Supplemental Methods

RNA-seq data analysis

The strand-specific RNA-seq data (SRA accession number SRP158448) used in this study were obtained from our previous study (Yang et al. 2019). We utilized three biological replicates per developmental stage. Raw reads were filtered using Trim Galore! and aligned to the pig genome with HISAT2 (v2.2.1, --rna-strandness RF) (Kim et al. 2015). FeatureCounts (v2.0.1) (Liao et al. 2014) was used to quantify the number of reads assigned to each gene based on the genomic coordinates of gene exons listed in the gene annotation file (Ensembl release 112). Gene expression levels were normalized by calculating TPM values and further corrected for library size using edgeR, resulting in a final gene expression matrix for all developmental stages.

Functional annotation of genomic features

To assign associated genes to genomic features (such as H3K27ac peaks, TEs, and SEs), we followed the basal plus extension rule of GREAT (McLean et al. 2010). To further refine our results, we filtered the associated genes based on their expression levels (TPM > 0.5 in at least one sample). Subsequently, we explored the functionality of a set of genomic features by annotating their associated genes with GO functions using clusterProfiler (v4.12.0) (Yu et al. 2012) R package.

WGBS data analysis

The WGBS data (SRA accession number SRP160645) used in this study were retrieved from our

previous study (Yang et al. 2021). To assess the degree of methylation at each CpG site, we calculated the proportion of methylated reads to total reads. For each genomic region, we determined the average methylation level by calculating the ratio of methylated reads across all internal CpG sites to the total reads. Non-methylated regions across the genome were identified using tileHMM R package (v1.0.5) (Humburg and Humburg 2009) and established methods (Bell et al. 2020).

Enrichment analysis of genomic features for genomic functional marks

Simulations of random peaks were used to determine the enrichment of a set of genomic features in a set of functional genomics marks (Liu et al. 2022) including: GWAS hits within ± 20 kb, *cis*-eQTLs, and conserved DNA elements. Briefly, we counted the number of features overlapping with the marks as the observed value (Obs). Then 1000 simulations were performed to assess the enrichment of these features for the marks. In each round, we sampled genomic intervals to match the number and length of the features and recorded the number of intervals that overlapped with the marks as Sim. After 1000 iterations, we calculated the mean of Sim as Rand. Finally, the enrichment fold was calculated as Obs/Rand, and its significance *p* was determined as the proportion of instances where Sim ≥ Obs.

Identification of eRNAs

Seqmonk (v1.47.2) was utilized to detect eRNAs using established methods (Carullo et al. 2020). The approach involved the following steps: (1) Defining distal H3K27ac peaks from this study as potential active enhancers; (2) Defining potential eRNA transcription regions as enhancer ranges

within ± 500 bp; (3) Utilizing strand-specific RNA-Seq data (Yang et al. 2019) to calculate the read count of potential eRNA transcription regions; (4) Inferring the bidirectional transcription behavior of eRNAs when the number of reads matched to positive strands accounted for 5% to 95% of the total reads.

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