

Appendix A Growth parameters for mutant and control sheep for GLM test**a.** Results about large fragment deletion, mutant, control by using GLM.

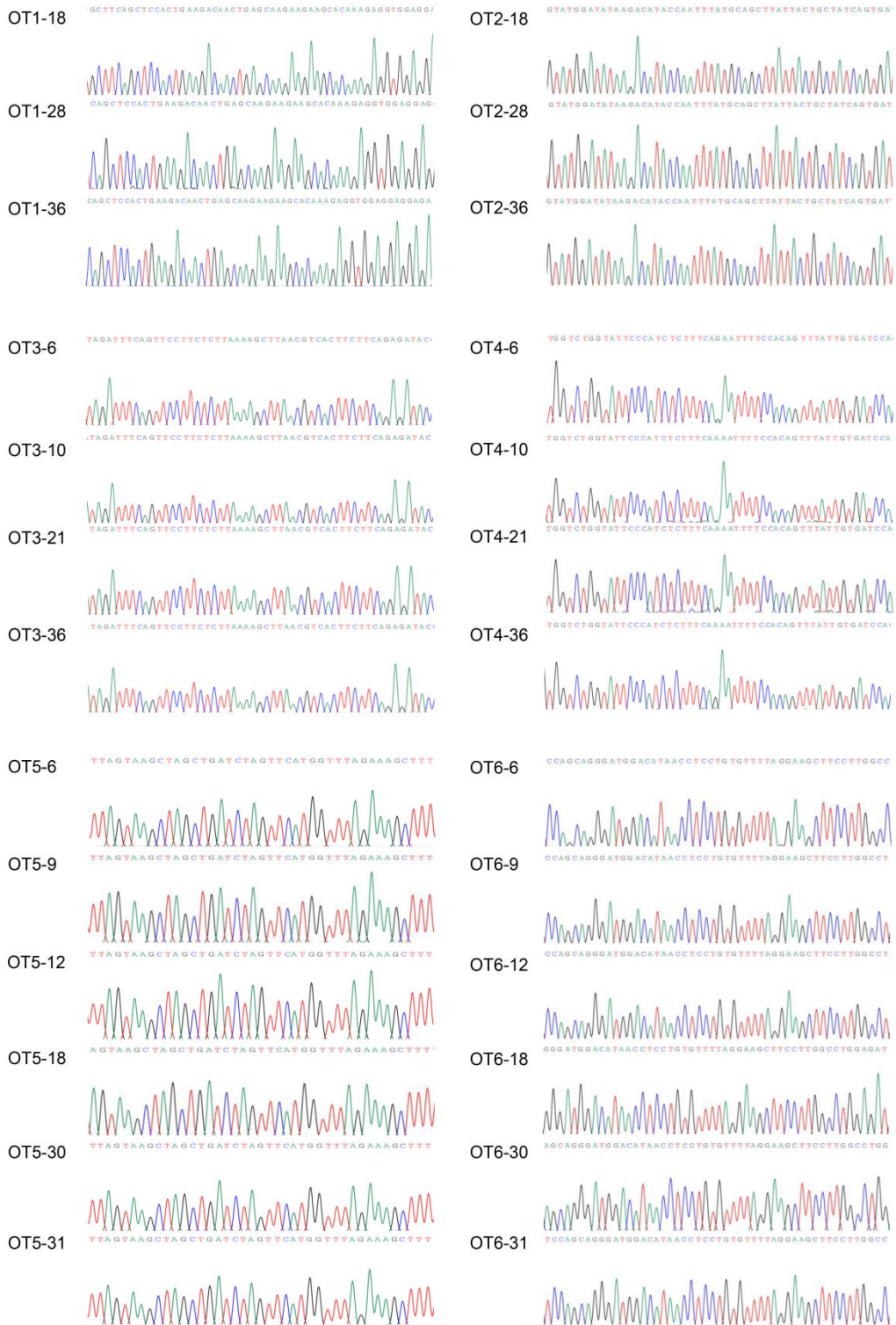
Growth parameter	Large fragment deletion (n=3; means±SD)	Mutant (n=10; means±SD)	Control (n=10; means±SD)	P-value
Birth weight (kg)	6.20±1.69 ^a	5.33±0.97 ^a	3.44±0.52 ^b	< 0.0001
BW at D15 (kg)	10.50±3.54 ^a	9.27±2.21 ^a	5.69±1.29 ^b	< 0.0001
BW at D30 (kg)	13.40±3.96 ^a	12.32±2.48 ^a	8.24±2.30 ^b	0.002
Weaning weight (D60, kg)	18.30±4.81 ^a	16.88±3.16 ^a	12.44±3.57 ^b	0.011
BW at D90 (kg)	25.25±3.46	23.93±3.31	20.42±5.54	0.143
BW at D120 (kg)	27.20±1.69	26.67±3.60	22.22±5.60	0.085
BW at D150 (kg)	28.60±2.69	27.94±2.95	24.79±6.67	0.318
BW at D180 (kg)	30.40±2.26	29.48±3.47	25.60±6.71	0.198

b. Results about large fragment deletion, few bases mutant, control by using GLM.

Growth parameter	Large fragment deletion (n=3; means±SD)	Single mutant (n=7; means±SD)	Control (n=10; means±SD)	P-value
Birth weight (kg)	6.20±1.69 ^a	5.09±0.72 ^b	3.44±0.52 ^c	< 0.0001
BW at D15 (kg)	10.50±3.54 ^a	8.91±1.99 ^a	5.69±1.29 ^b	0.001
BW at D30 (kg)	13.40±3.96 ^a	12.01±2.29 ^a	8.24±2.30 ^b	0.005
Weaning weight (D60, kg)	18.30±4.81 ^a	16.47±2.97 ^{ab}	12.44±3.57 ^b	0.027
BW at D90 (kg)	25.25±3.46	23.56±3.43	20.42±5.54	0.244
BW at D120 (kg)	27.20±1.69	26.53±4.03	22.22±5.60	0.162
BW at D150 (kg)	28.60±2.69	27.76±3.17	24.79±6.67	0.438
BW at D180 (kg)	30.40±2.26	29.21±3.81	25.60±6.71	0.312

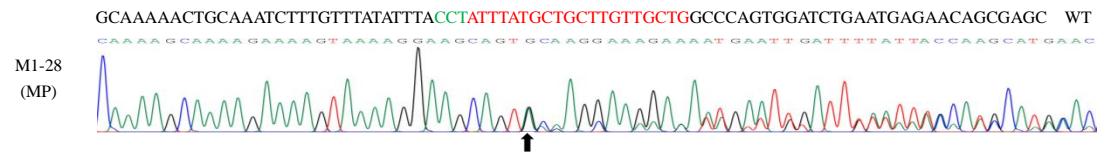
Appendix B Primers for amplifying off-targeted sites Cas9/sgRNA targeted *MSTN*

Gene targeted	Name of primer	Sequence (5'→3')	Amplicon (bp)
<i>MSTN</i> -sg1	<i>MSTN</i> OT1 Forward	CCCTGGGGATTCAGTGTTG	811
	<i>MSTN</i> OT1 Reverse	TGAGGGCTCGTCATTGCT	
	<i>MSTN</i> OT2 Forward	CAAAGAGTAGTGTTCCCTGGTG	613
	<i>MSTN</i> OT2 Reverse	CATTATGGGGCTTGTGGTT	
<i>MSTN</i> -sg2	<i>MSTN</i> OT3 Forward	TTGTCCCTTGTGGTGGTTC	505
	<i>MSTN</i> OT3 Reverse	TGTGCTAGAAACCAAGGTAAGG	
	<i>MSTN</i> OT4 Forward	CGTCCATCCCAGCGTTTC	400
	<i>MSTN</i> OT4 Reverse	CGCCAGGCTTCAGCAATA	
<i>MSTN</i> -sg3	<i>MSTN</i> OT5 Forward	TCCATTCACCCTTCCGCCTTAT	320
	<i>MSTN</i> OT5 Reverse	TTGGAAAAGACCCTGATGCT	
	<i>MSTN</i> OT6 Forward	GAACTGGGGTCTAAGCACTACG	689
	<i>MSTN</i> OT6 Reverse	TGGGAAAGCCCTAAGCAAC	

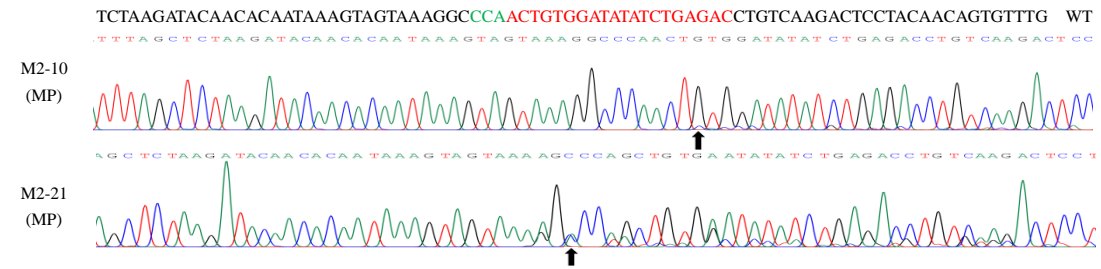


Appendix C Sequencing results of PCR products. Detection of sgRNA: Cas9-mediated off-target cleavage of *MSTN* by sanger sequencing.

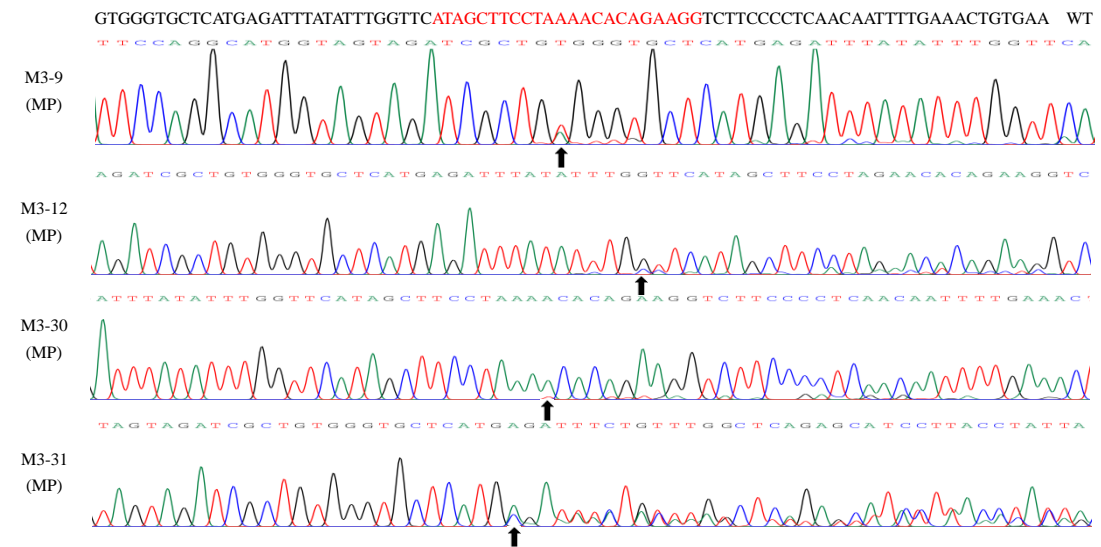
MSTN-sg1



MSTN-sg2



MSTN-sg3



Appendix D Sanger sequencing of modified loci was detected in positive sheep. Arrows indicate the sites of modified loci, MP, multiple peaks.

Appendix E sgRNA sequences and target sites

sgRNA	Targeting site	Location	strand
<i>MSTN</i> -sg1	CAGCAACAAGCAGCATAAATAGG	Chr2:118140452-118140474	-
<i>MSTN</i> -sg2	GTCTCAGATATATCCACAGTTGG	Chr2:118142714-118142736	-
<i>MSTN</i> -sg3	ATAGCTTCCTAAAACACAGAAGG	Chr2:118145427-118145449	+

Coordinates of sgRNA target sites are based on the sheep genome assembly Oar_v4.0

Appendix F Oligonucleotides for generating sgRNA expression vectors

Item	Sequence (5'→3')
<i>MSTN</i> sgRNA-1 top strand	TAGGCAGCAACAAGCAGCATAAAT
<i>MSTN</i> sgRNA-1 bottom strand	AAACATTTATGCTGCTTGTTGCTG
<i>MSTN</i> sgRNA-2 top strand	TAGGTCTCAGATATATCCACAGT
<i>MSTN</i> sgRNA-2 bottom strand	AAACACTGTGGATATATCTGAGA
<i>MSTN</i> sgRNA-3 top strand	TAGGATAGCTTCCTAAAACACAGA
<i>MSTN</i> sgRNA-3 bottom strand	AAACTCTGTGTTTTAGGAAGCTAT

Appendix G Primers for genotyping and amplifying Cas9/sgRNA targeted *MSTN* fragment

Gene targeted	Name of primer	Sequence (5'→3')	Amplicon (bp)
<i>MSTN</i> -sg1	<i>MSTN</i> E1 Forward	GTTTGGTGACTTGTGACAGA	886
	<i>MSTN</i> E1 Reverse	CGAATGCTATGCAAGCATTTC	
<i>MSTN</i> -sg2	<i>MSTN</i> E2 Forward	GACATGGAGGCGTTCGTTTCATT	422
	<i>MSTN</i> E2 Reverse	CTGGGAAGGTTACAGCAAGATCA	
<i>MSTN</i> -sg3	<i>MSTN</i> E3 Forward	GTCAGGCATTCAGATATTCA	821
	<i>MSTN</i> E3 Reverse	TGAACATAAATGGAATTTGA	