## A *MSTN*Del73C mutation with *FGF5* knockout sheep by CRISPR/Cas9 promotes skeletal muscle myofiber hyperplasia

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### Supplementary file 1 Production and slaughter information of *MSTN*Del73C mutation with *FGF5* knockout sheep

### Supplementary file 1A Summary of generation of sheep carrying biallelic mutations in dual genes via the CRISPR/Cas9 system

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| --- | --- | --- | --- | --- | --- | --- |
| Cas9/sgRNA(molar ratio) | Embryo injected | Recipients | Pregnancy | Alive lambs | Mutation | Biallelic mutation |
| MSTN | FGF5 | MSTN+FGF5 | MSTN | FGF5 | MSTN+FGF5 |
| 1:2 | 448 | 93 | 35 | 28 | 1 | 3 | 1 | 0 | 0 | 0 |
| 1:10 | 365 | 84 | 26 | 22 | 2 | 2 | 2 | 2 | 2 | 2 |
| 1:15 | 388 | 59 | 17 | 14 | 1 | 0 | 0 | 1 | 0 | 0 |

### Supplementary file 1B Muscle weight of different parts in WT and MF+/- sheep (g)

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| --- | --- | --- | --- |
| **Muscle Classification** | **WT (n=3)** | **MF+/- (n=4)** | ***P*-value** |
| Longissimus dorsi | 905.67±37.15 | 760.00±48.68 | 0.077 |
| Biceps brachii | 50.67±2.67 | 48.25±3.2 | 0.606 |
| Triceps brachii | 247.33±18.41 | 265.00±8.01 | 0.374 |
| Gluteus medius | 305.00±25.24 | 339.75±20.1 | 0.324 |
| Semimembranous | 199.33±21.84 | 194.00±18.5 | 0.859 |
| Semitendinosus | 435.33±41.73 | 400.00±19.71 | 0.438 |
| Biceps femoris | 712.00±57.65 | 619.50±78.39 | 0.417 |
| Quadriceps femoris | 604.67±46.23 | 612.50±29.5 | 0.886 |

### Supplementary file 1C The slaughter traits of muscles in WT and MF+/- sheep

|  |  |  |  |
| --- | --- | --- | --- |
| **Slaughter Indexes** | **WT (n=3)** | **MF+/- (n=4)** | ***P*-value** |
| Live weight (kg) | 56.33±3.088 | 50.15±2.058 | 0.14201 |
| Carcass weight(kg) | 32.23±2.436 | 28.5±1.588 | 0.23588 |
| Slaughter percentage (%) | 57.12±1.237 | 56.75±1.259 | 0.84403 |
| loin muscle area (cm2) | 17.17±1.58 | 13.95±1.757 | 0.24795 |
| Meat weight (kg) | 18.79±1.306 | 15.68±0.825 | 0.08707 |
| The proportion of meat in carcass | 0.58±0.005 | 0.55±0.018 | 0.18691 |
| The proportion of brisket and neck meat | 0.14±0.018 | 0.13±0.004 | 0.85322 |
| The proportion of loin meat | 0.11±0.005 | 0.09±0.011 | 0.33339 |
| The proportion of rib meat | 0.22±0.004 | 0.15±0.003 | 0.00003 |
| The proportion of foreleg meat | 0.18±0.009 | 0.21±0.012 | 0.20974 |
| The proportion of hind leg meat | 0.33±0.009 | 0.4±0.016 | 0.0252 |
| Neat percentage (%) | 0.58±0.005 | 0.55±0.018 | 0.18691 |

### Supplementary file 1D Meat quality of longissimus dorsi in WT and MF+/- sheep

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| --- | --- | --- | --- |
| **Meat Quality** | **WT (n=3)** | **MF+/- (n=4)** | ***P*-value** |
| pH45min | 6.37±0.136 | 6.38±0.043 | 0.95934 |
| pH24h | 5.58±0.038 | 5.49±0.038 | 0.230 |
| L\* | 32.14±2.165 | 29.81±2.165 | 0.326 |
| a\* | 13.44±0.452 | 15.29±0.452 | 0.084 |
| b\* | 7.52±0.990 | 6.22±0.990 | 0.224 |
| Drip loss (%) | 24.84±1.802 | 26.11±1.802 | 0.446 |
| Cooking loss (%) | 30.62±0.864 | 29.15±0.864 | 0.314 |

### Supplementary file 1E Shearing force of different parts in WT and MF+/- sheep (N)

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| --- | --- | --- | --- |
| **Muscle Classification** | **WT (n=3)** | **MF+/- (n=4)** | ***P*-value** |
| Longissimus dorsi | 42.7±5.497 | 40.5±6.080 | 0.808 |
| Biceps brachii | 37.7±2.125 | 40.2±8.675 | 0.806 |
| Triceps brachii | 49.8±7.174 | 55.5±5.023 | 0.530 |
| Gluteus medius | 53.2±4.946 | 68.0±7.206 | 0.181 |
| Semimembranous | 54.2±3.786 | 64.3±8.376 | 0.379 |
| Semitendinosus | 49.6±4.476 | 45.1±6.445 | 0.616 |
| Biceps femoris | 37.3±6.467 | 54.1±4.721 | 0.084 |
| Quadriceps femoris | 47.2±3139 | 57.0±7.298 | 0.325 |

### Supplementary file 1F Amino acid content of longissimus dorsi in WT and MF+/- sheep (%, DM basis)

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| --- | --- | --- | --- |
| **Amino Acid** | **WT (n=3)** | **MF+/- (n=4)** | ***P*-value** |
| Aspartic acid (Asp) | 5.62±0.449 | 6.22±0.348 | 0.334 |
| Threonine (Thr) | 2.68±0.211 | 3.00±0.162 | 0.271 |
| Serine (Ser) | 2.11±0.164 | 2.40±0.133 | 0.222 |
| Glutamic acid (Glu) | 10.71±0.848 | 12.18±0.61 | 0.207 |
| Proline (Pro) | 2.04±0.164 | 2.33±0.134 | 0.224 |
| Glycine (Gly) | 2.62±0.203 | 2.92±0.171 | 0.311 |
| Alanine (Ala) | 3.47±0.272 | 3.87±0.210 | 0.285 |
| Cystine (Cys) | 0.54±0.050 | 0.57±0.029 | 0.707 |
| Valine (Val) | 3.20±0.256 | 3.52±0.196 | 0.356 |
| Methionine (Met) | 1.70±0.139 | 1.87±0.101 | 0.353 |
| Isoleucine (Ile) | 3.03±0.246 | 3.38±0.183 | 0.295 |
| Leucine (Leu) | 5.09±0.406 | 5.62±0.318 | 0.346 |
| Tyrosine (Tyr) | 2.11±0.186 | 2.06±0.338 | 0.912 |
| Phenylalanine (Phe) | 3.15±0.279 | 3.48±0.207 | 0.376 |
| Lysine (Lys) | 5.32±0.437 | 5.97±0.358 | 0.297 |
| Histidine (His) | 2.22±0.200 | 2.38±0.179 | 0.577 |