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Generation of biallelic knock-out sheep via gene-editing and somatic cell nuclear transfer

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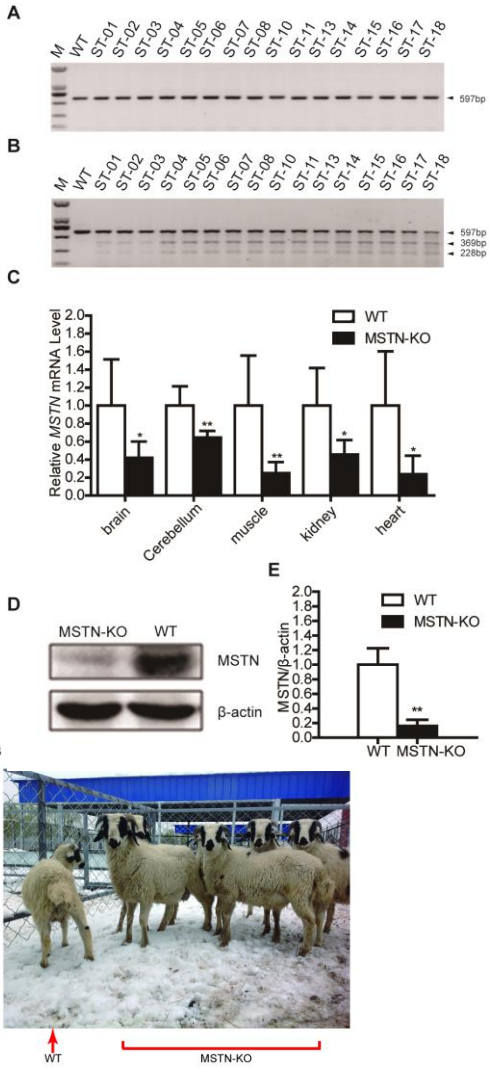
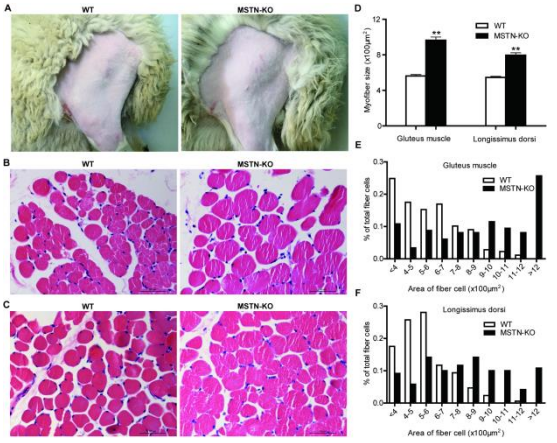
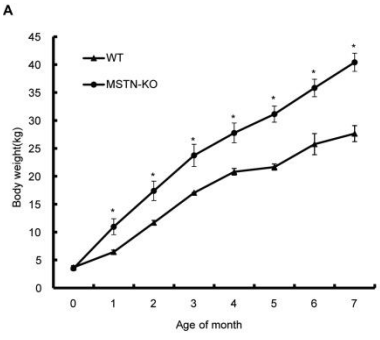
Abstract Transgenic sheep can be used to achieve genetic improvements in breeds and as an important large-animal model for biomedical research. In this study, we generated a TALEN plasmid specific for ovine MSTN and transfected it into fetal fibroblast cells of STH sheep. MSTN biallelic-KO somatic cells were selected as nuclear donor cells for SCNT. In total, cloned embryos were transferred into 37 recipient gilts, 28 (75.7%) becoming pregnant and 15 delivering, resulting in 23 lambs, 12 of which were alive. Mutations in the lambs were verified via sequencing and T7EI assay, and the gene mutation site was consistent with that in the donor cells. Off-target analysis was performed, and no off-target mutations were detected. MSTN KO affected the mRNA expression of MSTN relative genes. The growth curve for the resulting sheep suggested that MSTN KO caused a remarkable increase in body weight compared with those of wild-type sheep. Histological analyses revealed that MSTN KO resulted in muscle fiber hypertrophy. These findings demonstrate the successful generation of MSTN biallelic-KO STH sheep via gene editing in somatic cells using TALEN technology and SCNT. These MSTN mutant sheep developed and grew normally, and exhibited increased body weight and muscle growth.







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