

## *Supplementary Material*

### **DNA demethylation of myogenic genes may contribute to embryonic leg muscle development differences between Wuzong and Shitou geese**

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1      **Supplementary Data**

Supplementary Data1. Detailed scripts of WGBS and RNA-seq analysis of this study have been provided in Supplementary Data.

2      **Supplementary Figures and Tables**

2.1   **Supplementary Tables**

Supporting information captions:

**Table S1. Data filtering and aligning information of WGBS**

**Table S2. Distribution of CG, CHG and CHH methylation of the two goose breeds in gene, genome and transposable element regions.**

**Table S3. GO enrichment analysis information of differentially methylated genes (CG) between goose breeds at same stage.**

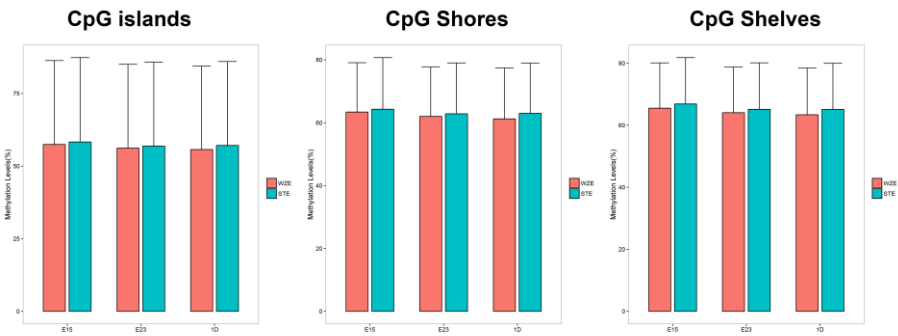
**Table S4. The methylation level of CG cites of *MyoD1* gene (upstream 2k, genebody and downstream 2k). The 13 CpGs analyzed by pyrosequencing are highlighted in yellow.**

**Table S5. The Pyrosequencing results of 13 methylation sites within the promoter region of *MyoD1*.**

**Table S6.    The primers for RT-qPCR**

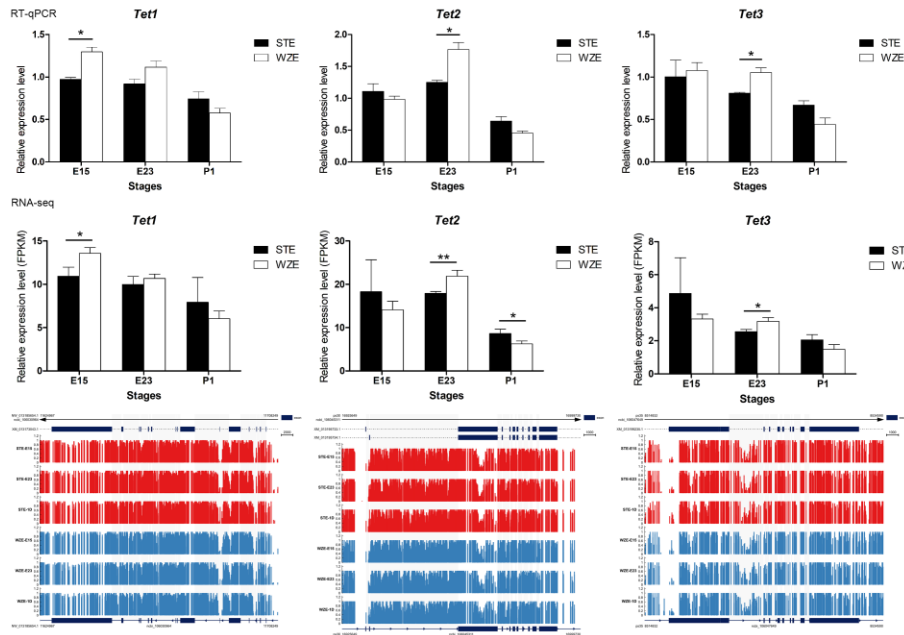
**Table S7. Design of pyrosequencing primers.**

2.2   **Supplementary Figures**

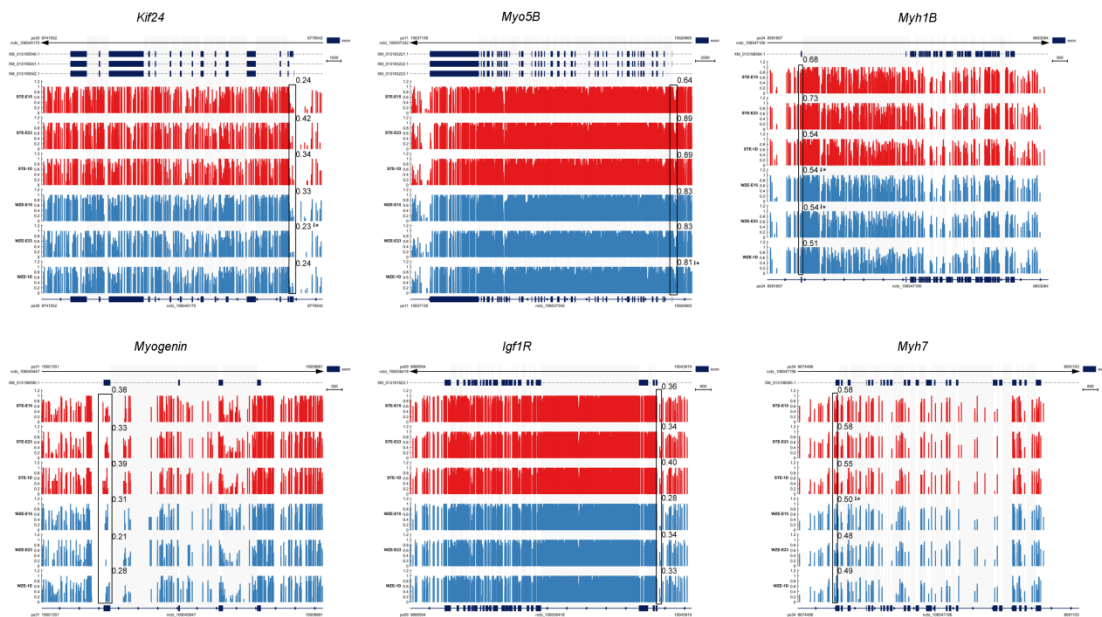


**Figure S1. DNA methylation comparison of CpG islands, shelves and shores.**The definition of CpG island is: CG content>50%, length>200 bp, observed CpG/expected

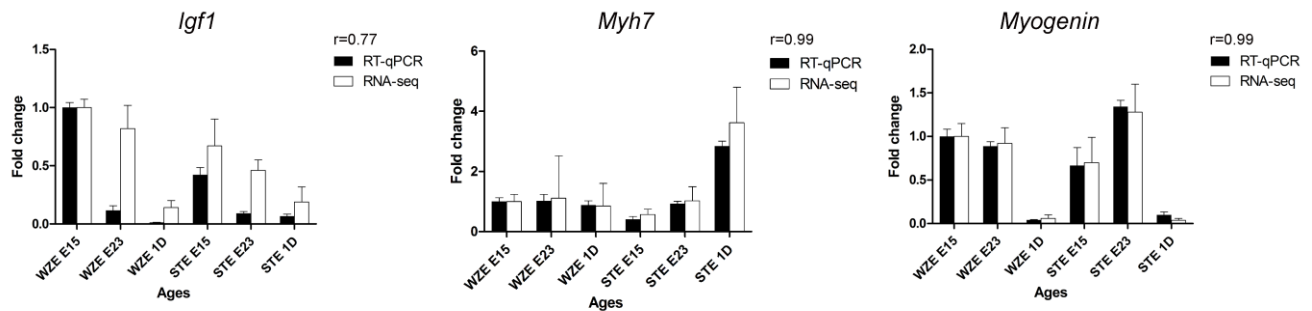
CpG>0.6. CpG shores refers to the area located within 2 kb upstream and downstream of CpG island; CpG shelves refers to the area located within 2 kb upstream and downstream of CpG shores (n=3).



**Figure S2. Gene expression patterns and DNA methylation level of *Tet1*, *Tet2* and *Tet3*.** RT-qPCR and RNA-seq gene expression patterns and of *Tet1*, *Tet2* and *Tet3* in STE and WZE during E15–P1 were analyzed (n=3). DNA methylation level of *Tet1*, *Tet2* and *Tet3* in WGBS data was illustrated by IGV software in STE and WZE during E15–P1 (n=3). \* $p < 0.05$ ; \*\* $p < 0.01$ .

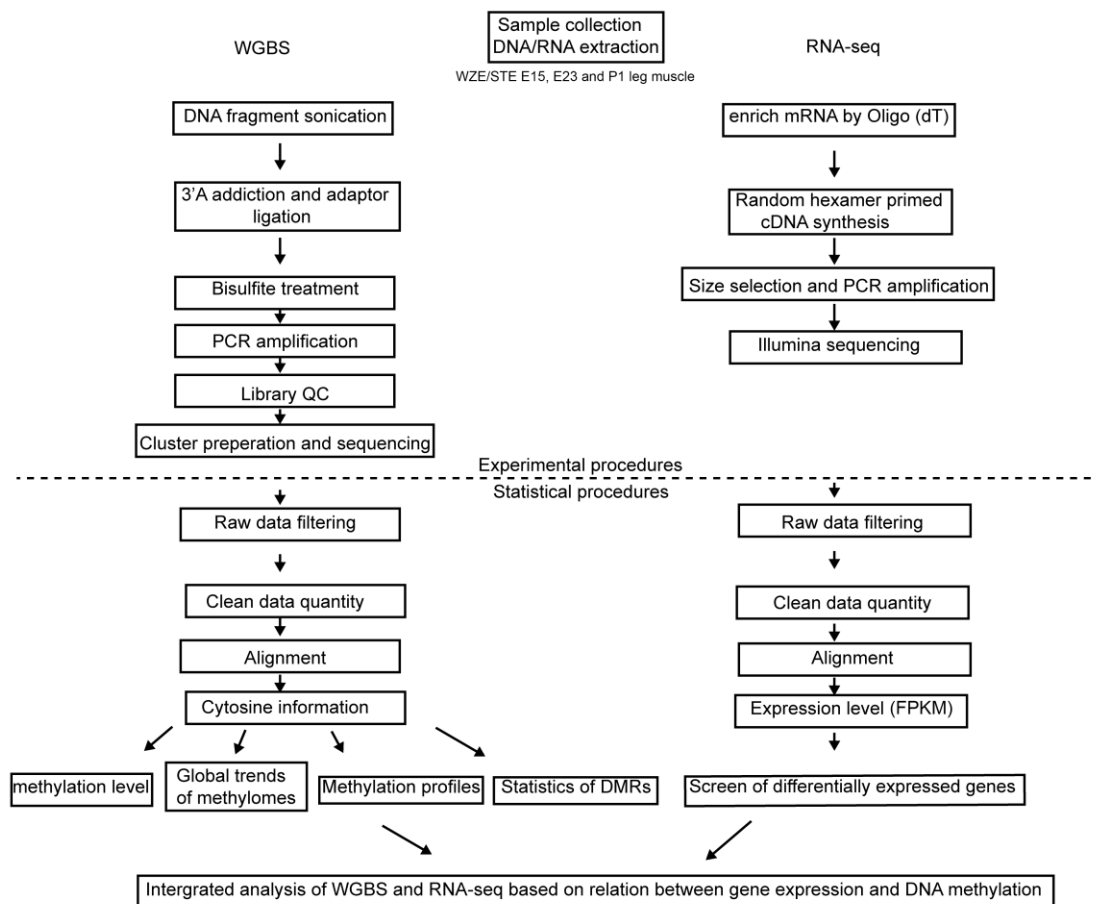


**Figure S3. Methylation level of myogenic related DEGs related to DNA methylation or vital myogenic genes in WGBS data illustrated by IGV software.** Regions around TSS are marked in black boxes with average DNA methylation level beside the boxes (n=3). Downward arrows indicate a lower DNA methylation level in comparison with STE.\* $p < 0.05$ .

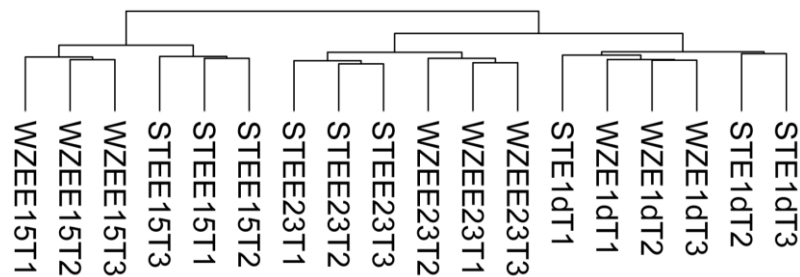


**Figure S4. Validation of RNA-seq data by RT-qPCR.**

RT-qPCR and RNA-seq validation of *Igf1*, *Myf7* and *Myogenin* expression (n=3). Pearson Correlation Coefficient (R) between RT-qPCR and RNA-seq was calculated.



**Figure S5. An overview of this study's design and analysis.** This figure summarized sample collection, sample handling, experimental procedures, and data processing procedures.



**Figure S6. Cluster analysis of RNA-seq data.** RNA-seq data of STE and WZE during E15 – P1 was used (n=3).