

Supplementary Material

Generation of gene-edited sheep with a defined Booroola fecundity gene (*FecB^B*) mutation in bone morphogenetic protein receptor type 1B (*BMPRI1B*) via clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) 9

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#37



Figure S1. Appearance of two lambs (#06 and #37) with BMPR1B disruption. Photos were taken by Shiwei Zhou in Apr 2018 after shearing.

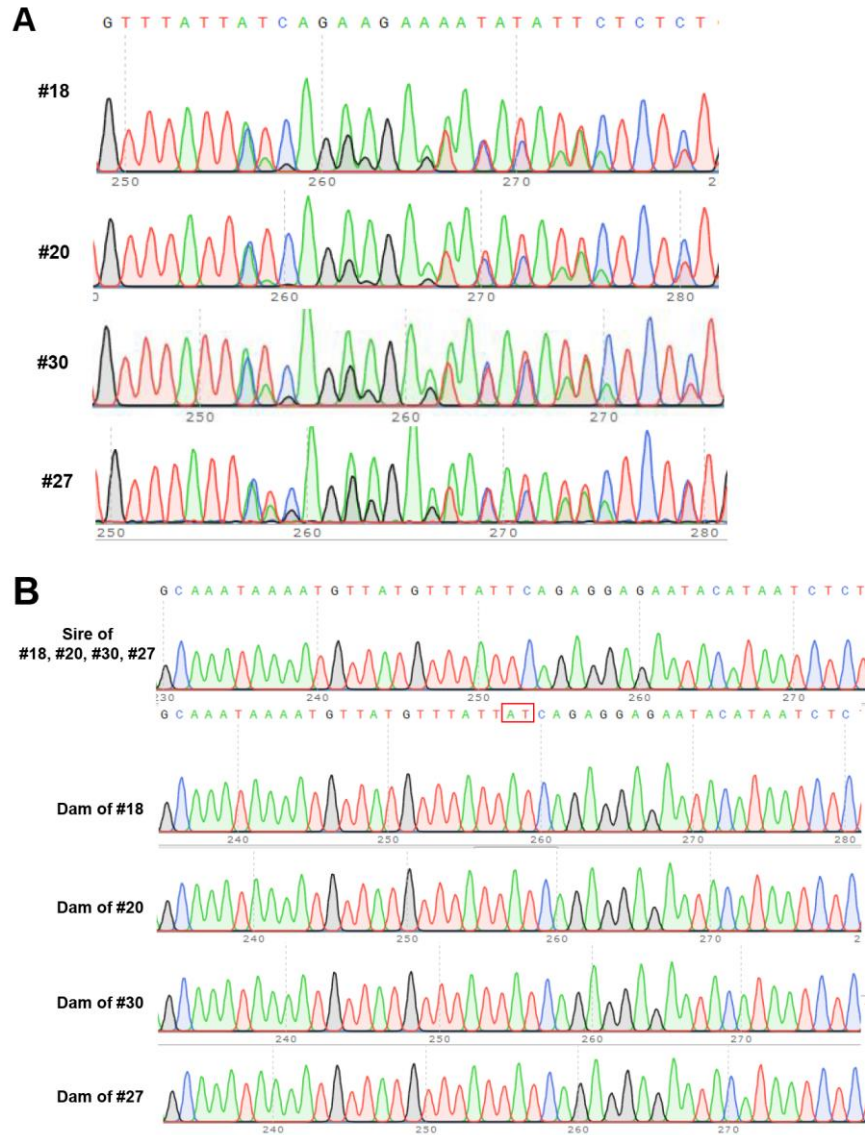


Figure S2. Sanger sequencing results of goat at the OT3 site. (A) Sequencing results of edited animals (#18, #20, #30) and an unedited control (#27). (B) Sequencing results of parents for edited animals (#18, #20, #30) and an unedited control (#27). The red box indicates 2 bp deletion in all the dam of #18, #20, #30, and #27.

Table S1 sgRNA of target sites in the ovine BMPR1B gene.

| sgRNA | Targeting site | Location | Strand |
|--------------|-------------------------|------------------------|--------|
| BMPR1B sgRNA | AGAGACAGAAATATATCAGACGG | Chr6:30695573-30695596 | + |

Coordinates of sgRNA target sites are based on the sheep genome assembly OAR v3.1.

Table S2 Oligonucleotides for generating sgRNA expression vectors.

| Name | Sequence |
|----------------------------|--|
| BMPR1B sgRNA top strand | ACCGAGAGACAGAAATATATCAGA |
| BMPR1B sgRNA bottom strand | AAACTCTGATATATTTCTGTCTCT |
| ssODN | CTACAGAGGAGGCCAGCTGGTTCCGAGAGACAGAAATATATCAG ACGGTGTGATGAGGCATGAAAACATCTT |

Table S3 Primers for genotyping and amplifying Cas9/sgRNA targeted BMPR1B fragment.

| Name | Sequence | Amplicon (bp) |
|----------|--------------------------|---------------|
| BMPR1B_F | CAGTTGTTTTCTTCTCTGAAGGAA | 709 |
| BMPR1B_R | AGTTAGAAATGATCTAACTGGAT | |

Table S4 Primers for genotyping and amplifying predicted off-target site fragments.

| Off-target site | Sequence | Amplicon (bp) |
|-----------------|-----------------------|---------------|
| BMPR1B -OT1F | AATATTTTTGCGTGTGGACAG | 290 |
| BMPR1B -OT1R | CAATTCCTCACACTACAGTT | |
| BMPR1B -OT2F | GATTTAGCTTGTGTTTCTTCC | 365 |
| BMPR1B -OT2R | GAGAATGTCAAAGCCCTTAAC | |
| BMPR1B -OT3F | GTTTTCTTTGGTTAGGTCTGC | 520 |
| BMPR1B -OT3R | CTGATTGGTTAGTTCCTGTGC | |
| BMPR1B -OT4F | CCCTACTGTGACTGCTTTCCA | 443 |
| BMPR1B -OT4R | ATGTTCTTAGCCACTACTGAT | |
| BMPR1B -OT5F | TCACACTGATGAGGAAAGGTC | 477 |
| BMPR1B -OT5R | ACCTTIGCAACTTTCATCACA | |
| BMPR1B -OT6F | AGCTTCTTAAATGGCTCCTCA | 478 |
| BMPR1B -OT6R | TCCCTTCTTTAGACATGCAC | |
| BMPR1B -OT7F | ACCGATAGAGACAGTGTGGC | 469 |
| BMPR1B -OT7R | ACACGAAGGCAGTAACAGCAA | |
| BMPR1B -OT8F | ATTTTGAAGAAAGCTCTGGTC | 601 |
| BMPR1B -OT8R | AAACTAGGCTGCTCTAATCTC | |
| BMPR1B -OT9F | CTCCCATGTTTTGTCTCAT | 579 |
| BMPR1B -OT9R | TTCATGTCGTGATTTCAAGCC | |

Table S5 List of predicted off-target sites.

| Position | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | Location | | | | | | | |
|----------|----|----|----|----|----|----|----|----|----|----|----|---|---|---|---|---|---|---|---|---|----------|-------|-----|--------|-------------|-------------|-------------|---|
| sgRNA | A | G | A | G | A | C | A | G | A | A | A | T | A | T | A | T | C | A | G | A | Chr. | Start | End | Strand | | | | |
| OT1 | A | G | A | G | A | C | A | G | A | A | A | G | A | T | A | T | A | C | A | G | A | A | G | G | 16 | 32,719,137 | 32,719,158 | - |
| OT2 | A | G | A | G | A | C | A | G | G | A | A | T | A | T | A | T | A | A | G | A | A | G | G | G | 2 | 8,106,479 | 8,106,500 | + |
| OT3 | A | G | A | G | G | C | A | G | A | A | A | T | T | A | T | C | A | G | A | C | G | G | G | 2 | 151,208,161 | 151,208,182 | + | |
| OT4 | A | G | A | G | A | C | A | C | A | A | A | T | A | T | G | T | C | A | G | A | A | G | G | G | 13 | 68,414,290 | 68,414,311 | - |
| OT5 | T | G | A | A | A | C | A | G | A | A | A | T | A | T | A | T | C | A | C | A | A | G | G | G | 1 | 208,231,801 | 208,231,822 | + |
| OT6 | A | G | A | A | C | C | A | G | G | A | A | T | A | T | A | T | C | A | G | A | A | A | G | G | 15 | 59,185,189 | 59,185,210 | + |
| OT7 | T | G | C | T | A | C | A | G | A | A | A | T | A | T | A | T | C | A | G | A | A | T | G | G | 11 | 50,956,905 | 50,956,926 | - |
| OT8 | A | A | A | G | C | C | A | A | A | A | A | T | A | T | A | T | C | A | G | A | A | G | G | G | 12 | 13,498,257 | 13,498,278 | + |
| OT9 | A | G | T | T | A | G | A | G | A | A | A | T | A | T | A | T | C | A | G | A | A | T | G | G | 2 | 208,282,633 | 208,282,654 | + |

Similar nucleotide to the target site was mark in red, the PAM sequence was marked as green color. Coordinates of sgRNA target sites are based on the sheep genome assembly OAR v3.1.