------|---------|------------------|------------------------------|----------|
| SL1       | 49.81          | 98.46                  | 93.50   | 87.87   | 99.22            | 44.04                        | 97.59    |
| SL2       | 50.38          | 98.29                  | 92.81   | 86.71   | 99.14            | 44.22                        | 97.62    |
| SL3       | 48.52          | 98.43                  | 92.75   | 86.52   | 99.25            | 43.25                        | 97.68    |
| SL4       | 50.39          | 98.26                  | 92.70   | 86.51   | 99.09            | 44.28                        | 97.67    |
| SL5       | 34.94          | 98.28                  | 94.62   | 89.82   | 99.30            | 31.09                        | 98.13    |
| SL6       | 49.92          | 98.27                  | 94.33   | 89.25   | 99.32            | 44.09                        | 98.28    |
| SL7       | 51.62          | 98.11                  | 94.25   | 89.14   | 99.25            | 45.55                        | 98.37    |
| SL8       | 49.26          | 98.38                  | 94.04   | 88.75   | 99.33            | 43.70                        | 97.71    |
| SL9       | 44.97          | 98.20                  | 93.48   | 87.85   | 99.22            | 39.88                        | 98.26    |
| SL10      | 46.69          | 98.37                  | 93.42   | 87.73   | 99.28            | 41.69                        | 97.69    |
| SL11      | 46.99          | 98.54                  | 93.37   | 87.71   | 99.28            | 41.65                        | 97.64    |
| SA1       | 16.24          | 95.46                  | 95.31   | 88.26   | 99.48            | 13.26                        | 97.34    |
| SA2       | 16.29          | 93.41                  | 94.59   | 85.37   | 99.24            | 9.90                         | 96.93    |
| SA3       | 16.21          | 95.02                  | 94.50   | 87.09   | 99.57            | 13.09                        | 97.23    |
| SA4       | 16.23          | 94.98                  | 95.35   | 88.43   | 99.49            | 13.17                        | 97.21    |
| SA5       | 16.20          | 92.80                  | 94.20   | 84.69   | 99.19            | 9.64                         | 97.06    |
| TC1       | 22.28          | 94.32                  | 97.50   | 92.23   | 97.92            | 18.73                        | 94.82    |
| TC2       | 6.62           | 87.99                  | 94.21   | 86.22   | 99.11            | 5.34                         | 79.81    |
| TC3       | 18.77          | 82.69                  | 93.77   | 83.35   | 99.62            | 14.48                        | 97.61    |
| TC4       | 14.95          | 81.37                  | 93.42   | 82.64   | 99.59            | 11.34                        | 97.37    |
| TC5       | 15.15          | 79.82                  | 93.22   | 82.34   | 99.58            | 11.26                        | 97.38    |
| TC6       | 18.66          | 81.23                  | 93.52   | 82.85   | 99.56            | 14.08                        | 98.14    |
| TC7       | 38.13          | 87.98                  | 94.99   | 87.07   | 98.14            | 29.61                        | 98.32    |
| TC8       | 6.73           | 89.43                  | 94.59   | 87.09   | 99.26            | 5.52                         | 94.71    |
| TC9       | 6.28           | 86.81                  | 93.97   | 85.77   | 99.07            | 4.99                         | 82.13    |
| TC10      | 9.23           | 94.87                  | 96.26   | 90.75   | 99.50            | 7.90                         | 97.58    |
| TCA1      | 10.01          | 91.67                  | 95.48   | 89.95   | 99.04            | 7.95                         | 92.09    |
| TCA3      | 27.74          | 94.88                  | 96.63   | 92.26   | 97.41            | 20.28                        | 97.69    |
| TCA5      | 10.09          | 92.39                  | 95.50   | 89.96   | 99.14            | 8.06                         | 86.94    |
| TCA6      | 62.09          | 96.57                  | 96.39   | 92.92   | 99.55            | 53.00                        | 98.00    |
| TCA7      | 17.74          | 94.82                  | 95.17   | 88.37   | 99.59            | 14.62                        | 97.56    |
| TCDQ1     | 25.99          | 96.64                  | 96.31   | 92.80   | 99.37            | 21.83                        | 97.75    |
| TCDQ2     | 10.06          | 92.05                  | 95.33   | 89.65   | 98.95            | 7.89                         | 74.54    |
|   |      |     |     |     |     |     |     |
|---|------|-----|-----|-----|-----|-----|-----|
| TCDQ3 | 26.42 | 96.20 | 96.20 | 92.58 | 99.50 | 23.01 | 97.82 |
| TCDQ4 | 40.20 | 87.05 | 96.17 | 91.50 | 99.05 | 28.75 | 98.31 |
| TCDQ5 | 14.31 | 93.26 | 95.93 | 90.84 | 99.10 | 11.26 | 92.51 |
| TCDQ6 | 45.39 | 91.07 | 94.94 | 88.88 | 98.77 | 33.02 | 98.70 |
| TCGZ1 | 10.44 | 92.00 | 95.50 | 90.00 | 99.00 | 8.26 | 86.81 |
| TCGZ10 | 10.39 | 92.52 | 96.07 | 91.20 | 98.98 | 8.06 | 85.33 |
| TCGZ4 | 22.17 | 95.64 | 95.98 | 92.20 | 99.12 | 19.38 | 97.67 |
| TCGZ5 | 15.90 | 92.89 | 95.96 | 90.90 | 97.57 | 12.14 | 82.37 |
| TCGZ6 | 34.58 | 86.39 | 96.50 | 92.09 | 98.81 | 24.12 | 98.20 |
| TCGZ7 | 44.39 | 92.31 | 95.06 | 89.09 | 98.91 | 33.90 | 98.04 |
| TCLZ1 | 11.24 | 84.48 | 94.99 | 89.20 | 98.27 | 8.03 | 81.04 |
| TCLZ2 | 12.68 | 90.52 | 95.29 | 89.66 | 97.84 | 9.46 | 76.44 |
| TCLZ3 | 10.22 | 87.47 | 93.84 | 86.89 | 97.81 | 7.50 | 93.90 |
| TCLZ4 | 23.33 | 93.68 | 96.35 | 91.71 | 97.07 | 16.84 | 78.40 |
| TCLZ5 | 21.37 | 91.22 | 94.93 | 88.88 | 99.02 | 16.44 | 96.97 |
| TCQH1 | 9.15 | 93.82 | 95.54 | 90.65 | 99.30 | 7.53 | 95.76 |
| TCQH10 | 19.06 | 95.43 | 96.01 | 90.19 | 99.55 | 15.47 | 97.63 |
| TCQH11 | 19.06 | 95.58 | 96.10 | 90.35 | 99.54 | 15.60 | 97.63 |
| TCQH5 | 13.07 | 77.23 | 95.07 | 89.51 | 97.99 | 8.40 | 89.50 |
| TCQH8 | 18.95 | 95.87 | 96.23 | 90.71 | 99.50 | 15.50 | 97.67 |
| TCQH9 | 18.66 | 96.03 | 96.29 | 90.83 | 99.50 | 15.34 | 97.64 |
| TCSN1 | 27.98 | 96.13 | 96.16 | 92.52 | 99.39 | 24.22 | 98.01 |
| TCSN3 | 32.23 | 86.34 | 96.53 | 92.16 | 99.27 | 23.08 | 98.20 |
| TCSN4 | 23.99 | 96.39 | 96.33 | 92.83 | 99.48 | 21.05 | 97.76 |
| TCSN5 | 24.60 | 87.73 | 95.85 | 90.76 | 98.92 | 17.87 | 94.28 |
| TCSN6 | 13.12 | 91.95 | 95.85 | 90.76 | 98.95 | 10.24 | 96.77 |
| TCSN7 | 14.76 | 94.84 | 96.69 | 91.67 | 99.66 | 11.74 | 97.28 |
| TCSN8 | 16.91 | 95.80 | 96.44 | 91.03 | 99.73 | 14.01 | 97.33 |
| TCSN9 | 15.18 | 94.82 | 96.52 | 91.22 | 99.50 | 12.54 | 97.38 |
| RJF1 | 17.34 | 85.03 | 94.24 | 83.23 | 98.85 | 13.49 | 97.62 |
| RJF2 | 28.60 | 95.77 | 93.80 | 88.26 | 99.57 | 24.34 | 98.66 |
| RJF3 | 18.08 | 89.17 | 95.45 | 85.92 | 99.63 | 14.85 | 96.94 |
| RJF4 | 21.71 | 91.40 | 95.90 | 87.03 | 99.56 | 18.18 | 97.12 |
| RJF5 | 40.23 | 93.06 | 95.18 | 88.59 | 99.58 | 34.01 | 97.47 |
Table S3. Summary for SNPs in 67 chickens. Related to Table 1.

| Category                   | Number   | Ratio  |
|----------------------------|----------|--------|
| Upstream                   |          |        |
| UTR5                       | 129,631  | 1.21%  |
| UTR5/UTR3                  | 5,702    | 0.05%  |
| Gene body                  |          |        |
| UTR body                   |          |        |
| UTR5                       | 129,631  | 1.21%  |
| UTR5/UTR3                  | 5,702    | 0.05%  |
| CDS                        |          |        |
| Missense                   | 55,480   | 0.52%  |
| Synonymous                 | 123,589  | 1.16%  |
| Nonsyn/Syn ratio (ω)       | 0.453    | -      |
| Stop gain                  | 467      | 0.00%  |
| Stop loss                  | 79       | 0.00%  |
| unknown                    | 1,114    | 0.01%  |
| Intronic                   | 5,626,745| 52.63% |
| Splicing                   | 456      | 0.00%  |
| UTR3                       | 236,288  | 2.21%  |
| Downstream                 | 141,610  | 1.32%  |
| Upstream/Downstream        |          |        |
| Intergenic                 | 4,200,109| 39.28% |
| ncRNA                      |          |        |
| exonic                     | 2,067    | 0.02%  |
| intronic                   | 8,288    | 0.08%  |
| splicing                   | 2        | 0.00%  |
| ts                         | 7,730,048| 72.30% |
| tv                         | 2,961,602| 27.70% |
| ts/tv                      | 2.61     | -      |
| Total                      | 10,691,650| 100.00%|
Table S4. Summary for InDels in 67 chickens. Related to Table 1.

| Category                  | Number   | Ratio   |
|---------------------------|----------|---------|
| Upstream                  | 28,102   | 1.35%   |
| Gene Body                 |          |         |
| UTR5                      | 24,097   | 1.16%   |
| UTR5/UTR3                 | 1,222    | 0.06%   |
| CDS                       |          |         |
| Frameshift deletion       | 4,169    | 0.20%   |
| Frameshift insertion      | 2,629    | 0.13%   |
| Non-frameshift deletion   | 2,571    | 0.12%   |
| Non-frameshift insertion  | 1,201    | 0.06%   |
| Stopgain                  | 162      | 0.01%   |
| Stoploss                  | 13       | 0.00%   |
| Unknown                   | 156      | 0.01%   |
| Intronic                  | 1,098,080| 52.65%  |
| Splicing                  | 667      | 0.03%   |
| UTR3                      | 53,160   | 2.55%   |
| Downstream                | 29,494   | 1.41%   |
| Upstream/Downstream       | 3,656    | 0.18%   |
| Intergenic                | 834,122  | 40.00%  |
| ncRNA                     |          |         |
| exonic                    | 395      | 0.02%   |
| intronic                  | 1,612    | 0.08%   |
| splicing                  | 1        | 0.00%   |
| Total                     | 2,085,509| 100.00% |
Table S5. Sample details for group abbreviations in the main text. Related to Table 1 and Figure 1.

| Sample ID | Groups                | group abbreviations | Cluster                       | Cluster abbreviations |
|-----------|-----------------------|---------------------|-------------------------------|-----------------------|
| SL1       | Sri Lankan            | SL                  | Sri Lankan sub-group 1        | SL1                   |
| SL3       | Sri Lankan            | SL                  | Sri Lankan sub-group 1        | SL1                   |
| SL7       | Sri Lankan            | SL                  | Sri Lankan sub-group 1        | SL1                   |
| SL9       | Sri Lankan            | SL                  | Sri Lankan sub-group 1        | SL1                   |
| SL2       | Sri Lankan            | SL                  | Sri Lankan sub-group 2        | SL2                   |
| SL4       | Sri Lankan            | SL                  | Sri Lankan sub-group 2        | SL2                   |
| SL5       | Sri Lankan            | SL                  | Sri Lankan sub-group 2        | SL2                   |
| SL6       | Sri Lankan            | SL                  | Sri Lankan sub-group 2        | SL2                   |
| SL8       | Sri Lankan            | SL                  | Sri Lankan sub-group 2        | SL2                   |
| SL10      | Sri Lankan            | SL                  | Sri Lankan sub-group 2        | SL2                   |
| SL11      | Sri Lankan            | SL                  | Sri Lankan sub-group 2        | SL2                   |
| SA1       | Saudi Arabian         | SA                  | -                             | -                     |
| SA2       | Saudi Arabian         | SA                  | -                             | -                     |
| SA3       | Saudi Arabian         | SA                  | -                             | -                     |
| SA4       | Saudi Arabian         | SA                  | -                             | -                     |
| SA5       | Saudi Arabian         | SA                  | -                             | -                     |
| TC1       | Tibetan chicken group 1 | TC1            | Tibetan chicken sub-group 1 in TC1 | TC1_sub1             |
| TC7       | Tibetan chicken group 1 | TC1            | Tibetan chicken sub-group 1 in TC1 | TC1_sub1             |
| TCAB1     | Tibetan chicken group 1 | TC1            | Tibetan chicken sub-group 1 in TC1 | TC1_sub1             |
| TCAB3     | Tibetan chicken group 1 | TC1            | Tibetan chicken sub-group 1 in TC1 | TC1_sub1             |
| TCLZ2     | Tibetan chicken group 1 | TC1            | Tibetan chicken sub-group 1 in TC1 | TC1_sub1             |
| TCSN1     | Tibetan chicken group 1 | TC1            | Tibetan chicken sub-group 1 in TC1 | TC1_sub1             |
| Code   | Group Description                  | Code   | Group Description                  | Code   | Group Description                  |
|--------|------------------------------------|--------|------------------------------------|--------|------------------------------------|
| TC2    | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TC3    | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TC4    | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TC5    | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TC8    | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TC9    | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TC10   | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCDQ3  | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCDQ4  | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCLZ3  | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCQH5  | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCQH8  | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCQH9  | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCQH10 | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCQH11 | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCSN6  | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCSN7  | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCSN8  | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCSN9  | Tibetan chicken group 1            | TC1    | Tibetan chicken sub-group 2 in TC1 | TC1_sub2|
| TCAB5  | Tibetan chicken group 2            | TC2    | Tibetan chicken sub-group 1 in TC2 | TC2_sub1|
| TCDQ1  | Tibetan chicken group 2            | TC2    | Tibetan chicken sub-group 1 in TC2 | TC2_sub1|
| TCDQ5  | Tibetan chicken group 2            | TC2    | Tibetan chicken sub-group 1 in TC2 | TC2_sub1|
| TCGZ1  | Tibetan chicken group 2            | TC2    | Tibetan chicken sub-group 1 in TC2 | TC2_sub1|
| TCGZ3  | Tibetan chicken group 2            | TC2    | Tibetan chicken sub-group 1 in TC2 | TC2_sub1|
| TCGZ4  | Tibetan chicken group 2            | TC2    | Tibetan chicken sub-group 1 in TC2 | TC2_sub1|
| Code   | Category                                      | Code | Category                                      | Code | Category                                      |
|--------|----------------------------------------------|------|----------------------------------------------|------|----------------------------------------------|
| TCGZ5  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 1 in TC2           | TC2_sub1 |
| TCGZ6  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 1 in TC2           | TC2_sub1 |
| TC6    | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCAB6  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCB7   | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCDQ2  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCDQ6  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCGZ10 | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCLZ1  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCLZ4  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCLZ5  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCQH1  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCSN3  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCSN4  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| TCSN5  | Tibetan chicken group 2                      | TC2  | Tibetan chicken sub-group 2 in TC2           | TC2_sub2 |
| RJF1   | Red junglefowl                               | RJF  | -                                            | -    |
| RJF2   | Red junglefowl                               | RJF  | -                                            | -    |
| RJF3   | Red junglefowl                               | RJF  | -                                            | -    |
| RJF4   | Red junglefowl                               | RJF  | -                                            | -    |
| RJF5   | Red junglefowl                               | RJF  | -                                            | -    |
Table S6. D-statistic between Tibetan chickens and SL chickens. Related to Figure 1.

|    | P1          | P2          | P3 | P4 | D value  | SE    | Z score |
|----|-------------|-------------|----|----|----------|-------|---------|
| TC1_sub1 | TC2_sub2 | SL         | RJF | -0.0589 | 0.0015 | -40.4315 |
| TC2_sub1 | TC2_sub2 | SL         | RJF | -0.0542 | 0.0014 | -38.2555 |
| TC1_sub2 | TC2_sub2 | SL         | RJF | -0.0484 | 0.0011 | -42.6085 |
| TC1_sub1 | TC1_sub2  | SL         | RJF | -0.0120 | 0.0010 | -11.9055 |
| TC2_sub1 | TC1_sub2  | SL         | RJF | -0.0049 | 0.0013 | -3.7505  |

Table S7. D-statistic between Tibetan chickens and SA chickens. Related to Figure 1.

|    | P1          | P2          | P3 | P4 | D value  | SE           | Z score |
|----|-------------|-------------|----|----|----------|---------------|---------|
| TC2_sub1 | TC1_sub1 | SA         | RJF | -0.05 | 0.00203783 | -24.59 |
| TC2_sub1 | TC2_sub2 | SA         | RJF | -0.046 | 0.00167179 | -27.64 |
| TC2_sub1 | TC1_sub2 | SA         | RJF | -0.04 | 0.00155033 | -25.8  |
| TC1_sub2 | TC1_sub1 | SA         | RJF | -0.011 | 0.00156117 | -7.046 |
| TC1_sub2 | TC2_sub2 | SA         | RJF | -0.006 | 0.00155738 | -3.66  |
Table S8. KEGG enrichment of PSGs in SA breed from the comparison between SA and TC2_Sub2. Related to Figures 2-6. KEGG pathway enrichment analysis was implemented by KOBAS 3.0 software. Ontologies with a cutoff value of 0.05 for \( P \)-values as being biologically significant.

| #Term               | ID    | PSGs number | P-Value  | PSGs                                                                                                                                 |
|---------------------|-------|-------------|----------|-------------------------------------------------------------------------------------------------------------------------------------|
| Metabolic pathways  | gga01100 | 78          | 1.46E-08 | **MSMO1, IDO2, G6PD, APIP, NDUFB8, ATP5J2, NDUFB5, GNPDA1, ALG6, PIGB, NDFS3, NOS2, TPK1, P4HA1, ME1, RRM2, PG51, GADL1, TBXAS1, PDHX, B3GNT5, HSD17B12, CYP2J21, CYP2J23, CYP2J22, AASS, PLA2G12B, ACSM3, AFMID, GCLC, HPGDS, DGK1, LPCAT2, GBE1, ETKN1, SYNJ2, ATP6V0D2, ST3GAL2, SUCLA2, GABT, TAT, PLCB2, SIIL, AMY2A, ACSS1, EXTL3, PTDSS1, AK9, ATP6V1H, PIGP, TPH1, MGAT2, RIMKLB, PIK3C2G, TK1, PRPS2, CDS2, NDUFA9, PAAH1B1, MAN1A1, MAN1A2, FTCD, GALNT12, LPI1, B3GALT5, SC5D, HEXA, NDUFA4, LOC101747660, MTHFD1L, AKN1D1, SDHD, CYP2J19, PPAT, POLE2, GFPT1, HILCS, PIKK, PLCB2, ADCY7, ADCY1, CAMK2D, GNAO1, ADCY9, MAP2K1, KIT, WNT8B, LEF1, RAF1, CREBBP, WNT7B |
| Melanogenesis       | gga04916 | 13         | 3.36E-05 | **PLCB2, ADCY7, ADCY1, CAMK2D, GNAO1, ADCY9, MAP2K1, KIT, WNT8B, LEF1, RAF1, CREBBP, WNT7B**                                          |
| Salmonella infection| gga05132 | 11         | 4.94E-05 | **IL18, NOS2, TJP1, DYNCI11, ARPC1B, ARPC1A, IFNGR2, LOC112529922, FLNB, ACTB, LOC112532987**                                    |
| Gap junction        | gga04540 | 12         | 5.24E-05 | **PLCB2, TJP1, TUBA3E, ADCY1, TUBB4B, LOC100858386, ADCY9,**                                                                      |
| Pathway                                      | Identifier | P-value | Genes                                                                 |
|----------------------------------------------|------------|---------|----------------------------------------------------------------------|
| MAPK signaling pathway                       | gga04010   | 9.00E-05| MAP2K1, ADCY7, PRKG1, ITPR2, RAF1, CACNA1I, RPS6KA2, TGFB3, FLNB, TNFRSF1A, CACNB2, CREB5, LOC100858386, MAP3K7, CACNA1G, CACNA1C, MAP2K1, LOC101750513, NLK, NF1, LOC112529922, FGF12, MAPKAPK5, DUSP16, RAF1, LOC112532987 |
| GnRH signaling pathway                       | gga04912   | 2.23E-04| PLCB2, ADCY7, ADCY1, CAMK2D, MPP2, CACNA1C, ADCY9, MAP2K1, LOC100858386, ITPR2, RAF1 |
| Vascular smooth muscle contraction           | gga04270   | 4.62E-04| PRKCH, PLCB2, ADCY7, ADCY1, PLA2G12B, CACNA1C, KCNMA1, ADCY9, MAP2K1, PRKG1, ITPR2, RAF1 |
| Influenza A                                  | gga05164   | 6.29E-04| IL18, SOCS3, TNFRSF1A, CREB5, IL12B, MAP2K1, IFNAR1, TLR7, IFNGR2, ACTB, CPSF4, RAF1, CREBBP, AGFG1 |
| Wnt signaling pathway                        | gga04310   | 8.32E-04| PLCB2, LRP6, TBL1XR1, PPARD, MAP3K7, DKK1, CAMK2D, WNT8B, NFATC2, LEF1, NLK, CREBBP, WNT7B |
| Adrenergic signaling in cardiomyocytes       | gga04261   | 2.05E-03| PLCB2, ADCY7, ADCY1, LOC112533460, CAMK2D, CACNB2, CREB5, CACNA1C, ADCY9, ATP1B3, KCNQ1, PPP2R5A |
| Arachidonic acid metabolism                  | gga00590   | 2.54E-03| CYP2J21, TBXAS1, CYP2J23, CYP2J22, PLA2G12B, CYP2J19, HPGDS |
| Linoleic acid metabolism                     | gga00591   | 3.76E-03| PLA2G12B, CYP2J19, CYP2J21, CYP2J23, CYP2J22 |
| Adherens junction                            | gga04520   | 4.38E-03| TJP1, NLK, MAP3K7, PTPRM, LEF1, ACTB, CREBBP, CTNA3 |
| Calcium signaling pathway                    | gga04020   | 6.56E-03| CACNA1I, NOS2, ADCY7, ADCY1, ERBB4, CAMK2D, CACNA1C, PLCB2, ADCY9, ITPR2, CACNA1G, LOC112533510, PHKG1 |
| Oocyte meiosis                               | gga04114   | 6.95E-03| RPS6KA2, ADCY7, ADCY1, CAMK2D, ADCY9, MAP2K1, AR, ITPR2, PPP2R5A |
| Cytokine-cytokine receptor interaction       | gga04060   | 1.05E-02| IFNAR1, IL18, EDA, ACVR1, IFNGR2, TNFRSF1A, EDA2R, IL12B, KIT, TGFB3, CCR6, CCR9, CCL20 |
| Glycosphingolipid biosynthesis               | gga00603   | 1.20E-02| HEXA, ST3GAL2, B3GALT5 |
| Pathway                                      | ID   | p-value | Genes                                                                 |
|----------------------------------------------|------|---------|-----------------------------------------------------------------------|
| Globo series                                 |      |         |                                                                       |
| Glutathione metabolism                       | gga00480 | 1.74E-02 | HPGDS, GSTAL2, RRM2, GSTA2, GCLC                                     |
| Glycerophospholipid metabolism               | gga00564 | 1.88E-02 | PTDSS1, LPIN1, PLA2G12B, CDS2, DGK1, LPCAT2, ETNK1, PGS1              |
| Progesterone-mediated oocyte maturation       | gga04914 | 2.31E-02 | RPS6KA2, ADCY7, ADCY1, ADCY9, PDE3B, MAP2K1, RAF1                    |
| Purine metabolism                            | gga00230 | 2.61E-02 | AK9, ADCY7, PRPS2, ADCY1, PDE3A, ADCY9, PDE3B, PPAT, RRM2, PDE4B, POLE2 |
| Base excision repair                         | gga03410 | 2.69E-02 | PCNA, POLE2, LIG1, LIG3                                              |
| ErbB signaling pathway                       | gga04012 | 2.76E-02 | AREG, PTK2, ERBB4, CAMK2D, MAP2K1, PAK6, RAF1                       |
| Oxidative phosphorylation                    | gga00190 | 2.80E-02 | NDUFA4, NDUFB8, ATP5J2, NDUFB5, ATP6V1H, NDUFA9, NDUFS3, ATP6V0D2, SDHD |
| Endocytosis                                  | gga04144 | 2.84E-02 | RAB11FIP3, AMPH, TGFbeta, ERBB4, SNX4, GRK6, GRK7, ARPC1A, KIT, ZFYVE9, DNM3, ARPC1B, CHMP1A, AP2B1, AGAP1 |
| mTOR signaling pathway                       | gga04150 | 3.56E-02 | RPS6KA2, RRAGA, TNFRSF1A, GRB10, ATP6V1H, MAP2K1, WNT8B, LRP6, RAF1, WNT7B |
| Apoptosis                                    | gga04210 | 3.98E-02 | TUBA3E, TNFRSF1A, MAP2K1, TRADD, XIAP, ITPR2, ACTB, RAF1, HTRA2      |
| Phagosome                                    | gga04145 | 4.87E-02 | TUBA3E, DYNC1I1, TUBB4B, THBS2, ATP6V1H, NOX3, LAMP2, ATP6V0D2, ACTB |
Table S10. KEGG enrichment of PSGs in SL breed from the comparison between SL and TC2_Sub2. Related to Figures 2-4. KEGG pathway enrichment analysis was implemented by KOBAS 3.0 software. Ontologies with a cutoff value of 0.05 for $P$-values as being biologically significant.

| Term                              | ID     | PSG number | $P$-value | PSGs                                                                                                                                 |
|-----------------------------------|--------|------------|-----------|-------------------------------------------------------------------------------------------------------------------------------------|
| Adrenergic signaling in cardiomyocytes | gga04261 | 18         | 5.99E-07  | PPP2R3A, ADCY1, ATP2B2, PLCB4, MYH15, ADCY2, CAMK2D, CACNA1C, ADCY8, MAPK14, CACNA1S, AKT3, CREM, MAPK13, CACNA2D3, PPP2R5C, ATP2B4, LOC101750560, CACNA1C, PLCB4, ADCY1, ADCY2, CAMK2D, ADCY8, LOC100858386, EGFR, MAPK14, GNRH1, MAPK13, CACNA1S |
| GnRH signaling pathway            | gga04912 | 12         | 3.88E-05  | MSMO1, AOC3, TRAK2, CHPF2, MBOAT2, P4HA1, SGMS2, TPK1, PLCB4, BCO1, URAH, PAPSSI, SGPL1, UROC1, GCNT3, CES1L1, HKDC1, NDST2, DNMT1, DGKI, PFKP, PLCH2, PAICS, ITPKB, GALNTL6, SPTLC3, NT5C1A, MRI1, GOT2, BCKDHB, EPRS, PTS, DGKE, LSS, EXTL3, ACACA, TBXAS1, LOC107051134, ACSS3, IDH2, SEPHSI, HK1, COMT, ALOX15B, GALNT9, PIGV, LOC112533497, LAMA3, NAT8L, MCEE, MAN1A2, CMP1, TRIT1, B Pant1, CDS2, G6PC, AGPAT5, PPAT, POLE3, ANPEP, MGAT4C, PRIM2, SDHD, GCSH, CACNA1B, ATP2B2, PLCB4, ADCY1, ADCY2, CAMK2D, ADCY8, SLC25A4, CACNA1C, EGFR, LOC112533510, CACNA1S, TACR2, ITPKB, PHKA2, TACR3, ATP2B4 |
| Metabolic pathways                | gga01100 | 64         | 5.23E-05  | MSMO1, AOC3, TRAK2, CHPF2, MBOAT2, P4HA1, SGMS2, TPK1, PLCB4, BCO1, URAH, PAPSSI, SGPL1, UROC1, GCNT3, CES1L1, HKDC1, NDST2, DNMT1, DGKI, PFKP, PLCH2, PAICS, ITPKB, GALNTL6, SPTLC3, NT5C1A, MRI1, GOT2, BCKDHB, EPRS, PTS, DGKE, LSS, EXTL3, ACACA, TBXAS1, LOC107051134, ACSS3, IDH2, SEPHSI, HK1, COMT, ALOX15B, GALNT9, PIGV, LOC112533497, LAMA3, NAT8L, MCEE, MAN1A2, CMP1, TRIT1, BPNT1, CDS2, G6PC, AGPAT5, PPAT, POLE3, ANPEP, MGAT4C, PRIM2, SDHD, GCSH, CACNA1B, ATP2B2, PLCB4, ADCY1, ADCY2, CAMK2D, ADCY8, SLC25A4, CACNA1C, EGFR, LOC112533510, CACNA1S, TACR2, ITPKB, PHKA2, TACR3, ATP2B4 |
| Calcium signaling pathway         | gga04020 | 17         | 7.50E-05  | MSMO1, AOC3, TRAK2, CHPF2, MBOAT2, P4HA1, SGMS2, TPK1, PLCB4, BCO1, URAH, PAPSSI, SGPL1, UROC1, GCNT3, CES1L1, HKDC1, NDST2, DNMT1, DGKI, PFKP, PLCH2, PAICS, ITPKB, GALNTL6, SPTLC3, NT5C1A, MRI1, GOT2, BCKDHB, EPRS, PTS, DGKE, LSS, EXTL3, ACACA, TBXAS1, LOC107051134, ACSS3, IDH2, SEPHSI, HK1, COMT, ALOX15B, GALNT9, PIGV, LOC112533497, LAMA3, NAT8L, MCEE, MAN1A2, CMP1, TRIT1, BPNT1, CDS2, G6PC, AGPAT5, PPAT, POLE3, ANPEP, MGAT4C, PRIM2, SDHD, GCSH, CACNA1B, ATP2B2, PLCB4, ADCY1, ADCY2, CAMK2D, ADCY8, SLC25A4, CACNA1C, EGFR, LOC112533510, CACNA1S, TACR2, ITPKB, PHKA2, TACR3, ATP2B4 |
| Pathway Description                                      | Code   | P-value   |
|---------------------------------------------------------|--------|-----------|
| Neuroactive ligand-receptor interaction                 | gga04080 | 9.80E-05 |
| MAPK signaling pathway                                 | gga04010 | 1.62E-04 |
| Insulin signaling pathway                              | gga04910 | 1.38E-03 |
| Gap junction                                           | gga04540 | 2.52E-03 |
| Adherens junction                                       | gga04520 | 3.63E-03 |
| AGE-RAGE signaling pathway in diabetic complications   | gga04933 | 4.63E-03 |
| Melanogenesis                                          | gga04916 | 4.97E-03 |
| Progesterone-mediated oocyte maturation                | gga04914 | 6.31E-03 |
| Purine metabolism                                      | gga00230 | 9.03E-03 |
| Vascular smooth muscle contraction                     | gga04270 | 1.13E-02 |
| Butirosin and neomycin                                 | gga00524 | 1.36E-02 |

Pathway genes:

- **Neuroactive ligand-receptor interaction**: GABRQ, GABRG1, GABBR2, UTS2R, GABRA3, S1PR2, S1PR1, ADRA2C, GRM8, GLP1R, MC1R, GRID2, MC3R, MTNR1B, MTNR1A, TACR3, TACR2, PARD3, GLRA2, GRIK2, GRIK4, SSTR1, CRHR1, TGFb3, LOC101750560, CACNA2D3, CACNA1S, MAP4K4, DUSP6, CACNA1B, LOC100858386, EGFR, PTPRR, MAPK14, CACNA1C, FLNC, AKT3, MAPK13, FGFR2, FGF12, FGF14, FGF13, PTEN, CASP3
- **MAPK signaling pathway**: PPP1R3E, ACACA, LOC101750560, HKDC1, RPS6KB1, HK1, G6PC, PPARG1A, GSK3B, AKT3, SORBS1, PHKA2
- **Insulin signaling pathway**: TJP1, PLCB4, ADCY1, ADCY2, ADCY8, LOC100858386, EGFR, TUBB3, GUCY1A2
- **Gap junction**: TJP1, EGFR, SORBS1, PARD3, PTPRB, CDH1, CTNNA3, IQGAP3
- **Adherens junction**: TJP1, PLCB4, ADCY1, ADCY2, CAMK2D, ADCY8, GSK3B, ASIP, WNT7B
- **AGE-RAGE signaling pathway in diabetic complications**: TGFb3, PLCB4, CASP3, PIM1, MAPK14, AKT3, MAPK13, COL3A1, LOC101750560
- **Melanogenesis**: MC1R, PLCB4, ADCY1, ADCY2, CAMK2D, ADCY8, GSK3B, ASIP, WNT7B
- **Progesterone-mediated oocyte maturation**: LOC101750560, ADCY2, MAD1L1, ADCY8, MAPK14, AKT3, MAPK13, ADCY1
- **Purine metabolism**: PAPSS1, ADCY1, ADCY2, ADCY8, GUCY1A2, PAICS, PPAT, POLE3, PDE5A, NT5C1A, PRIM2, URAH
- **Vascular smooth muscle contraction**: PRKCH, PLCB4, ADCY1, ADCY2, CACNA1C, ADCY8, RAMP2, GUCY1A2, CACNA1S
- **Butirosin and neomycin**: HKDC1, HK1
| Pathway                                  | Gene ID | P-value | Enzymes/Proteins                                                                 |
|------------------------------------------|---------|---------|---------------------------------------------------------------------------------|
| biosynthesis                             |         |         |                                                                                 |
| Propanoate metabolism                    | gga00640| 4       | 1.96E-02 ACSS3, BCKDHB, MCEE, ACACA                                            |
| Galactose metabolism                     | gga00052| 4       | 2.43E-02 HKDC1, HK1, G6PC, PFKP                                                |
| Fructose and mannose metabolism          | gga00051| 4       | 2.68E-02 HKDC1, PFKFB3, HK1, PFKP                                             |
| Ubiquitin mediated proteolysis           | gga04120| 9       | 3.37E-02 WWP2, UBE2N, UBE2E3, PARK2, HERC1, PPIL2, LOC107051050, UBE2S, UBE2F |
| Salmonella infection                      | gga05132| 6       | 3.37E-02 IL18, TJP1, DYNC1H1, MAPK14, MAPK13, FLNC                          |
| FoxO signaling pathway                   | gga04068| 9       | 3.66E-02 SIRT1, TGFB3, LOC101750560, EGFR, S1PR1, G6PC, MAPK14, AKT3, MAPK13 |
| Glycerolipid metabolism                  | gga00561| 5       | 4.25E-02 MBOAT2, AGPAT5, DGKE, DGKI, LOC112533497                             |
| Dorso-ventral axis formation             | gga04320| 3       | 4.76E-02 ETS1, EGFR, SPIRE2                                                   |
| Wnt signaling pathway                    | gga04310| 8       | 4.78E-02 PLCB4, LOC107051757, LRP5, CAMK2D, INVS, DKK2, GSK3B, WNT7B          |
Table S12. The list of chickens living in tropical climates. Related to Figures 4 and 5.

| Sample ID   | Sampling location |
|-------------|-------------------|
| India1      | India             |
| India11     | India             |
| India13     | India             |
| India15     | India             |
| India17     | India             |
| India19     | India             |
| India21     | India             |
| India23     | India             |
| India25     | India             |
| India27     | India             |
| India29     | India             |
| India3      | India             |
| India31     | India             |
| India33     | India             |
| India35     | India             |
| India37     | India             |
| India39     | India             |
| India41     | India             |
| India43     | India             |
| India45     | India             |
| India47     | India             |
| India49     | India             |
| India5      | India             |
| India51     | India             |
| India55     | India             |
| India57     | India             |
| India59     | India             |
| India61     | India             |
| India7      | India             |
| India9      | India             |
| Xcelris_10A | India             |
| Xcelris_10B | India             |
| ypt2082     | Pakistan          |
| ypt2086     | Pakistan          |
| ypt2087     | Pakistan          |
| ypt2091     | Pakistan          |
| ypt2092     | Pakistan          |
| Code       | Location                                      |
|------------|-----------------------------------------------|
| ypt2093    | Pakistan                                      |
| ypt2094    | Pakistan                                      |
| ypt2095    | Pakistan                                      |
| ypt2098    | Pakistan                                      |
| ypt2100    | Pakistan                                      |
| ypt2101    | Pakistan                                      |
| ypt2102    | Pakistan                                      |
| ypt2103    | Pakistan                                      |
| ypt2105    | Pakistan                                      |
| ypt2106    | Pakistan                                      |
| ypt2107    | Pakistan                                      |
| ypt2108    | Pakistan                                      |
| ypt2109    | Pakistan                                      |
| ypt2110    | Pakistan                                      |
| ypt2111    | Pakistan                                      |
| ypt2112    | Pakistan                                      |
| ypt2114    | Pakistan                                      |
| ypt2115    | Pakistan                                      |
| ypt2116    | Pakistan                                      |
| ypt2117    | Pakistan                                      |
| ypt2118    | Pakistan                                      |
| ypt2119    | Pakistan                                      |
| ypt2120    | Pakistan                                      |
| ypt2121    | Pakistan                                      |
| ypt2122    | Pakistan                                      |
| ypt2124_L4_I052 | Sri Lanka                                   |
| ypt2126_L3_I053 | Sri Lanka                                   |
| ypt2127_L1_I036 | Sri Lanka                                   |
| ypt2128_L6_I054 | Sri Lanka                                   |
| ypt2144_L6_I055 | Sri Lanka                                   |
| ypt2145_L6_I056 | Sri Lanka                                   |
| ypt2146_L6_I001 | Sri Lanka                                   |
| Xcelris_97 | Bihar(hybrid), India                          |
| Xcelris_5A | border area of AP, TN & KNT (VELLORE), India  |
| Xcelris_5B | border area of AP, TN & KNT (VELLORE), India  |
| 67S_L1_I011 | Esfahan, Iran                                |
| 69S        | Esfahan, Iran                                |
| 70S        | Esfahan, Iran                                |
| 71S_L3_I023 | Esfahan, Iran                                |
| 72S_L1_I024 | Esfahan, Iran                                |
| 73S_L1_I025 | Esfahan, Iran                                |
| Sample Code     | Location         |
|----------------|------------------|
| 74S_L4_I026    | Esfahan, Iran    |
| ypt957         | Esfahan, Iran    |
| ypt958         | Esfahan, Iran    |
| ypt959         | Esfahan, Iran    |
| ypt960         | Esfahan, Iran    |
| ypt961         | Esfahan, Iran    |
| Xcelris_12A    | Kashmir, India   |
| 35S_L5_I041    | Mahhad, Iran     |
| ypt912         | Mahhad, Iran     |
| ypt913         | Mahhad, Iran     |
| ypt915         | Mahhad, Iran     |
| ypt916         | Mahhad, Iran     |
| ypt917         | Mahhad, Iran     |
| Xcelris_6A     | Punjab, India    |
| Xcelris_6B     | Punjab, India    |
| 127S_L2_I045   | Shiraz, Iran     |
| 128S_L1_I042   | Shiraz, Iran     |
| 129S_L3_I043   | Shiraz, Iran     |
| 130S_L4_I044   | Shiraz, Iran     |
| 17S_L3_I024    | Shiraz, Iran     |
| 18S_L7_I025    | Shiraz, Iran     |
| 19S_L4_I026    | Shiraz, Iran     |
| 20S_L7_I027    | Shiraz, Iran     |
| 82S            | Shiraz, Iran     |
| 83S_L3_I033    | Shiraz, Iran     |
| ypt909_L5_I004 | Shiraz, Iran     |
| ypt910_L3_I005 | Shiraz, Iran     |
| ypt911_L6_I006 | Shiraz, Iran     |
| ypt924_L3_I021 | Shiraz, Iran     |
| ypt925_L2_I022 | Shiraz, Iran     |
| ypt926_L2_I023 | Shiraz, Iran     |
Table S13. Population frequencies at the focal *EPAS1* SNP in 67 chickens. Related to Figure 2.

| Allele/genotype | SA frequency (5) | SL frequency (11) | RJF (5) | TC (46) |
|-----------------|------------------|-------------------|---------|---------|
| A               | 0.700            | 0.046             | 0.1     | 0       |
| T               | 0.300            | 0.955             | 0.9     | 0       |
| AA              | 2                | 0                 | 0       | 0       |
| TT              | 0                | 10                | 4       | 46      |
| AT              | 3                | 1                 | 1       | 0       |
| Missing         | 0                | 0                 | 0       | 0       |
| Sample Number   | 5                | 11                | 5       | 46      |
Table S14. Population frequencies at the focal EPAS1 SNP in 845 chickens. Related to Figure 2.

| Site / Mutation | Intron | Allele / genotype | Domesticated Chickens | TCs (112) | RJs (149) | ALL (845) |
|-----------------|--------|-------------------|-----------------------|-----------|-----------|-----------|
|                 |        |                   | TRCs (109)           | NTRCs (475) | \(0.130\) | \(0.021\) | \(0.000\) | \(0.023\) | \(0.032\) |
| Chr3:26726127   | 6th    | C                 | 80                    | 424        | 106       | 127       | 737       |
| T->C            |        | T                 | 7                     | 14         | 0         | 6         | 27        |
|                 |        | CC                | 9                     | 2          | 0         | 0         | 11        |
|                 |        | TT                | 0                     | 0          | 0         | 0         | 0         |
|                 |        | CT                | 13                    | 35         | 6         | 16        | 70        |
|                 |        | Missing           | 13                    | 35         | 6         | 16        | 70        |
|                 |        | A                 | 0.17                  | 0.065      | 0.065     | 0.065     | 0.065     |
|                 |        | T                 | 0.85                  | 0.945      | 0.945     | 0.945     | 0.945     |
| Chr3:26731748   | 6th    | AA                | 8                     | 7          | 0         | 5         | 20        |
| T->A            |        | TT                | 80                    | 428        | 103       | 125       | 736       |
|                 |        | AT                | 20                    | 38         | 1         | 8         | 67        |
|                 |        | Missing           | 1                     | 2          | 8         | 11        | 22        |
|                 |        | G                 | 0.18                  | 0.057      | 0.057     | 0.057     | 0.057     |
|                 |        | A                 | 0.82                  | 0.943      | 0.943     | 0.943     | 0.943     |
| Chr:26731832    | 6th    | GG                | 9                     | 8          | 0         | 2         | 19        |
| A->G            |        | AA                | 77                    | 425        | 103       | 133       | 738       |
|                 |        | AG                | 21                    | 38         | 1         | 7         | 67        |
|                 |        | Missing           | 2                     | 4          | 8         | 7         | 21        |
Table S15. Comparisons of genotypes of TRCs and NTRCs with the background of RJFs and all chickens using Chi-square tests. Related to Figure 2.

| Site     | Groups       | TRCs (109) | NTRCs (475) | TCs (112) | RJFs (149) | ALL (845) |
|----------|--------------|------------|-------------|-----------|------------|-----------|
| Chr3 : 26726127 | TRCs (109)  | -          | 2.19E-12    | 5.81E-08  | 6.03E-06   | 1.66E-10  |
|          | NTRCs (475) | 2.19E-12   | -           | 3.57E-02  | 8.34E-01   | 1.06E-01  |
| Chr3 : 26731748 | TRCs (109)  | -          | 2.16E-08    | 3.56E-09  | 3.54E-04   | 1.32E-07  |
|          | NTRCs (475) | 2.16E-08   | -           | 1.75E-03  | 5.19E-01   | 3.05E-01  |
| Chr3 : 26731832 | TRCs (109)  | -          | 1.32E-09    | 4.93E-10  | 1.33E-07   | 1.02E-09  |
|          | NTRCs (475) | 1.32E-09   | -           | 1.32E-03  | 2.20E-01   | 5.15E-01  |
Transparent Methods

Whole-genome sequence quality filtering

We used the genome sequences for 36 high-altitude Tibetan chickens (NCBI accession: SRP067615) that we had previously generated, and downloaded 32 additional accessions from the NCBI database, including accession PRJNA241474, containing genome sequences for 6 red junglefowl and 10 Tibetan chickens, and accession PRJNA453469, containing sequences for 11 Sri Lankan indigenous chickens and 5 Saudi Arabian indigenous chickens. Approximately 1.67 Tb of whole-genome sequences based on Illumina paired-end technology was used in this study. To avoid reads with artificial bias that mainly resulted from base-calling duplicates and adapter contamination, fastp software (Chen et al., 2018) was used to perform quality control with the default parameters. Consequently, we obtained 1.56 Tb (~20.25-fold per individual) of high-quality paired-end reads, including 95.13% and 88.98% nucleotides with phred quality ≥ Q20 (with an accuracy of 99.0%) and ≥Q30 (with an accuracy of 99.9%), respectively.

Read mapping and genomic variant calling

After sequence quality filtering, we first mapped the remaining high-quality sequences to the chicken reference genome (GRCg6a, GCF_000002315.6) using BWA software (v. 0.7.17-r1188) (Abasht et al., 2009) with the command ‘mem -t 10 -k 32 -M’. Second, we converted SAM format to BAM format using the package SAMtools (v.1.9) (Wahlberg et al., 2009). Third, we sorted BAM files using the package Sambamba (v. 0.6.8) (Tarasov et al., 2015). Finally, the sorted bam file was marked in duplicate using the command “MarkDuplicates” in the package picard (v. 2.18.15, http://broadinstitute.github.io/picard). Subsequently, we performed individual gVCF calling according to the best practices using the Genome Analysis Toolkit (GATK, version v4.1.2.0) (McKenna et al., 2010) with the HaplotypeCaller-based method and then population SNP calling by merging all gVCFs with the commands
“GenomicsDBImport” and “GenotypeGVCFs”. To obtain credible population SNP and InDel sets, we performed a screening process as follows:

(a) For filtering SNPs, the hard filter command ‘VariantFiltration’ was applied to exclude potential false-positive variant calls with the parameter ‘--filterExpression "QD < 2.0 || MQ < 40.0 || FS > 60.0 || SOR > 3.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0"’.

(b) For filtering InDels, the hard filter command ‘VariantFiltration’ was applied to exclude potential false-positive variant calls with the parameter ‘"QD < 2.0 || FS > 200.0 || SOR > 10.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0”’.

(c) Screening of biallelic variants was performed with a Hardy–Weinberg equilibrium p-value >= 0.01. After this filtering step, we obtained 23,746,923 high-quality SNPs and 2,854,845 high-quality InDels.

(d) Variants were filtered out when the proportion of samples within the population lacking the variant was > 10% and the minor allele frequency (MAF) was < 0.01. Consequently, a total of 10,691,650 biallelic high-quality SNPs and 2,085,509 biallelic high-quality InDels were retained for subsequent analyses.

**Annotation of genomic variants**

Genomic variant annotation was performed according to the chicken genome using the package ANNOVAR (Wang et al., 2010). Based on the genome annotation, genomic variants were categorized as being in exonic regions (overlapping with a coding exon), intronic regions (overlapping with an intron), splice sites (within 2 bp of a splicing junction), upstream and downstream regions (within a 1-kb region upstream or downstream from the transcription start site), and intergenic regions. The functional consequences of the variants in coding regions were further grouped into synonymous, nonsynonymous, frameshift, nonframeshift, stop-gain, stop-loss and unknown. Unknown function indicates various errors in the gene structure definition in the database file.
Phylogenetic tree and population structure

To investigate the genetic relationships between 67 chickens, we constructed a phylogenetic tree using the maximum likelihood (ML) algorithm with the GTRGAMMA model and 100 bootstrap iterations based on PGVs using the RAxML program (version: 8.2.12) (Stamatakis, 2014). The population genetic structure was examined via an expectation maximization algorithm, as implemented in the program FRAPPEv1.170 (Tang et al., 2005). The number of assumed genetic clusters K ranged from 2 to 9, with 10,000 iterations for each run. We also conducted PCA to evaluate genetic structure using GCTA software (Yang et al., 2011).

Genome-wide selective sweep scanning

Based on chicken population SNP data sets, four methods were applied to identify genome-wide selection signals.

Firstly, by comparing the 10 pairwise genetic differentiation ($F_{ST}$) (Weir and Cockerham, 1984) between these populations including breeds/cluster of SA, SL, TC1_Sub2, TC2_Sub2 and RJFs with a sliding window (40-kb windows sliding in 20-kb steps), we employed a modified population branch statistic (PBS) approach (Yi et al., 2010; Zhan et al., 2014), which showed very powerful to detect incomplete selective sweeps over short divergence times. Our approach was designed to take advantage of three outgroup and used to identify SA/SL breeds specific selection genomic regions. Based on the original PBS algorithm, we specifically modified the PBS formula to detect SA breed as follows:

$$\frac{(T^{SA-TC2_2} + T^{SA-TC1_2} + T^{SA-RJF} - T^{TC1_2-TC2_2} - T^{TC1_2-RJF} - T^{TC2_2-RJF})}{3}$$

Where T represents the population divergence time in units scaled by the population size, which is the negative log transformed $(1 - F_{ST})$ between two populations. The same formula was also used to identify selection sweep of the SL breed. We considered as the candidate selection regions when PBS values of the comparative
sliding windows at a significance of $P < 0.01$ (Z-test). Therefore, we identified 24.18 Mb (2.30% of the genome) and 15.58 Mb (1.48% of the genome), encompassing 723 and 464 genes, in SA and SL breeds, respectively.

Second, nucleotide diversity ($\theta_\pi$) (Nei and Li, 1979) were calculated based on a sliding window (40-kb windows sliding in 20-kb steps) in two populations, A and B. The statistic $\log_2(\theta_{\pi}^A / \theta_{\pi}^B)$ was then calculated with respect to A and B populations. A negative value (1% outlier) suggests selection in population A, a positive value (1% outlier) suggests selection in population B.

Third, the test of cross-population composite likelihood ratio (XP-CLR) (Chen et al., 2010) was performed with the following parameters: sliding window size, 0.1 cM; grid size, 10 k; maximum number of SNPs within a window, 300; and correlation value for 2 SNPs weighted with a cutoff of 0.99. The general genetic maps for chickens used in this study were 2.8 cM/Mb for chr1-9 and 6.4 cM/Mb for chr10-28 and chr32 (Axelsson et al., 2005). Using the TC2_Sub2 cluster as the control population, we performed the XP-CLR test for SA and SL breeds, respectively. The thresholds for identifying candidate selection sweep regions were top 1% of XP-CLR score.

Fourth, the sign of cross-population extended haplotype homozygosity (XP-EHH) indicated the direction of selection for the two alleles in two populations with default parameters (Sabeti et al., 2007). For the XP-EHH score between two populations of A and B, a positive value (top 1% of all) suggests selection in population A, whereas a negative value (bottom 1% of all) suggests selection in population B.

By integrating the four methods, we identified candidate regions supported by at least one method. Subsequently, all candidate selection regions were assigned to corresponding SNPs and genes.

**Genotype frequency analysis**

To infer the patterns of genotype frequency changes, we set a priority order with
TRCs (tropical chickens), NTRCs (non-tropical chickens), and Tibetan chickens. Genotype frequencies for SNPs were first calculated for each population, and then a linear regression was performed to examine the patterns with the priority order. The equation for the linear regression was as follows: $Y = aX + b$, where $Y$ is the estimated value of genotype frequency, $X$ was the observed value of genotype frequency, $a$ was the slope, and $b$ was the intercept.

**Functional enrichment analysis for PSGs**

We submitted the PSGs to PANTHER for Gene Ontology (GO) enrichment analysis of GO biological process (GO-BP), molecular function (GO-MF) and cellular component (GO-CC) terms (Gene Ontology et al., 2013) with the background of all known chicken genes using the binomial distribution test (Mi et al., 2013). KEGG is a database resource for understanding the high-level functions and utilities of biological systems, such as cells, organisms and ecosystems, based on molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies (http://www.genome.jp/kegg/). KEGG pathway enrichment analysis of PSGs was implemented by KOBAS 3.0 software (Kanehisa et al., 2008; Mao et al., 2005) based on the statistical significance of the binomial distribution test results. For ontologies, we considered a cutoff value of 0.05 for P-values as being biologically significant (Mi et al., 2013).

**Heat stress assay**

The C2C12 cell line, an immortalized mouse myoblast cell line, was purchased from Shanghai Life Science Research Institute, Chinese Academy of Science. Cells were grown in high-glucose Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA), 100 μg/mL streptomycin and 100 U/mL penicillin. Cells were maintained in a...
humidified atmosphere containing 5% CO₂ at 37 ℃ for normal growth. For the heat stress assay, we cultured the cells under two temperature conditions: 42 ℃ for heat stress and 37 ℃ for the control. Heat stress treatment at 42 ℃ was performed for 3, 6 and 12 hours. Then, the cells were harvested at each time point for further qRT-PCR to investigate gene expression.

RNA extraction and real-time PCR
Cellular total RNA was extracted using RNAiso Plus reagent (Takara, Japan) according to the manufacturer’s protocols and reverse transcribed to cDNA using EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgen Biotech, Beijing, China) following the manufacturer’s recommendations. The expression levels of each gene were normalized to that of ACTB, which was used as an endogenous control. We performed quantitative real-time polymerase chain reaction (qRT-PCR) on a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) using TransStart Top Green qPCR SuperMix (Transgen Biotech, Beijing, China) following the manufacturer’s protocols. The qRT-PCR measurements were evaluated in triplicate. The fold change in expression levels was determined using the \(2^{-\Delta\Delta Ct} \) method. Data were analyzed by GraphPad Prism software 7.0 (GraphPad, USA), and \( p < 0.05 \) was considered statistically significant.

Identification of molecular convergence
Convergent sites included both “parallel” and “convergent” sites that were previously defined (Zhang and Kumar, 1997). We used 11 species, which included six birds and five mammals, to identify convergent amino acid substitutions for adaptation to tropical climate. Of these species, three also experience desert stress, namely, the southern ostrich (\( S. camelus \)) (Zhang et al., 2015), which is abundant in dry areas with low rainfall in Africa; the yellow-throated sandgrouse (\( P. gutturalis \), NCBI accession
GCF_000699245.1), which was collected from the Sharjah Breeding Center in the United Arab Emirates (Zhang et al., 2014); and Bactrian camels (Jirimutu et al., 2012), which live in desert regions. Other species were used as control branches, such as the White throated tinamou (Tinamus guttatus, GCA_002215935.1) (Zhang et al., 2014), the domestic pigeon (Columba livia, GCF_000337935.1) (Shapiro et al., 2013), turkey (Meleagris gallopavo, GCF_000146605.3) (Dalloul et al., 2010), human (Homo sapiens, GCF_000001405.39), the mouse (Mus musculus, GCF_000001635.26) and cattle (Bos taurus, GCF_002263795.1). Amino acid sequences for each node of the 4,844 single-copy orthologs were deduced under the “aaml” model using CODEML in PAML (Yang, 1997) based on the phylogenetic relationship (Fig. 6D). Convergent substitutions between the two divergent lineages of species must meet the following criteria: $S_1 \neq S_2; S_3 = S_4; S_3 \neq S_1$ and $S_4 \neq S_2$. The criteria for parallel substitutions are $S_1 = S_2; S_3 = S_4; S_3 \neq S_1$ and $S_4 \neq S_2$, where $S_1, S_2, S_3,$ and $S_4$ represent ancestral amino acids for the interior nodes in the tree (Fig. 6E). Comparisons of observed convergent sites versus those generated under random expectation were performed under the JTT-f_{gene} amino acid substitution models for each gene, using scripts provided in a previous study (Zou and Zhang, 2015).

References
Abasht, B., Sandford, E., Arango, J., Settar, P., Fulton, J.E., O'Sullivan, N.P., Hassen, A., Habier, D., Fernando, R.L., Dekkers, J.C., et al. (2009). Extent and consistency of linkage disequilibrium and identification of DNA markers for production and egg quality traits in commercial layer chicken populations. BMC genomics 10 Suppl 2, S2.
Axelsson, E., Webster, M.T., Smith, N.G., Burt, D.W., and Ellegren, H. (2005). Comparison of the chicken and turkey genomes reveals a higher rate of nucleotide divergence on microchromosomes than macrochromosomes. Genome Res 15, 120-125.
Chen, H., Patterson, N., and Reich, D. (2010). Population differentiation as a test for selective sweeps. Genome Res 20, 393-402.
Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34, i884-i890.
Dalloul, R.A., Long, J.A., Zimin, A.V., Aslam, L., Beal, K., Blomberg Le, A., Bouffard, P., Burt, D.W., Crasta, O., Crooijmans, R.P., et al. (2010). Multi-platform next-generation sequencing of the domestic turkey (Meleagris gallopavo): genome assembly and analysis. PLoS Biol 8, e1000475.

Gene Ontology, C., Blake, J.A., Dolan, M., Drabkin, H., Hill, D.P., Li, N., Sitnikov, D., Bridges, S., Burgess, S., Buza, T., et al. (2013). Gene Ontology annotations and resources. Nucleic Acids Res 41, D530-535.

Jirimutu, Wang, Z., Ding, G., Chen, G., Sun, Y., Sun, Z., Zhang, H., Wang, L., Hasi, S., Zhang, Y., et al. (2012). Genome sequences of wild and domestic bactrian camels. Nat Commun 3, 1202.

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., Katayama, T., Kawashima, S., Okuda, S., Tokimatsu, T., et al. (2008). KEGG for linking genomes to life and the environment. Nucleic Acids Res 36, D480-484.

Mao, X., Cai, T., Olyarchuk, J.G., and Wei, L. (2005). Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. Bioinformatics 21, 3787-3793.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., et al. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20, 1297-1303.

Mi, H., Muruganujan, A., Casagrande, J.T., and Thomas, P.D. (2013). Large-scale gene function analysis with the PANTHER classification system. Nat Protoc 8, 1551-1566.

Nei, M., and Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76, 5269-5273.

Sabeti, P.C., Varilly, P., Fry, B., Lohmueller, J., Hostetter, E., Cotsapas, C., Xie, X., Byrne, E.H., McCarroll, S.A., Gaudet, R., et al. (2007). Genome-wide detection and characterization of positive selection in human populations. Nature 449, 913-918.

Shapiro, M.D., Kronenberg, Z., Li, C., Domyan, E.T., Pan, H., Campbell, M., Tan, H., Huff, C.D., Hu, H., Vickrey, A.I., et al. (2013). Genomic diversity and evolution of the head crest in the rock pigeon. Science 339, 1063-1067.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312-1313.

Tang, H., Peng, J., Wang, P., and Risch, N.J. (2005). Estimation of individual admixture: analytical and study design considerations. Genet Epidemiol 28, 289-301.

Tarasov, A., Vilella, A.J., Cuppen, E., Nijman, I.J., and Prins, P. (2015). Sambamba: fast processing of NGS alignment formats. Bioinformatics 31, 2032-2034.

Wahlberg, P., Carlborg, O., Foglio, M., Tordoir, X., Syvanen, A.C., Lathrop, M., Gut, I.G., Siegel, P.B., and Andersson, L. (2009). Genetic analysis of an F(2)
intercross between two chicken lines divergently selected for body-weight. BMC genomics 10, 248.

Wang, K., Li, M., and Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38, e164.

Weir, B.S., and Cockerham, C.C. (1984). Estimating F-Statistics for the Analysis of Population Structure. Evolution 38, 1358-1370.

Yang, J., Lee, S.H., Goddard, M.E., and Visscher, P.M. (2011). GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet 88, 76-82.

Yang, Z.H. (1997). PAML: a program package for phylogenetic analysis by maximum likelihood. Comput Appl Biosci 13, 555-556.

Yi, X., Liang, Y., Huerta-Sanchez, E., Jin, X., Cuo, Z.X., Pool, J.E., Xu, X., Jiang, H., Vinckenbosch, N., Korneliussen, T.S., et al. (2010). Sequencing of 50 human exomes reveals adaptation to high altitude. Science 329, 75-78.

Zhan, S., Zhang, W., Niitepold, K., Hsu, J., Haeger, J.F., Zalucki, M.P., Altizer, S., de Roode, J.C., Reppert, S.M., and Kronforst, M.R. (2014). The genetics of monarch butterfly migration and warning colouration. Nature 514, 317-321.

Zhang, G., Li, C., Li, Q., Li, B., Larkin, D.M., Lee, C., Storz, J.F., Antunes, A., Greenwold, M.J., and Meredith, R.W. (2014). Comparative genomics reveals insights into avian genome evolution and adaptation. Science 346, 1311-1320.

Zhang, J., Li, C., Zhou, Q., and Zhang, G. (2015). Improving the ostrich genome assembly using optical mapping data. GigaScience 4, s13742-13015-10062-13749.

Zhang, J.Z., and Kumar, S. (1997). Detection of convergent and parallel evolution at the amino acid sequence level. Mol Biol Evol 14, 527-536.

Zou, Z.T., and Zhang, J.Z. (2015). Are convergent and parallel amino acid substitutions in protein evolution more prevalent than neutral expectations? Mol Biol Evol 32, 2085-2096.