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Introgression of Eastern Chinese and Southern Chinese haplotypes contributes to the improvement of fertility and immunity in European modern pigs

--Manuscript Draft--

Manuscript Number:	GIGA-D-19-00160R2
Full Title:	Introgression of Eastern Chinese and Southern Chinese haplotypes contributes to the improvement of fertility and immunity in European modern pigs
Article Type:	Research
Funding Information:	National Natural Science Foundation of China (31525023) — Prof. Jun Ren National Key Research Project of China (2016ZX08006-5) — Prof. Jun Ren
Abstract:	Pigs were domesticated independently from European and Asian wild boars nearly 10,000 years ago. Chinese indigenous pigs have been historically introduced to improve Europe local pigs. However, the geographic origin and biological functions of introgressed Chinese genes in modern European pig breeds remain largely unknown. Here we explored whole-genome sequencing data from 266 Eurasian wild boars and domestic pigs to produce a fine-scale map of introgression between French Large White (FLW) and Chinese pigs. We show that FLW pigs had historical admixture with both Southern Chinese (SCN) and Eastern Chinese (ECN) pigs approximately 200–300 years ago. Moreover, a set of SCN haplotypes was shown to be beneficial for improving disease resistance and those with ECN haplotypes are favorable for improved reproductive performance in FLW pigs. In addition, we confirm human-mediated introgression events at the AHR locus, at which the haplotype of most likely ECN origin contributes to increased fertility of FLW pigs. This study advances our understanding of the breeding history of global domestic pigs and highlights the importance of artificial introgression in the formation of phenotypic characteristics in domestic animals.
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Response to Reviewers:	Dear Dr. Hongling Zhou,

Thank you for giving us the opportunity to further revise our manuscript entitled “Population genomic data reveal origin and phenotypic effect of Chinese haplotypes introgressed into European modern pigs” (GIGA-D-19-00160.R1). We are grateful to the reviewer’s constructive comments that help us to improve this manuscript. According to the reviewer’s suggestions, we explored a new data set retaining all informative SNPs to reanalyze the AHR region. The results are in agreement with the reviewer’s assumption that interspecies hybridization most likely did not occur at this locus. For details, please see the point-to-point respond to the reviewer’s comments. We revised the manuscript accordingly and now submit it to your journal. We sincerely hope that the revised manuscript would satisfy you and the reviewer. Your consideration of acceptance for publication will be greatly appreciated. Thanks again for your kind help and effort to our work. Please do not hesitate to contact me if you have any other questions or comments.

Best regards,
Jun Ren

Respond to Reviewers:

The manuscript has certainly improved compare to the previous version. I do like the overall paper and would like to see it published, however I feel some of my criticisms were not accurately dealt with. I will expand a bit on these to clarify what I meant and how they could help to improve the manuscript.

Respond: Thank you for your positive comment on the improvement of our manuscript. We greatly appreciate your further comments that enabled us to improve this paper.

-Looking at Figure 2a, the mean of the rIBD seems centered around 0. What I would conclude from that is that the majority of the genomes of FLW contains equal contributions from SCN and EWB, and indeed 'a positive rIBD indicated potential introgression' (line 486-487). I find it surprising that such a large part of the FLW genomes contains this high SCN signature. Looking at the different panels in Fig1 I don't see evidence of such high haplotype sharing. Could it be that the distribution is Z-transformed? If not, what would be your explanation of the rIBD value centered around 0 for figure 2a? Could you discuss this is the manuscript as well?

Respond: To address this concern, we carefully checked the statistical data and made a close examination on the introgression signals between FLW and SCN, and on those between FLW and EWB. Although the Manhattan plot (Figure 2a) looks like roughly equal contributions from SCN and EWB to FLW genomes, the frequency distribution of rIBD values clearly show that FLW contains a larger fraction of EWB genomes than SCN genomes. We show the distribution plot as a supplementary figure (supplementary figure S4a in the manuscript). In this figure, the median and mean of rIBD values were -0.023 and -0.026. It should be mentioned that the distribution was not Z-transformed.

-I am still not convinced that the introgression at the AHR locus is coming from interspecies hybridization. I feel the data does not support that conclusion. I have two reasons to doubt this statement:
1) The clustering of a Chinese wild boar within the haplotype group of FLW at the AHR locus. This can be seen in Fig 5a, 5b, Supp12. In your response you argue that this is probably due to introgression from domestic pigs into wild boar, but there is no evidence provided that this is more likely than the haplotype (or a similar one) being present (be it at low frequency) in the Chinese wild boars. Please note also that the sampling of Chinese wild boar is rather low, and represents multiple locations in the wild, spanning a large geographical area. Therefore, only low haplotype frequencies within this group would be expected anyway.
2) Filtering for minor allele frequency >0.05 removed many OUT-specific alleles. If you remove all alleles that occur less than 25 times (when using 266 re-sequenced animals) the out-specific branch length is strongly reduced. This introduced a bias in your OUT animals towards ancestral alleles that are present in outgroup animals as

	well as in sus scrofa. In line 318 you mention that the nucleotide differences between the XVI haplotype and OUT haplotypes are only 7, but I would really like to know the differences without a filter for minor allele frequency. I find it highly unlikely that all out species contain the exact same haplotype at this locus, since they diverged millions of years ago. Therefore, I believe this is an artefact of the filtering. Even though not filtering for MAF may introduce some false positive variants within your dataset, those results can provide valuable information of how distinct these haplotypes really are. Also note that ABBA-BABA tests relyon an excess of derived lineage-specific alleles, and when these are filtered out such proportions are distorted. I strongly suggest to redo the analysis at the AHR locus using a less stringent filtering on MAF, because of the unequal sampling in your dataset. Perhaps if you retain all alleles that are observed at least twice (so homozygous within one animal, or two heterozygotes) you already have a less biased view on the origin of the haplotypes at this locus. Your results indeed support Asian pig-derived haplotypes into FLW, and I think these results are worthwhile, but I would remove the conclusions about interspecies hybridization. If indeed interspecies hybridization occured before introgression into FLW, could you reconstruct a scenario how this should have happened? Was the introgression directly into the domestic lineage, or into a wild ancestor of ECN? Respond: We are thankful to these constructive comments. According to your suggestions, we reanalyzed the AHR region using a new data set containing all SNPs that were observed at least twice in the 266 re-sequenced animals. The result is in agreement with your expectation. First, a number of OUT-specific alleles were added to this region (Figure 5a in the revision). Second, the most frequent haplotype (XVIII) appeared 99 times in the 266 sequenced individuals, including 30 FLW pigs, 24 Large White pigs from other countries, 17 Erhualian pigs, 26 Tibetan pigs and two Asian wild boars (Figure 5b in the revision). Last, the OUT-specific alleles increased the distance between this major haplotype and five OUT haplotypes XXVI, XXVII, XXVIII, IV and I from 7 to 11, 16 35, 31, 97, respectively (supplementary Figure 13 in the revision). Altogether, these findings support our conclusion of introgression of Asian (most likely East Chinese pigs) haplotypes into FLW pigs, but do not support our previous assumption of interspecies hybridization at the AHR locus. Hence, we removed the conclusion about interspecies hybridization from the manuscript and revised this manuscript accordingly. We highlight all corrections in red and show these new findings in the new version of Figure 5 and supplementary files in the revised manuscript. Minor comment: -In table S2 and S3 you have regions of potential introgression on chromosome 23, which doesn't exist in pigs. You probably mean the X-chromosome? Respond: Yes. We have changed “23” to “X” in the two tables.
Additional Information:	
Question	**Response**
Are you submitting this manuscript to a special series or article collection?	No
Experimental design and statistics Full details of the experimental design and statistical methods used should be given in the Methods section, as detailed in our Minimum Standards Reporting Checklist. Information essential to interpreting the data presented should be made available in the figure legends.	Yes

Have you included all the information requested in your manuscript?	
Resources A description of all resources used, including antibodies, cell lines, animals and software tools, with enough information to allow them to be uniquely identified, should be included in the Methods section. Authors are strongly encouraged to cite Research Resource Identifiers (RRIDs) for antibodies, model organisms and tools, where possible. Have you included the information requested as detailed in our Minimum Standards Reporting Checklist?	Yes
Availability of data and materials All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories (where available and ethically appropriate), referencing such data using a unique identifier in the references and in the “Availability of Data and Materials” section of your manuscript. Have you have met the above requirement as detailed in our Minimum Standards Reporting Checklist?	No
If not, please give reasons for any omissions below. as follow-up to "**Availability of data and materials** All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories	All sequence data will be submitted to a publicly available repositories when this manuscript is acceptable for publication in GigaScience.

(where available and ethically appropriate), referencing such data using a unique identifier in the references and in the “Availability of Data and Materials” section of your manuscript. Have you have met the above requirement as detailed in our Minimum Standards Reporting Checklist? "	

Introgression of Eastern Chinese and Southern Chinese haplotypes contributes to the improvement of fertility and immunity in European modern pigs

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Abstract

Pigs were domesticated independently from European and Asian wild boars nearly 10,000 years ago. Chinese indigenous pigs have been historically introduced to improve Europe local pigs. However, the geographic origin and biological functions of introgressed Chinese genes in modern European pig breeds remain largely unknown. Here we explored whole-genome sequencing data from 266 Eurasian wild boars and domestic pigs to produce a fine-scale map of introgression between French Large White (FLW) and Chinese pigs. We show that FLW pigs had historical admixture with both Southern Chinese (SCN) and Eastern Chinese (ECN) pigs approximately 200–300 years ago. Moreover, a set of SCN haplotypes was shown to be beneficial for improving disease resistance and those with ECN haplotypes are favorable for improved reproductive performance in FLW pigs. In addition, we confirm human-mediated introgression events at the *AHR* locus, at which the haplotype of most likely ECN origin contributes to increased fertility of FLW pigs. This study advances our understanding of the breeding history of global domestic pigs and highlights the importance of artificial introgression in the formation of phenotypic characteristics in domestic animals.

Introduction

Integrated genomic and archaeological evidence have illuminated the fact that the wild boar (*Sus scrofa*) originated in the Islands of Southeast Asia about 5 million years ago and then dispersed throughout Eurasia. Approximately 1 million years ago, geographic isolation caused by glacial events hampered the continuous gene flow among Eurasian wild boars, causing European and Asian wild boars to differentiate from each other [1-4]. About 10,000 years ago, European and Asian wild boars were domesticated independently in the Near East and China, respectively [3, 5, 6]. After long-term artificial selection and natural selection, abundant genetic resources of domestic pigs appeared in China, accounting for about one-third of global breeds [7, 8]. Chinese pigs are distributed in diverse geographic regions and have different breed features. For example, Erhualian (EHL) and Meishan pigs in East China are known for their prolificacy, with a litter size of more than 15, and for their thick skin. Luchuan (LUC) and Bama pigs in South China have inferior reproductive performance (8–10 piglets per parity), have thin skin and excellent heat resistance [7]. These pig breeds not only play a critical role in the Chinese pig industry, but also have contributed to the development of international commercial breeds, such as the Large White (LW) [9, 10].

Chinese pigs were introduced to Europe mainly during three historical periods [7]. From 1685 to 1757, the Qing Dynasty set up four foreign trade ports: two in East China (Shanghai and Ningbo) and two (Zhangzhou and Guangzhou) in South China. Europe (especially England) had frequent trade with China through these four ports, mainly via the East India Company. This raises the possibility that Eastern Chinese (ECN) and Southern Chinese (SCN) pigs may have been transported to European countries during this period. From 1757 to 1841, only the Guangzhou port in South China was permitted access to foreign trade, and a ban was imposed on maritime trade or intercourse with foreign countries in 1757. It is well documented that SCN pigs had been introduced to England for the hybridization of local pigs during this period,

contributing to the formation of Berkshire [9] and LW pigs [10]. In 1978, the Chinese government launched the reform and open-door policy. Since then, ECN pigs, including Meishan, Jinhua, and Jiaxing Black, have been introduced into France, America, and Japan for the development of prolific synthetic lines [7].

Recently, whole-genome re-sequencing analysis has confirmed the human-mediated translocation of Chinese pigs into Europe that provided genetic variations for the selective breeding of modern commercial LW pigs [11]. However, it remains unknown if SCN or ECN pigs or both were introduced to Europe, because previous studies used a limited number of Chinese pigs from different locations as a whole population. French Large White (FLW) pigs are known for their excellent reproductive performance. A remarkable genetic improvement of litter size has been witnessed in FLW pigs over the past decades, but the molecular mechanisms underlying the fecundity remain unclear, although the fecundity is speculated to be related to the recent introgression of highly prolific Chinese pigs such as ECN pigs [7]. Further studies are required to test this speculation.

In this study, we explored whole-genome sequencing data of 266 Eurasian pigs to show that both SCN and ECN haplotypes were introgressed into LW pigs ~200–300 years ago. Some of the introgressed haplotypes have been under preferential selection to improve fertility and immunity in FLW pigs. For instance, the prolificacy-associated *AHR* haplotype was most likely introgressed from ECN pigs to FLW pigs through human-driven transportation. These findings advance our understanding of the breeding history and genetic mechanisms underlying breed characteristics of global domestic pigs.

Results

Whole-genome sequencing data

We obtained whole-genome sequencing data of 266 animals from 25 populations (**supplementary table S1**), including 36 highly prolific FLW pigs from the nucleus populations of two breeding companies. The 36 pigs were selected due to their total number born (TNB) piglets of more than 19 and distant genetic relationship between each individual (**supplementary fig. S1**). High-depth re-sequencing was conducted on a Hiseq 2000 or 2500 sequencer (Illumina, USA). After filtering raw data (see Methods), we called 32.7 million single nucleotide polymorphisms (SNPs) from the 266 individuals. For the 28 LW pigs whose sequence data were retrieved from the public NCBI database (see Methods), we used the Illumina Porcine SNP60 chip [12] data set to identify their origin. We demonstrated that 14 individuals belonged to the American Large White (ALW) lineage, and the other 14 individuals belonged to the Dutch Large White (NLW) line (**supplementary fig. S2**).

Genetic differentiation between SCN and ECN pigs

Eurasian wild boars began to differentiate as early as ~1 million years ago [2, 3], and Chinese and European wild boars were independently domesticated about 10,000 years ago [1, 3]. The remarkable genetic differentiation between Chinese and Western pigs was reflected in the results from principal component analysis (PCA), phylogenetic analysis and admixture analysis (**fig. 1**). In our PCA analysis, the first principal component (PC1) accounted for 16.32% of the total eigenvalue, which clearly separated the Chinese pig from the Western pig. The second principal component (PC2) showed the differentiation among Chinese pigs, especially between SCN and ECN pigs (PC2 = 3.78%, **fig. 1a**). In a neighbor joining tree between individuals (**fig. 1b**) and populations (**fig. 1c**), Chinese and Western pigs defined two separate clades. For Chinese domestic pigs, SCN and ECN pigs formed two different branches. The clustering pattern was similar to the maximum likelihood tree revealed with TreeMix analysis, in which two Sumatras wild boars, one *Sus barbatus*, one *Sus verrucosus*, one *Sus cebifrons*,

one *Sus celebensis*, and one *Phacochoerus africanus* were treated as an outgroup (OUT), and the interpretation of the maximum likelihood tree reached 99.9% (**supplementary fig. S3**). In an admixture analysis, Chinese pigs and European pigs showed two distinct ancestral lineages when K = 2, although there were gene flows between the two groups, especially the North Chinese pig, that clearly mixed with European pig lineages, whereas LW (including FLW) pigs showed signatures of admixture with Chinese pigs. ECN pigs represented by Jinhua (JH) pigs and SCN pigs represented by Luchuan pigs appeared as the two ancestral lineages of Chinese pigs when K = 3 (**fig. 1d**). Altogether, these findings not only confirmed the independent domestication of Chinese and European pigs, but also revealed that SCN pigs and ECN pigs have marked genetic differentiation and represent two ancient lineages of the Chinese domestic pig.

SCN and ECN pigs were introgressed into Europe between 220 and 310 years ago

To determine whether SCN and ECN pigs were introduced into Europe via human-mediated transportation, we performed relative identity-by-descent (rIBD) analysis using whole genome sequencing data (see Methods). We detected 5,107 and 5,024 50-kb regions with signatures of potential introgression from SCN (**supplementary table S2**) or ECN (**supplementary table S3**) pigs into FLW pigs, respectively (**figs. 2a and 2b, supplementary fig. S4**). The introgressed DNA from SCN and ECN pigs differed greatly in FLW pigs, with an overlap of only 6.0% introgression regions (**fig. 2c**) and 2.9% genes within these regions (**fig. 2d**). We thus performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis on the genes located in the introgressed regions. The genes within the regions of inferred introgression with SCN pigs and ECN pigs were enriched in the immune-related signaling and fertility pathways, respectively (**fig. 2e**). We further used ALDER software [13] to estimate the time of admixture between FLW and SCN or ECN pigs,

144 which yielded an estimate of 53 ± 9 (265 ± 45 years) and 54 ± 9 (270 ± 45 years) generations
145 ago, respectively. This estimation was consistent with historical records stating that SCN pigs
146 were deliberately transported to England at the onset of the first Industrial Revolution and
147 contributed to the breeding of LW pigs [11]. In addition, these results supported our speculation
148 that ECN pigs were also introduced into Europe to improve the productivity of local pigs
149 between 1685 and 1757.
150
151 **The introgressed *GOLM1-NAA35* haplotype from SCN pigs has been under selection to**
152 **enhance the disease resistance of FLW pigs**
153 We detected seven genomic regions with strong signatures of introgression from SCN pigs in
154 the genomes of FLW pigs (rIBD value > 0.2; **supplementary table S4**). Two adjacent genes
155 (3,511 bp apart), *GOLM1* and *NAA35*, were located in one of these seven regions. The *GOLM1*
156 gene encodes a type II Golgi transmembrane protein, which is mainly synthesized in the rough
157 endoplasmic reticulum, assists in processing proteins in the Golgi and is responsive to viral
158 infections [14]. In 2016, Li *et al*. [15] reported that the *GOLM1-NAA35* locus markedly
159 modulated the cytokine interleukin-6 (IL-6) production by human immune cells in response to
160 multiple pathogens. Given the important role of the *GOLM1-NAA35* locus in disease resistance,
161 we chose this locus for further study.
162 We first made a close examination of the rIBD results for a 2-Mb region encompassing the
163 *GOLM1-NAA35* locus (SSC10: 33.20–33.58 Mb on Sscrofa10.2 and 29.15-29.50 Mb on
164 Sscrofa11.1). We found that the frequency of shared IBD haplotypes between FLW and SCN
165 pigs at the *GOLM1-NAA35* locus was significantly higher than those in the surrounding regions
166 (**fig. 3a**). Moreover, we observed remarkably elevated genetic differentiation (F_{ST}) between
167 FLW pigs and European wild boars, in contrast to the particularly decreased F_{ST} between FLW
168 and SCN pigs in the *GOLM1-NAA35* region we observed (**fig. 3b**). In addition, there were four

main *GOLM1-NAA35* haplotypes in FLW pigs. Most individuals (32 out of 36) carried haplotypes similar to those of SCN pig (**fig. 3c**).

Next, we used 3,447 SNPs in the *GOLM1-NAA35* region to construct an NJ tree (**supplementary fig. S5**). We found that most FLW pigs (n = 32) clustered with SCN pigs to form a branch that was separated from ECN pigs and European pigs, whereas only a small number of FLW pigs (n = 4) clustered with European pigs, which was in stark contrast to a genome-wide NJ-tree (**fig. 1a**). We further constructed a haplotype network using 298 SNPs at the *GOLM1-NAA35* locus (**fig. 3d**). We clearly identified haplotype VII as being the main haplotype in FLW pigs, and this haplotype appeared 37 times in all populations, including 23 times in FLW pigs, 8 times in LW pigs, and 6 times in SCN pigs. The SCN-major haplotype VIII and haplotype VII differed at only four different sites, whereas the unique haplotypes (XIX, XXIII and X) of European wild boars and haplotype VII differed at more than 180 sites (**supplementary fig. S6**). These results corroborate the historical introgression of SCN pigs into FLW pigs and illuminate that haplotype VII at the *GOLM1-NAA35* locus in FLW pigs originated from SCN pigs.

We noted that the introgressed haplotype VII was present in other LW pigs at low frequencies but was absent in other European domestic pigs. This was conceivable because all LW populations originated in England where SCN pigs were introduced during the first Industrial Revolution (early 19th century) [7]. Moreover, this introgressed haplotype appeared one time in European wild boars. Considering the outdoor grazing of early European pigs, we believe that European wild boars had admixture with European domestic pigs, after which this haplotype was introgressed from European domestic pigs into European wild boars.

The haplotype heatmap of the *GOLM1-NAA35* region shows that the SCN-originated haplotype VII was frequently present in FLW pigs (**fig. 3c**), which suggested that this haplotype may be selected for in FLW pigs. To verify this hypothesis, we first compared the linkage

disequilibrium (LD) values (r^2) of the *GOLM1-NAA35* region and an upstream (3 Mb) region with the same size as the *GOLM1-NAA35* locus. We found that the LD level in the *GOLM1-NAA35* region of the FLW population ($r^2_{0.3}$ = 192.3 kb) was significantly higher than that of all other populations (**supplementary fig. S7a)**, whereas the LD value ($r^2_{0.3}$) in the upstream region was only 17.3 kb, which was similar to most populations **(supplementary fig. S7b**). Subsequently, we performed LD analysis for 10,000 81.9-kb regions randomly sampled across the genomes of 36 FLW pigs (**supplementary fig. S7c**). We found that the LD value (r^2) in the *GOLM1-NAA35* region ranked in the top 2.6% of the 10,000 bootstrap results, which was a significant outlier (P = 0.02) and suggested that the introgressed *GOLM1-NAA35* haplotype likely underwent a preference selection in FLW pigs, resulting in a local increase of LD level in this target region. XP-EHH analysis also showed evidence of selection at the *GOLM1-NAA35* region in FLW pigs but not in other LW pigs (**fig. 3e**).

To examine whether the *GOLM1-NAA35* haplotypes were associated with serum IL-6 content in FLW pigs, we collected venous blood from 54 healthy adult FLW sows at the same physiological stage and determined the IL-6 levels in the serum of each individual using an enzyme-linked immunoassay (ELISA) (**supplementary table S5**). Meanwhile, we defined the *GOLM1-NAA35* haplotypes for each individual using two tag SNPs and then tested the association between these haplotypes and IL-6 content. We found that individuals homozygously carrying the introgressed haplotype (*QQ*) had significantly higher IL-6 concentrations than heterozygotes individuals (*Qq*) (P = 0.015, **fig. 3f**). Altogether, a sensible explanation for the introgression at the *GOLM1-NAA35* locus is that the *GOLM1-NAA35* haplotype was historically introgressed from SCN pigs into LW pigs and then has been under preferential selection to improve the effective production of IL-6 in response to pathogens and consequently enhance the resistance to infectious disease of FLW pigs.

Historically, South China was renowned as a land of plague with a humid and stuffy environment. It was notorious for local infectious diseases, including malignant malaria that caused high transmission and mortality rates before the Southern Song Dynasty (1127–1279 AD). This hostile environment imposed severe physiological challenges on the inhabitants in South China [7]. Native inhabitants like humans and pigs are believed to have evolved the adaptive mechanisms to address this harsh environment, likely via selection of immune-related genes during the long history of colonization of this area. It is thus conceivable that those genes, including *GOLM1-NAA35* within the introgression regions from SCN pigs, are enriched in immune-related signaling pathway genes. Interestingly, a recent genomic analysis unraveled a list of genes related to immune response under selection in southern Han Chinese, including *G6DP* associated with resistance to malaria [16].

The introgressed *KATNAL1* haplotype from ECN pigs has been preferentially selected to increase the fertility of FLW boars

In FLW pigs, a 200 kb region on chromosome 11 (6.68-6.88 Mb on Sscrofa10.2 and 6.92-7.12 Mb on Sscrofa11.1) showed the strongest (the highest rIBD value) signal of admixture with ECN pigs, and it contained only one gene, *KATNAL1*. *KATNAL1* regulates microtubule dynamics in testicular support cells, affecting the separation and binding of microtubules. Promoting the rapid reorganization of testicular support cell microtubule arrays is an essential process for spermatogenesis and male fertility [17]. Thus, *KATNAL1* plays an important role in spermatogenesis. Given the top introgression signal at the *KATNAL1* locus and the role of *KATNAL1* in boar fertility, we conducted an in-depth analysis focusing on the *KATNAL1* region using the same method as used for the *GOLM1-NAA35* locus.

We found that the frequency of the shared IBD haplotype between FLW and ECN pigs in the *KATNAL1* region was particularly higher than that in the surrounding segments (**fig. 4a**).

There was a remarkable local increase of F_{ST} between FLW pigs and European wild boars and a significant decrease of F_{ST} between FLW pigs and ECN pigs in the *KATNAL1* region **(fig. 4b**). FLW pigs had four main haplotypes in this region. Most individuals (30 out of 36) carried haplotypes highly similar to the ECN haplotypes, and the others were similar to European wild boars and European domestic pigs (**fig. 4c**). Additionally, 30 FLW pigs and ECN pigs were clustered into one large clade while only six FLW pigs were grouped with European pigs in an NJ tree that was constructed with 529 SNPs in the *KATNAL1* gene (**supplementary fig. S8**). Meanwhile, we constructed a haplotype network using these 529 SNPs (**fig. 4d**) and analyzed the nucleotide differences among different haplotypes (**supplementary fig. S9**). The most frequent haplotype (XXVII) appeared 57 times in the 266 tested individuals, including 35 FLW pigs, 18 ECN pigs, 2 ALW pigs and 2 SCN pigs. This haplotype and its closest ECN haplotype (XXV, at five different sites, **supplementary fig. S9**) were divergent from the European pig haplotype groups (**fig. 4d**). These results further demonstrated that the *KATNAL1* haplotypes were introgressed from ECN pigs into FLW pigs.

We performed LD bootstrap sampling and XP-EHH analysis to detect evidence of selection at the *KATNAL1* locus in FLW pigs. First, we compared the LD value (r^2) of the *KATNAL1* region and those of 10,000 randomly selected genomic regions with the same size as the *KATNAL1* gene (43.4 kb). We found that the LD level in the *KATNAL1* region ($r^2_{0.3}$= 437.5 kb) was a significant (P = 0.02) outlier, ranking in the top 2.5% of 10,000 bootstrap results (**supplementary fig. S10**). We also detected a significant selection signal at the *KATNAL1* locus in FLW pigs but not in other LW pigs using XP-EHH (**fig. 4e**). These results suggest that the introgressed *KATNAL1* haplotype from ECN pigs was preferentially selected for in FLW pigs.

Given the important role of *KATNAL1* in male fertility, the fecundity of ECN pigs and historical selection for fecundity in FLW pigs, we speculated that the introgressed *KATNAL1*

haplotype could contribute to the improvement of male reproductive performance and thus underwent selection in FLW pigs since introgression. To test this hypothesis, we analyzed the association between the *KATNAL1* haplotypes and FLW boar fertility that was represented by the average estimated breeding value (EBV) for TNB of mating sows. We detected a significant difference in boar fertility between 17 homozygous carriers of the introgressed haplotype (*QQ*) and 14 carriers of non-ECN pig haplotypes (*qq*) (P = 0.036; **fig. 4f**). The EBV for TNB (EBV-TNB) of *QQ* individuals was 0.018, with a difference of 0.32 (which equates to an increase of 0.32 piglets born per parity) compared with *qq* individuals. As TNB is a complex multi-locus trait, an increase of 0.32 piglets born is substantial for current pig breeding programs. This indicated that the introgressed *KATNAL1* haplotype has been favored and intensively selected by breeders, contributing to the formation of excellent reproductive traits in FLW pigs.

AHR haplotypes that associate with increased litter size were likely introgressed from ECN pigs into LW pigs

In 2014, Bosse *et al.* [11] found that Chinese haplotypes in a 6.8-Mb region on chromosome 9 containing the *AHR* gene were introgressed into European pigs and were preferentially selected to increase fertility during the development of LW pigs. We also conducted a shared haplotype test (rIBD) between 121 Chinese pigs and 64 LW pigs in this 6.8-Mb region. We confirmed the presence of Chinese-derived haplotypes in European pigs including FLW pigs, with a strong introgression signal at the *AHR* locus (SSC9: 92.25–97.45 Mb in Sscrofa10.2 and 83.90–88.40 Mb in Sscrofa11.1) (**supplementary fig. S11**). To explore the geographic origin of the introgressed Chinese *AHR* haplotypes, we first constructed a phylogenetic tree of all sequenced individuals around the *AHR* region, and surprisingly found that most of domestic pigs were clustered together with small genetic distance but were divergent from European and Asian wild boars (**supplementary fig. S12a**). We further reconstructed and visualized haplotypes

around the *AHR* gene (86.5–86.6 Mb on Sscrofa11.1 and 95.4–95.56 Mb on Sscrofa10.2) and found that most haplotypes of LW pigs were highly similar to those of Chinese EHL pigs and Tibetan pigs (**fig. 5a**). In an NJ-tree of this region, 15 FLW pigs gathered with EHL pigs and Tibetan pigs, defining a branch distinct from other Chinese breeds (**supplementary fig. S12b**). Moreover, the most frequent haplotype (XVIII) appeared 99 times in all 266 sequenced individuals, including 30 FLW pigs, 24 other LW pigs, 17 EHL pigs, 26 Tibetan pigs and 2 Asian wild boars (**fig. 5b**). The nucleotide difference between this haplotype (XVIII) and Chinese haplotype XVII was only 6, in contrast to 70 between this haplotype and EWB haplotype XLII (**supplementary fig. S13**). In addition, FLW pigs and EHL pigs had the smallest F_{ST} values with the exception of Tibetan pigs and other LW pigs (**supplementary fig. S12c**). Given the geographic distance between Tibet and Europe and the lack of any historical records describing the importation of Tibetan pigs into Europe, we argue that Chinese derived *AHR* haplotypes in FLW pigs were most likely introgressed from ECN pigs such as EHL pigs through human-mediated transportation about 200–300 years ago.

We noted that the introgressed haplotype XVIII was desirable for increasing the EBV-TNB of both FLW pigs (**fig. 5c**) and EHL pigs (**fig. 5d**). By genotyping the haplotype tag SNPs and one-way analysis of variance (see Methods), we found that homozygous carriers of the introgressed *AHR* haplotype (XVIII) had 0.24 higher EBV-TNB than heterozygous carriers (P = 0.001, **supplementary table S6**) in EHL pigs. Moreover, the introgressed *AHR* haplotype was significantly associated with increased EBV-TNB in FLW sows, with an additive effect value of 0.25 (P = 2.39 e^{-05}; **fig. 5c, supplementary table S7**), which was in agreement with the report of Bosse *et al*.[11]. Similar to the *KATNAL1* and *GOLM1-NAA35* regions, the LD value of FLW pigs at the *AHR* gene region ranked in the top 7% (significant outlier) of all 10,000 bootstrap values (P = 0.03, **supplementary fig. S14**). We also detected a significant iHS selection signal within the FLW pig population (**supplementary fig. S15**). These findings

enabled us to conclude that the introgressed *AHR* haplotype had been under a preferential selection to improve the fertility of FLW pigs.

Discussion

Introgression of both SCN and ECN pig DNA contributed to the genetic improvement of European modern pig breeds

European and Asian domestic pigs were independently domesticated from European and Asian wild boars, respectively, nearly 10,000 years ago [3, 5, 6]. In this study, population genetics analyses confirmed striking genetic differences between Chinese and European domestic pigs and uncovered obvious genetic differentiation between SCN and ECN pigs, which represent two ancestral lineages of Chinese pigs. Of note, we identified Chinese haplotypes in FLW pigs, which were introgressed from both SCN and ECN pigs. We inferred that the introgression events occurred 220–310 years ago, which was in accordance with historical records that SCN pigs were transported to England through the Guangzhou port during the first Industrial Revolution [7]. Our results also supported the speculation that ECN pigs were introduced into Europe, likely through the Shanghai and Ningbo ports, in the decades before the Qing Dynasty imposed a ban on the sea in 1757. Thus, we believe that both SCN and ECN pigs were introduced to Europe to improve the production performance of local breeds, contributing to the development of modern European commercial pig breeds. Taking the *GOLM1-NAA35* and *KATNAL1* loci as examples, the introgressed *GOLM1-NAA35* haplotype from SCN pigs was beneficial for improving disease resistance in FLW pigs, and the introgressed *KATNAL1* haplotype from ECN pigs was favorable for boar fertility and provided genetic variations for the development of high-fecundity FLW pigs. In addition, we show that the *AHR* haplotype associated with increased sow litter size was introduced from ECN pigs into European pigs, such as the Large White breed, through human-mediated transportation and hybridization some

200–300 years ago. It has further experienced preferential selection, presumably during the past decades, and is present at high frequency in FLW pigs, contributing to the improvement of the reproductive performance of this breed. It shows that human-driven crossbreeding play important roles in the development of global pig breeds, illustrating a complex breeding history of domestic pigs. These findings not only advance our understanding of the breeding history of modern European commercial pig breeds but also provides insights into the genetic mechanisms underlying economically important traits in pigs.

Materials and Methods

Samples

All procedures used for this study and involving animals were in compliance with guidelines for the care and utility of experimental animals established by the Ministry of Agriculture of China. The ethics committee of Jiangxi Agricultural University approved this study. This study utilized genome-wide re-sequencing data from 266 animals (**supplementary table S1**), of which 153 pigs were re-sequenced for this study and 113 genome sequence datasets were downloaded from public databases (Registration Nos. PRJEB1683 [18], PRJEB9922 [19], PRJNA260763 [20], PRJNA398176 [21], PRJNA213179 [22] and PRJNA488327 [23]). Among the 153 pigs, 36 were FLW sows and were collected from the Guangdong WENS Food Company (24 individuals) and Jiangxi Lvhuan Animal Husbandry Company (12 individuals). The 36 FLW sows were selected according to the following criteria. First, we calculated the relationship coefficients of all individuals in the nucleus populations of the two companies using DMU software [24] and pedigree records. Then we selected sows with a small relationship coefficient and excellent litter sizes (TNB more than 16). Finally, we chose 36 prolific individuals with distant kinship according to a phylogenetic relationship network constructed by Cytoscape v3.2.1 (Cytoscape, RRID:SCR_003032) [25] (**supplementary fig.**

S1). In total, there were 27 wild boars from China and Europe, 7 outgroup individuals, 121 pigs from Chinese indigenous breeds, and 111 pigs from European commercial breeds. According to the geographic distribution, Chinese domestic pigs were divided into ECN (37) pigs, SCN (20) pigs, SWCN (36) pigs, and NCN (28) pigs (see **supplementary table S1** for details). In addition, whole-genome sequence data of 28 LW pigs was downloaded from public databases, with 14 individuals submitted by Seoul National University [20] and another 14 individuals submitted by Wageningen University [18]. To identify the source of these 28 LW pigs, we downloaded the Illumina 60K chip SNP data set of 76 LW pigs [26], including 20 Dutch Large White pigs (NLW), 16 Danish Large White pigs (DLW), 20 Chinese Large White pigs (CLW), and 20 American Large White pigs (ALW). Next, we retrieved the same 60K chip SNPs from the whole-genome sequence data sets of the 28 LW pigs. We filtered out SNPs with an MAF less than 0.05, a call rate less than 90%, and an LD (r^2) value more than 0.3 using PLINK v1.9 (PLINK, RRID:SCR_001757) [27], and we performed PCA and NJ-tree analyses using the remaining SNPs to identify the origin of the 28 LW pigs (**supplementary fig. S2**).

Whole-genome sequencing and SNP calling

We extracted genomic DNA from the ear tissues of 153 pigs using a routine phenol/chloroform protocol, and eligible samples were delivered to the Novogene company (Beijing, China). Sequencing was performed on Hiseq 2000 or 2500 instruments (Illumina, La Jolla, CA USA; Illumina HiSeq 2500 System, RRID:SCR_016383). The sequencing libraries were constructed with 125 bp paired ends (PE125), a 500 bp average insert fragment size, and a fragment size less than 800 bp. The genome sequencing coverage of each individual was at least 20×, with a minimum data of 60 G.

Quality control: We obtained the raw sequencing data from Hiseq sequencing platform using raw image data. We obtained clean data for performing downstream analysis after

performing the following steps: (1) removal of the linker sequence, (2) retention of reads with Q20 of more than 90% (the probability of base recognition correct rate higher than 99%) and Q30 of more than 85% (the probability of base recognition correct rate higher than 99.9%)[28], (3) culling of short repeat DNA segments, and (4) filtering reads with three consecutive "N".

Mutation detection: We established a reference genome index of Sscrofa 10.2 [6] using the index function in BWA v0.7.12 (BWA, RRID:SCR_010910) [29]. We blasted paired-end reads against the index using an algorithm from BWA and obtained binary bam files from sam files by SAMtools v1.4 (SAMTOOLS, RRID:SCR_002105) [30]. We used samblaster v0.1.22 (SAMBLASTER, RRID:SCR_000468) [31] to reject redundancy information and calculated the alignment rate between re-sequencing data and the reference genome, as well as coverage and sequencing depth. We sorted binary bam files via GATK v3.7 (GATK, RRID:SCR_001876) [32]. We used the HaplotypeCaller function for mutation detection across each chromosome of each individual and obtained a SNP data set of the 266 individuals by deleting InDel information. We filtered out SNPs with an MAF less than 0.01 and a call rate less than 90% using PLINK v1.9 [27]. We used the remaining 32.7 million SNPs in the data set for subsequent statistical analysis.

Population genetic analysis

First, we generated a SNP data set with an MAF more than 0.05 and a call rate more than 90% from autosomal SNPs from 259 pigs (*Sus scrofa*) excluding seven OUT individuals. Second, we pruned SNPs with an LD (r^2) decay of more than 0.3 in each window with 50 SNPs using the command indep-pairwise (50 10 0.3) in PLINK v1.9 [27]. Then four principal components of each individuals were estimated using --pca command in GCTA software [33]. The average shared allele (1-Dst) distance matrix between individuals was constructed using the command --distance-matrix in PLINK v1.9. A rootless NJ tree was constructed via phylip v3.69 (PHYLIP,

RRID:SCR_006244) [34] and was visualized with FigTree v1.42 (FigTree, RRID:SCR_008515). We also explored the unbiased estimation method proposed by Weir and Cockerham to calculate the genetic differentiation (F_{ST}[35]) matrix between 14 Chinese pig breeds and 6 European pig breeds using the --fst command in PLINK v1.9 ([27]. Then, we constructed an interbreed NJ tree using phylip v3.69 [34]. ADMIXTURE (ADMIXTURE, RRID:SCR_001263) [36] was used to estimate the ancestral lineage composition under default parameters. First, we removed the OUT group and populations with fewer than five individuals. Then we randomly selected six individuals from the remaining 21 populations and filtered out SNPs with an MAF of less than 0.05, an LD (r^2) of more than 0.3, and call rates less than 90%. Finally, we used a data set with 125 individuals and 658,601 SNPs to analyze the ancestral lineage composition patterns. In addition, we utilized TreeMix v1.12 [37] to infer the genetic differentiation among populations. We set OUT as the outgroup population, excluding populations with fewer than six samples and SNPs with an MAF less than 0.05 and a call rate less than 90%. We used the data set with 19,282,590 SNPs to estimate genetic differentiation among 21 populations under no migration events via TreeMix v1.12 [37].

Introgression analysis

We detected the introgression signals between Chinese pigs (ECN and SCN pigs) and FLW pigs using an IBD sharing approach [11]. First, we used a data set with 266 individuals and approximately 20 million SNPs to phase haplotypes using the fastPhase function [38] in Beagle v4.0 and to detect IBD fragments in each individual using the fastIBD function [39]. Then we divided the whole genome into numbers of 50 kb windows (25 kb sliding) and calculated the shared IBD haplotype numbers between two populations (FLW vs. European wild boars (EWB), FLW vs. ECN, and FLW vs. SCN) in each window. We phased the haplotypes and detected the IBD regions independently 10 times and then normalized the IBD values (nIBD).

The nIBD values ranged from 0 (no shared IBD detected) to 1 (all individuals shared the IBD haplotype). Finally, we used the rIBD (relative frequency of IBD) statistic to measure the shared IBD between FLW pigs and SCN or ECN pigs, respectively ($rIBD_{FLW\text{-}SCN} = nIBD_{FLW\text{-}SCN} - nIBD_{FLW\text{-}EWB}$, $rIBD_{FLW\text{-}ECN} = nIBD_{FLW\text{-}ECN} - nIBD_{FLW\text{-}EWB}$), where a positive rIBD indicated potential introgression and 5% empirical distribution in the far right tail were set as the significance threshold. For genomic regions showing strong rIBD introgression signals in FLW pigs, we further estimated F_{ST} between FLW pigs and European wild boars, as well FLW pigs and Chinese pigs (SCN pigs or ECN pigs), respectively. We also constructed haplotype networks using SNPs with a MAF of greater than 0.05 and call rates of greater than 90% at the *GOLM1-NAA3* (298 SNPs) and *KATNAL1* (529 SNPs) loci, and using all SNPs (217 SNPs) that were observed at least twice in the 266 re-sequenced individuals at the *AHR* locus. We explored the fastPhase function with 1000 iterations in Beagle v4.0 (BEAGLE, RRID:SCR_001789) [39] to phase haplotypes and used the "haploNet" command in the R package "pegas" [40] to calculate the pairwise differences between haplotypes. We selected SNPs with an MAF of greater than 0.05, a call rate of greater than 90% and an LD (r^2) < 0.3 using PLINK v1.9 [27], and then explored the selected SNPs to estimate the admixture time between populations via ALDER v1.0.3 under default parameters [13]. In short, we used the "convert" function in EIGENSTRAT [41] to convert the data format. We set FLW as a mixed population, EWB and SCN as one reference population, EWB and ECN as another reference population, and five years as one generation.

Signature of selection

We used the data set that excluded SNPs with an MAF of less than 0.05 and a call rate less than 90% in the whole-genome SNPs data set of 36 FLW pigs to calculate the correlation coefficient (r^2) of each SNP pair in a target region using the commands --r2 inter-chr --ld-

window-r2 0 in PLINK v1.9 [40], and we used the average r^2 as the LD value in the region. Meanwhile, we randomly selected 10,000 regions with the same size as the target region across the genome, and we calculated the average r^2 of each region in the 36 FLW pigs. Finally, we visualized the density curve of the 10,000 bootstrap values using R. Furthermore, we used commands --ihs [42] and --xpehh [43] under default parameters in selscan [44] software to detect the signatures of selection in 50 kb windows with a step size of 25 kb in FLW pigs.

Haplotype association analysis

The *GOLM1-NAA35* locus: We detected the serum IL-6 levels in 54 mature FLW sows at an age of 2–2.5 years from the same farm using the Porcine IL-6 ELISA Kit (Shanghai Keshun Biological Technology, China). The concentration of each individual was determined from the averaged repeat of three trials per individual. Meanwhile, we selected two tag SNPs to distinguish the introgressed haplotypes (VII and VIII) from the other haplotype in the *GOLM1-NAA35* region in FLW pigs (**fig. 3e**). The tag SNPs were genotyped by Sanger sequencing PCR products amplified with specific primers (**supplementary table S5**). A Student's *t*-test was used to detect the association between haplotypes and the serum IL-6 concentrations (log2 (IL-6 values)).

The *KATNAL1* locus: We collected 765 FLW sows and 31 FLW boars from the Jiangxi Lvhuan Farming Group. First, we filtered parities with litter size less than five piglets. Then we set estrus, year, season, parity and pregnancy duration as fixed effects, and mating boars and random sow effects as random effects. We then estimated the EBV for TNB of 765 FLW pigs via DMU software [24] and pedigree information. Next, we genotyped eight tagged SNPs to distinguish each *KATNAL1* haplotype in the 31 FLW boars by PCR amplification and Sanger sequencing with primers listed in **supplementary table S8**. We denoted the introgressed XXVII haplotype from ECN pigs as Q (**fig. 4e**) and the other haplotypes as q (**supplementary**

table S9). Finally, we used Student's *t*-test to test the association between *KATNAL1* haplotypes and the average EBV-TNB of mating sows of the 31 FLW boars.

The *AHR* locus: We genotyped two tagged SNPs representing the *AHR* haplotypes for 344 FLW sows by PCR amplification and Sanger sequencing with primers listed in **supplementary table S6**. We identified 230 *QQ* sows homozygous for the introgressed haplotype, 36 *Qq* sows and 78 *qq* sows who were missing the introgressed haplotypes (**supplementary table S6**). Then we tested the association between the *AHR* haplotypes and the EBV-TNB of the 344 sows using single-factor analysis of variance. Furthermore, we collected 221 Erhualian sows with multiparity records from Jiangsu Provence and calculated the EBV-TNB of these sows using DMU software and pedigree information as mentioned above. We genotyped a tag SNP in the *AHR* region by Sanger sequencing PCR products with specific primers (**supplementary table S7**). We detected 176 *QQ* sows homozygous for the introgressed haplotype and 45 heterozygous (Qq) sows. We used a Student's *t*-test to examine the association between *AHR* haplotypes and EBV-TNB in Erhualian sows.

Funding

This study is supported by the Natural Science Foundation of China (31525023) and the National Key Research Project of China (2016ZX08006-5).

Abbreviations

ALW: American Large White; NLW: Dutch Large White; DLW: Danish Large White; EBV: estimated breeding value ECN: Eastern Chinese; EHL: Erhualian ; ELISA: enzyme-linked immunoassay; FLW: French Large White; GO: Gene Ontology; JH: Jinhua; KEGG: Kyoto Encyclopedia of Genes and Genomes; LD: linkage disequilibrium; LUC: Luchuan; LW:

Large White; OUT: outgroup; PCA: principal component analysis; rIBD: relative identity-by-descent; SCN: Southern Chinese; SNPs: single nucleotide polymorphisms.

Author Contributions

J.R. and L.H. designed the study and analyzed the data. J.R., H.C. and L.H. wrote the paper. H.C., M.H., and B.Y. performed the bioinformatic analyses. H.C., M.H., Z.D. Z.W. and Y.H. collected data and performed sequencing and genotyping experiments.

Acknowledgements

We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Availability of supporting data and materials

On top of the public datasets used, previously unpublished raw sequencing is available via NCBI Bioproject PRJNA550237. All other supporting data and materials are available in the *GigaScience* GigaDB database [45].

Competing financial interests

The authors declare no competing financial interests

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Figure Legends

Fig. 1. Population relationship and structure. (**a**) Principal component analysis of Chinese and European pigs. ECN, East Chinese pigs; NCN, North Chinese pigs; SCN, South Chinese pigs; SWCN, Southwest Chinese pigs; EUD, European domestic pigs. (**b**) Neighbor-joining (NJ) tree based on an identity-by-state matrix among individuals. (**c**) NJ tree based on an Fst matrix between populations. (**d**) Population structure of Chinese and European pigs revealed by ADMIXTURE analysis. MIN, Min pigs; HT, Hetao pigs; LWH, Laiwu pigs; EHL, Erhualian pigs; MS, Meishan pigs; JH, Jinhua pigs; GST, Tibetan pigs (gansu)； SCT, Tibetan pigs (Sichuan); YNT, Tibetan pigs (Yunnan); TT, Tibetan pigs (Tibet); WZS, Wuzhishan pigs; LUC, Luchuan pigs; BMX, Bamaxiang pigs; XIANG, Xiang pigs; AWB, Asian wild boars; OUT, outgroup; EWB, European wild boars; HMP, Hampshire; DU, Duroc; LR, Landrace; PI, Pietrain; WDU, White Duroc; WLW, Dutch Large White pigs; KLW, Korea Large White pigs; FLW, French Large White pigs.

Fig. 2. Introgressed Chinese haplotypes in French Large White pigs. (**a**) Manhattan plot of rIBD values between French Large White (FLW) and South Chinese (SCN) pigs (positive value) or European wild boars (EWB) (negative value). The red dashed line indicates the top 5% significance threshold. (**b**) Manhattan plot of rIBD values between FLW and East Chinese (ECN) pigs (positive value) or EWB (negative value). (**c**) Venn diagram of introgressed DNA (50 Kb windows) from SCN and ECN pigs in FLW pigs. (**d**) Venn diagram of genes in the introgressed regions from SCN and ECN pigs in FLW pigs. (**e**) Significantly enriched GO processes and KEGG pathways of introgressed genes in the introgressed regions from SCN and ECN pigs under selection in FLW pigs.

690 **Fig. 3. Introgression at the *GOLM1-NAA35* locus.** (**a**) rIBD values in a 2 Mb region
691 harboring the *GOLM1-NAA35 g*ene. The brown dashed line indicates the 5% threshold line,
692 and the *GOLM1-NAA35* region is indicated by grey dashed lines. (**b**) Genetic differentiation
693 index (F_{ST}) between French Large White (FLW) and European wild boar (EWB) or South
694 Chinese (SCN) pigs. (**c**) Haplotype heatmap in the *GOLM1-NAA35* region. Major and minor
695 alleles in FLW pigs are indicated by beige and light blue, respectively. (**d**) Haplotype network
696 in the *GOLM1-NAA35* region. Each circle represents a haplotype, and the size of the circle is
697 proportional to the haplotype frequency. The line width and length represent the difference
698 between haplotypes. Different colors represent pigs from different geographical regions.
699 SWCN, Southwest Chinese pigs; NCN, North Chinese pigs; AWB, Asian (Chinese) wild boars;
700 ECN, East Chinese pigs; EUD, European domestic pigs. (**e**) Selection signals in the *GOLM1-*
701 *NAA35* region by XP-EHH analysis between FLW and other Large White pigs. The brown
702 dashed line indicates the 5% threshold line. (**f**) Serum interleukin 6 (IL-6) contents of FLW
703 pigs homozygous (QQ) or heterozygous (Qq) for the introgressed *GOLM1-NAA35* haplotypes.
704 Student's *t*-test was employed to compute the *P*-value ($P = 0.015$).
705
706 **Fig. 4. Introgression at the *KATNAL1* locus.** (**a**) rIBD values in a 2 Mb region encompassing
707 the *KATNAL1* gene. The brown dashed line indicates the 5% threshold line, and the *KATNAL1*
708 region is indicated by grey dashed lines. (**b**) Genetic differentiation index (F_{ST}) between French
709 Large White (FLW) and European wild boar (EWB) or East Chinese (ECN) pigs. (**c**) Haplotype
710 heatmap of the *KATNAL1* region. Major and minor alleles in FLW pigs are indicated by beige
711 and light blue, respectively. (**d**) Haplotype network in the *KATNAL1* region. The legend is the
712 same as in Figure 3. (**e**) Selection signals by XP-EHH analysis between FLW and other Large
713 White pigs. The brown dashed line indicates the 5% threshold line. (**f**) Estimated breeding
714 values for total number of piglets born (TNB EBV) of FLW sows that mated with FLW boars

homozygous (QQ) or heterozygous (Qq) for the introgressed haplotypes. Student's *t*-test was employed to compute the *P*-value ($P = 0.036$).

Fig. 5. Human-mediated introgression at the *AHR* locus. (**a**) Haplotype heatmap of a 100 kb region encompassing the *AHR* gene on chromosome 9 (86.5-86.6 Mb on Sscrofa11.1 and 95.4-95.56 Mb on Sscrofa10.2). Major and minor alleles in FLW pigs are indicated by beige and light blue, respectively. (**b**) *AHR* haplotype network. Each pie chart represents one unique haplotype, and the radius of the pie chart is proportional to the two times of $\log_2$ (number of chromosomes with that haplotype). The width and length of the edges are proportional to the $\log_2$ (number of pairwise differences between the joined haplotypes) plus one, and the thinnest edge represents a difference of one mutation. The full names of pig codes are given in the legend of Figure 3. (**c**) French Large White sows carrying the homozygous archaic *AHR* haplotype show significantly ($P = 2.39 \times 10^{-5}$) lower estimated breeding values for total number born EBV (TNB_EBV), compared with those who do not carry the archaic haplotype. (**d**) Erhualian sows homozygously carrying the archaic haplotype (QQ) have higher ($P = 0.0096$) TNB_EBV than heterozygous carriers (qq).

Figure 1

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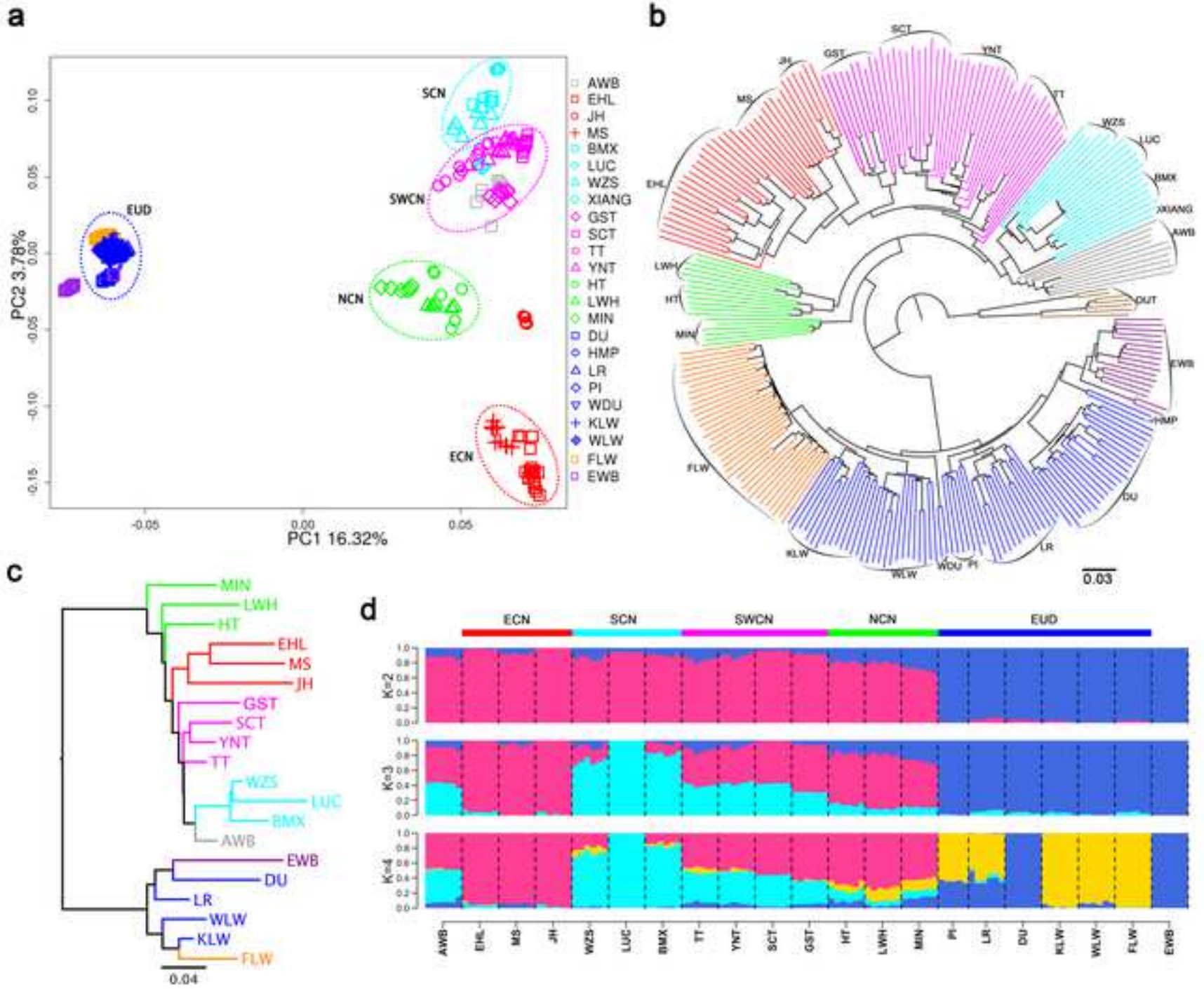

Figure 2

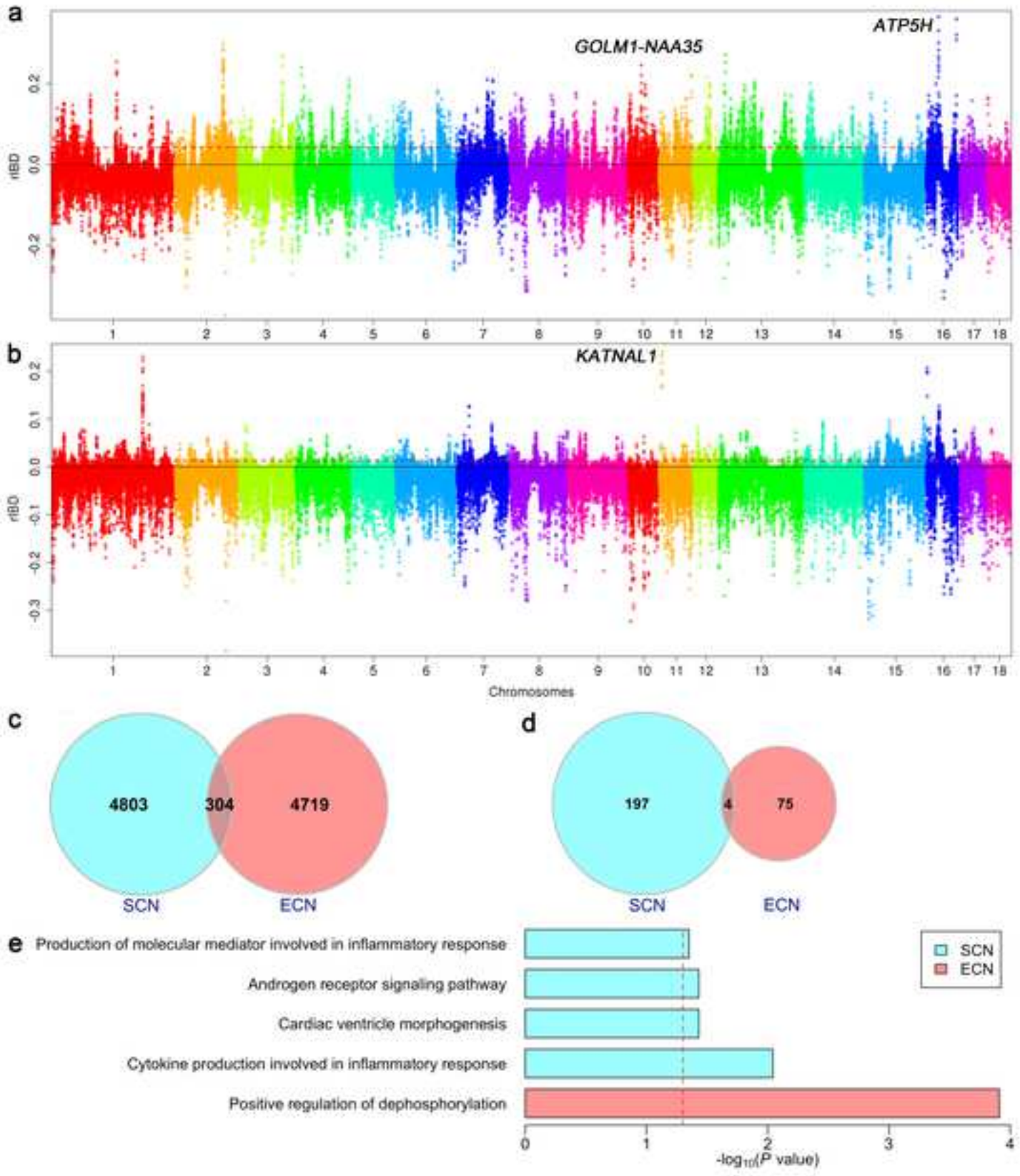

Figure 3

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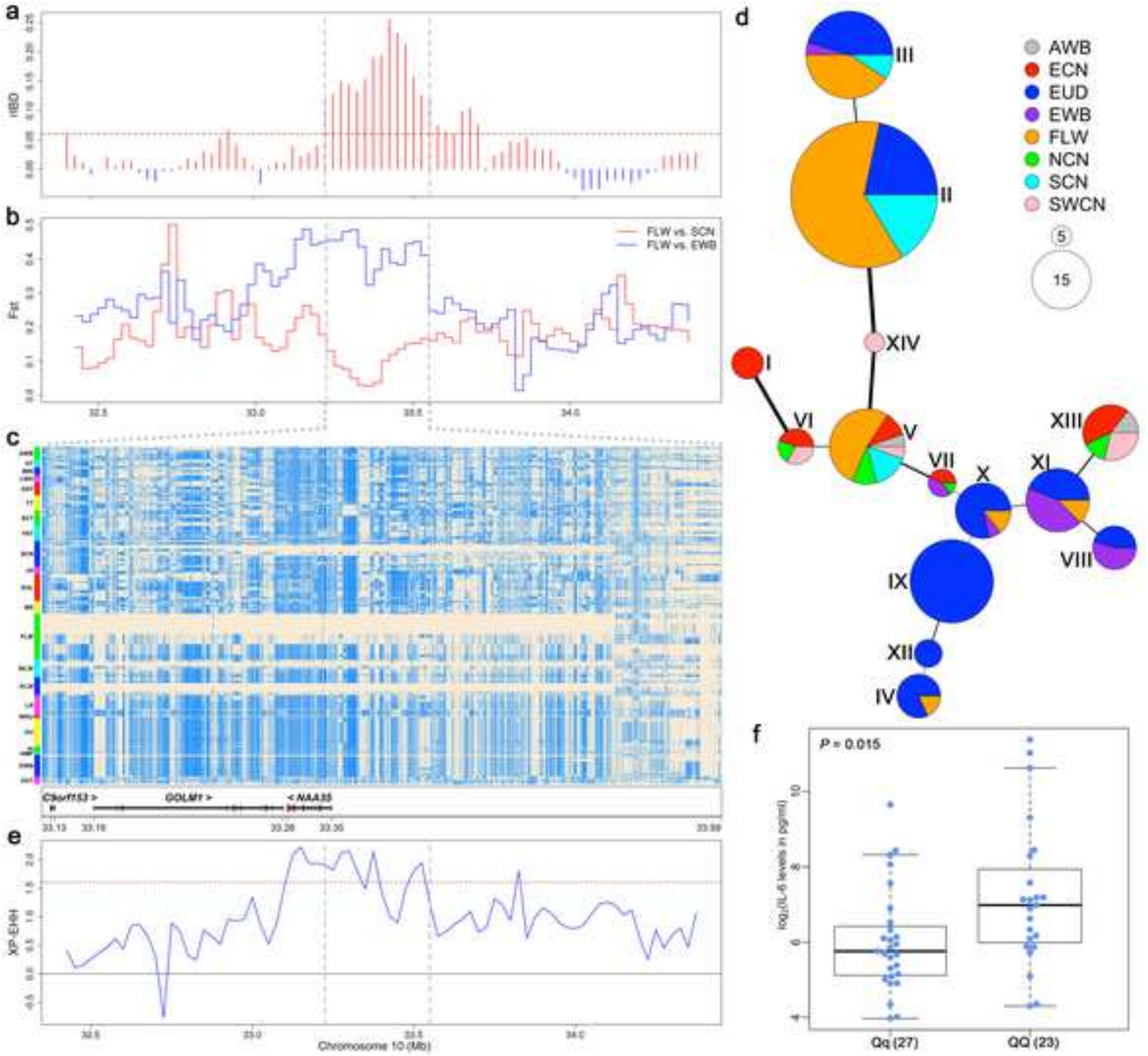

Figure 4

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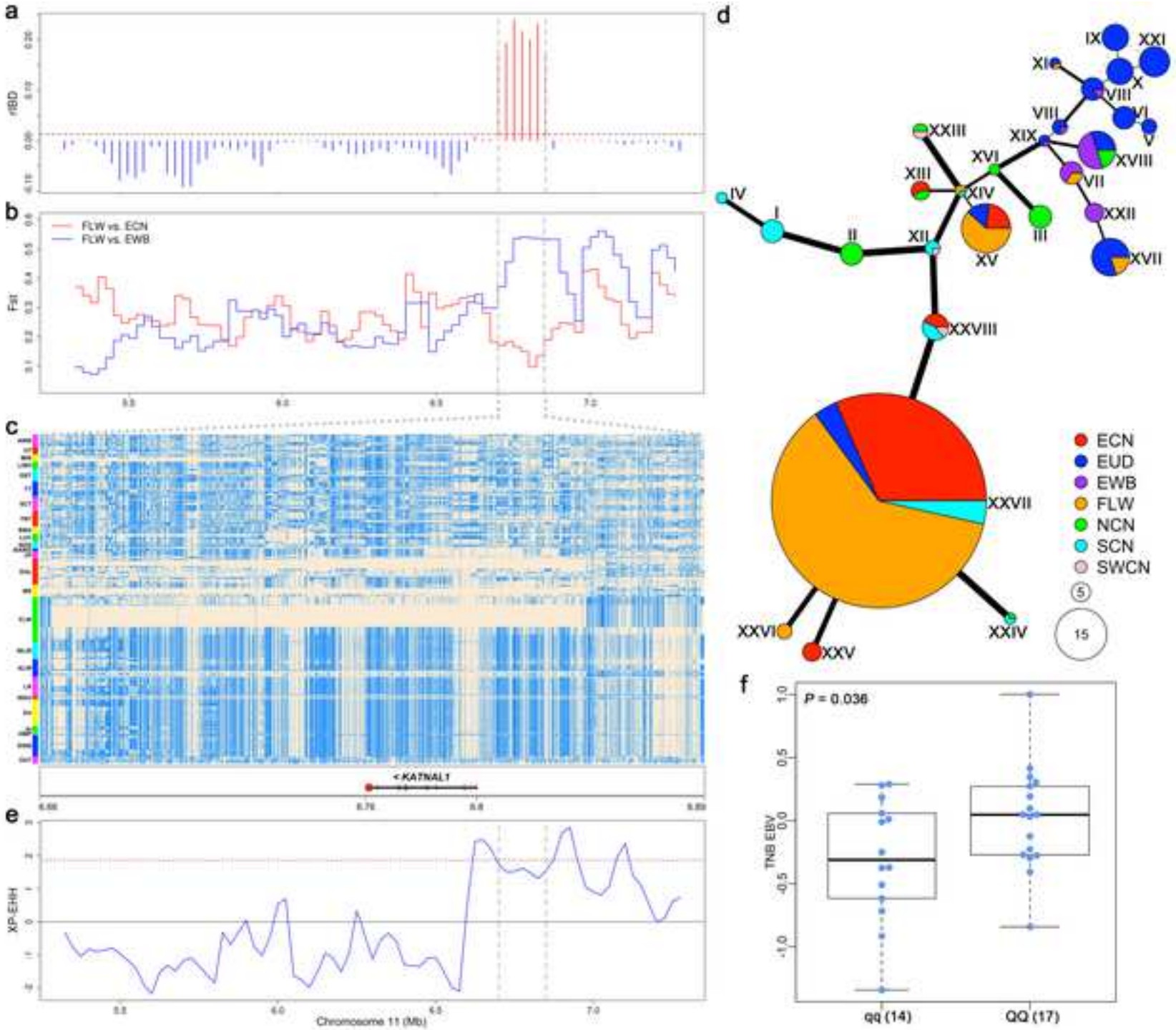

Figure 5

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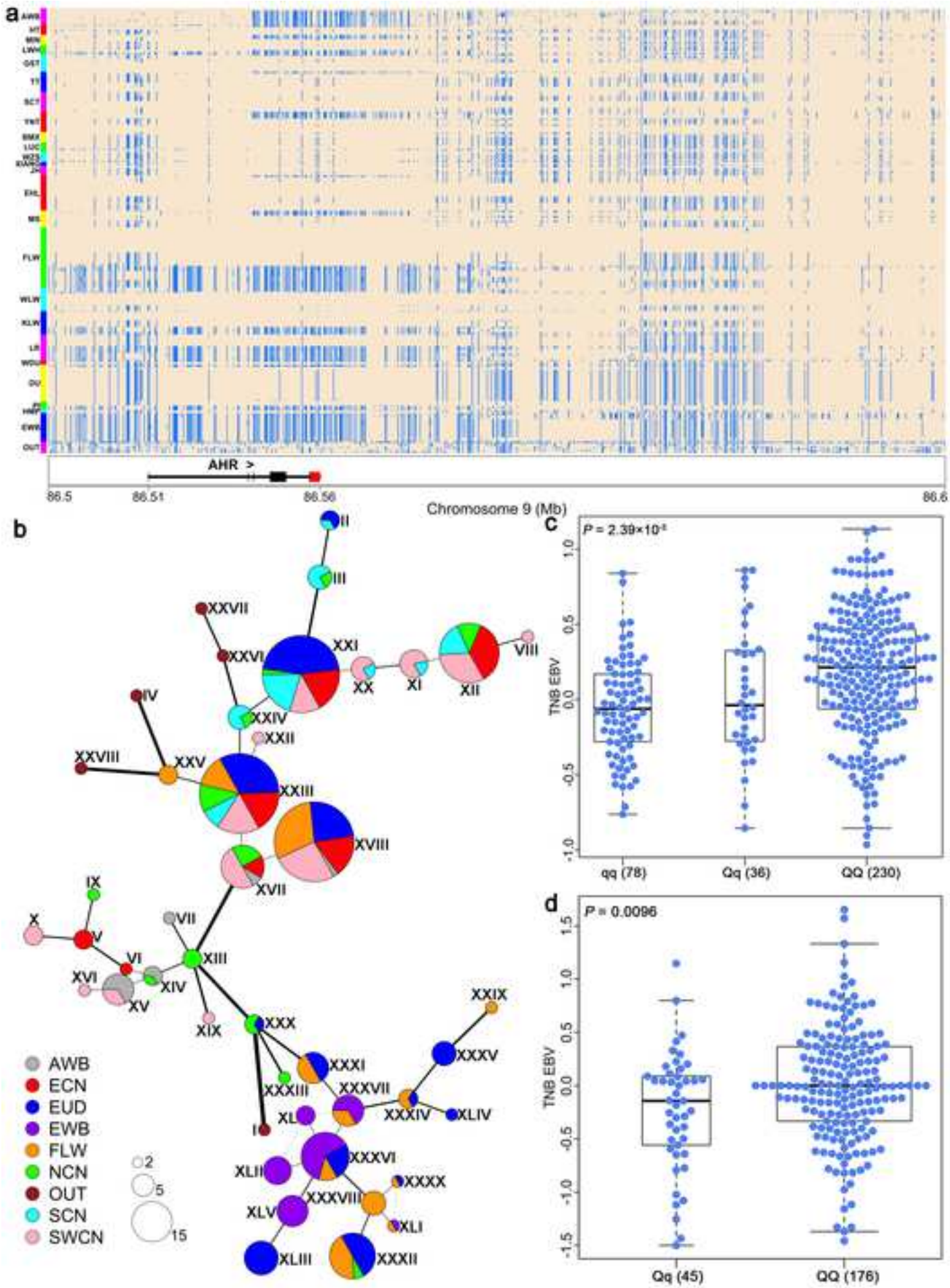

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