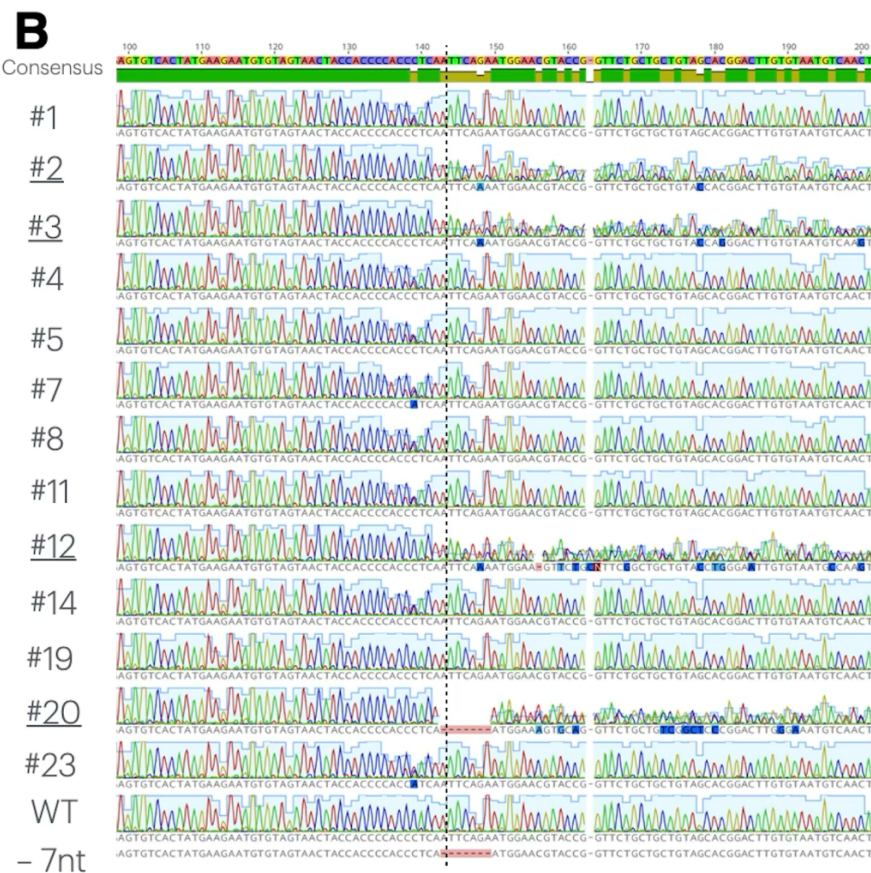
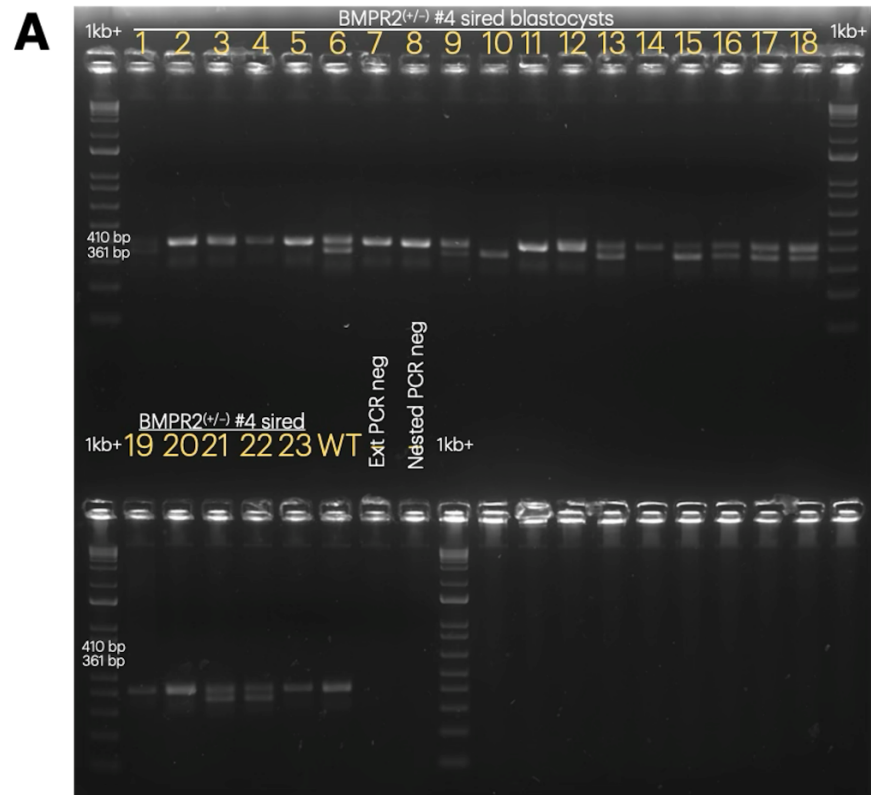


Supplemental Figures and Tables



Supplemental Figure 1. Radiographs of Lamb #6 taken on day of life 1. (A) Arthrogryposis of both forelimbs, with hyperextension of the metacarpal phalangeal joint and hyperflexion of the distal interphalangeal joints; (B) mandibular brachygnathia.

Supplemental Figure 2. Analysis of 23 sheep blastocysts produced through in vitro production with Lamb #4 sperm and oocytes derived from slaughterhouse-collected wild type (WT) ovaries. (A) Nested PCR targeting *BMPR2* was performed to identify 11/23 (47.8%) embryos with the -49 bp deletion. (B) Embryos with only the WT-sized band were Sanger sequenced to identify the 4/23 (17.4%) embryos (underlined number) with both WT and the 7 bp deletion alleles. Vertical dashed line indicates canonical cut site of Cas9 with single guide RNA 1.



Supplemental Table 1. Predicted nuclei acid sequences of edited and wild type (WT) *BMPR2* alleles. Snapgene software (www.snapgene.com) predicted the amino acid sequences of *BMPR2* edits described in Table 1 and Figure 2. *indicates stop codon, in-frame insertions are bolded, frame-shifted amino acids are underlined, in-frame deletion locations indicated by /.

WT and WT (CAT)	MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC YGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVVTTTPPSIQNGTYRFCCCSTDLCNVNFTENFP PPDTPPLSPPHSFNRDETI I IALASVSVLAVLIVALCFGYRMLTGDRKQGLHSMNMMEAAASEPSL DLNKLLELIGRGYGA VYKGS LDERP VAVKVFSXANRQNFINEKNIYRVPLMEHDNIARFIVGD ERV TADGRMEYLLVMEYYPNGSLCKYLSLHTSDWVSSCRLAHSVTRGLAYLHTELPRGDHYKPAIS HRDLNSRNVLVKNDGTCVISDFGLSMKLTGNXLVRPGEEDNAAISEVGTIRYMAPEVLEGA VNL RD CESALKQVDMYALGLIYWEIFMRCTDLFPGXSVPEYQMAFQTEVGNHPTFEDMQVLVSR EKQRPKF PEAWKENS L A V R S L K E T I E D C W D X D A E A R L T A Q C A E E R M A E L M M I W E R N K S V S P T V N P M S T A M Q N E RNL SHNRVPKIGPYPDYSSSSYIEDSIHHTDSIVKNISSEHSMSSTPLTIGEKNRNSINYERQQA QARIPSPETS V T S L S T N T T T T N T T G L T P S T G M T T I S E V P Y P D E T S L H A T N V S Q P V G P T P V C L Q L T E EDLETNKLDPK EVDKNLKESSDENLMEHSLKQFSGPDPLSSTSSSLPYPLIKLAVEVTGQQDFTQA ANGQACLI PDVPPTQIYPLPKQQLPKRPTSLPLNTKNSTKEPRLKFGSKHKS NLKQVETGVAKMN TINAAEPHIVTVMNGVAGRNVNSHTATTQYANGVVP SGQTANTVAHRAQEMLQNQF IGEDTRL NINSSPDEHEP LLRREQQAGHDEGVLDRLVDRRERPLEGGRTNSNNNNSNPCSEQEVPTQGV PSTV ADPGPSKPRRAQRPN SLDLSATNVLDGSSLQLGDSTQDGKSGSGEKIKKRVKTPYSLKRWRPSTWV ISTEPLDCEVNNNGKDRAVHKSSTTVYLADGGTATTMVSKDIGMNCL*
#1 Allele 2 (+21)	MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC YGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVVTTTPPS SPCTPPSI QNGTYRFCCCSTDLCNV NFTENFPPDTPPLSPPHSFNRDETI I IALASVSVLAVLIVALCFGYRMLTGDRKQGLHSMNMMEA AASEPSLDLNKLLELIGRGYGA VYKGS LDERP VAVKVFSXANRQNFINEKNIYRVPLMEHDNI ARFIVGDERVTADGRMEYLLVMEYYPNGSLCKYLSLHTSDWVSSCRLAHSVTRGLAYLHTELPRGD HYKPAISHRDLNSRNVLVKNDGTCVISDFGLSMKLTGNXLVRPGEEDNAAISEVGTIRYMAPEVLE GAVNLRDCESALKQVDMYALGLIYWEIFMRCTDLFPGXSVPEYQMAFQTEVGNHPTFEDMQVLVSR EKQRPKFPEAWKENS L A V R S L K E T I E D C W D X D A E A R L T A Q C A E E R M A E L M M I W E R N K S V S P T V N P M STAMQNERNL SHNRVPKIGPYPDYSSSSYIEDSIHHTDSIVKNISSEHSMSSTPLTIGEKNRNSI NYERQQAQARIPSPETS V T S L S T N T T T T N T T G L T P S T G M T T I S E V P Y P D E T S L H A T N V S Q P V G P T P VCLQLTEEDLETNKLDPK EVDKNLKESSDENLMEHSLKQFSGPDPLSSTSSSLPYPLIKLAVEVTG QQDFTQAANGQACLI PDVPPTQIYPLPKQQLPKRPTSLPLNTKNSTKEPRLKFGSKHKS NLKQVE TGVAKMNTINAAEPHIVTVMNGVAGRNVNSHTATTQYANGVVP SGQTANTVAHRAQEMLQNQF IGEDTRLNINSSPDEHEP LLRREQQAGHDEGVLDRLVDRRERPLEGGRTNSNNNNSNPCSEQEVPT QGV PSTVADPGPSKPRRAQRPN SLDLSATNVLDGSSLQLGDSTQDGKSGSGEKIKKRVKTPYSLKR WRPSTWVISTEPLDCEVNNNGKDRAVHKSSTTVYLADGGTATTMVSKDIGMNCL*
#2 Allele 2 (-4,+3)	MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC YGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVVTTTPPTFRMERTGSAAVARTCVMSTLLRIFH <u>LQTQHHSVHLIHLTEMRO*</u>
#3 Allele 3 (+1)	MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC YGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVVTTTPPSNSEWNVPVLLL*
#4 Allele 2 (-49)	MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC YGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVVTTTPPSMSTLLRIFHLQTQHHSVHLIHLTEM RO*
#4 Allele 3 (-7) and #6 Allele 2 (-7)	MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC YGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVVTTTPPSMERTGSAAVARTCVMSTLLRIFHLQ <u>TQHHSVHLIHLTEMRO*</u>
#5 Allele 2 (-9,+1)	MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC YGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVVTTTPPSTNVPVLLL*
#5 Allele 2 (-15)	MTSSPRRPRRVPSLLWTVLLVSAAAAAQNQERLCAFKDPYQQDLGIGESRISHENGITILCSKGSTC YGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVV T / IQNGTYRFCCCSTDLCNVNFTENFPPDTP TPLSPPHSFNRDETI I IALASVSVLAVLIVALCFGYRMLTGDRKQGLHSMNMMEAAASEPSLDLN LKLELIGRGYGA VYKGS LDERP VAVKVFSXANRQNFINEKNIYRVPLMEHDNIARFIVGDERVT ADGRMEYLLVMEYYPNGSLCKYLSLHTSDWVSSCRLAHSVTRGLAYLHTELPRGDHYKPAISHRDL

	NSRNVLVKNDGTCVISDFGLSMKLTGNXLVRPGEEDNAAISEVGTIRYMAPEVLEGA VNL RDCESA LKQVDMYALGLIYWEIFMRCTDLFPGXSVPEYQMAFQTEVGNHPTFEDMQVLVSREKQRPKFPEAW KENSLAVRSLKETIEDCWDXDAEARLTAQCAEERMAELMMIWERNKSVSPTVNPMTAMQNERNLS HNRRVPKIGPYPDYSSSSYIEDSIHHTDSIVKNISSEHSMSSTPLTIGEKNRNSINYERQQAQARI PSPETSVTSLSTNTTTTNTTGLTPSTGMTTISEVPYPDETS LHATNVSQPVGPTPVCLQLTEEDLE TNKLDPKVEVDKNLKESSDENLMEHSLKQFSGPDPLSSTSSSLPYPLIKLAVEVTGQQDFTQAANGQ ACLIPDVPPTQIYPLPKQONLPKRPTSLPLNTKNSTKEPRLKFGSKHKSNLKQVETGVAKMNTINA AEPHIVTVTMNGVAGRNVNSHTATTQYANGVVP SGQTANTVAHRAQEMLQNQFIGEDTRLNINS SPDEHEPLLRRQQAGHDEGVLDRLVDRRERPLEGGRTNSNNNSNPCSEQEVPTQGV PSTVADPG PSKPRRAQRPNSLDLSATNVLDGSSLQLGDSTQDGKSGSGE KIKKRVKTPYSLKRWRPSTWVISTE PLDCEVNNNGKDRAVHSKSSTTVYLADGGTATTMVSKDIGMNCL*
#8 Allele 2 (+94)	MTSSPRRPRRVPSLLWTVLLVSAAAASQNQERLCAFKDPYQQDLGIGESRISHENG TILCSKGSTC YGLWEKSKGDINLVKQGCWSHIGDPQECHYEECVVTTTPPSIQNGTYRFCCCSTDLCNVN <u>YEEMCS</u> NYHPTINSEWNVPVLLL*

Supplemental Table 2. Composition of Synthetic Oviductal Fluid-HEPES (SOF-HEPES) using reagents that are embryo cell culture quality and prepared using embryo cell culture quality water.

Component	Final Concentration
NaCl	107.7 mM
KCl	7.16 mM
KH₂PO₄	1.19 mM
MgCl₂ 6H₂O	0.49 mM
Sodium Lactate	5.3 mM
CaCl₂ H₂O	1.71 mM
Fructose	0.5 mM
HEPES	21 mM
MEM-NEAA	1X
BME-EAA	1X
NaHCO₃	4 mM
Sodium Pyruvate	0.33 mM
Glutamax	1 mM
BSA	1 mg/mL
Gentamicin	5 µg/mL

Supplemental Table 3. PCR primers, reaction conditions, cycling conditions, expected wild type (WT) product size and quantitative PCR amplification efficiency (E). Primers were 10 μ M except where indicated. GoTaq Green, Promega. KAPAG2 Robust, Roche. Q5 High-Fidelity, New England Biolabs. Apex Taq RED, Genesee Scientific.

Purpose	Primer	Reagents/rxn	Rxn conditions	WT Amplicon Size (bp)	E (%)
Nested_1 for Blastocysts Sanger Sequencing/ TIDER	Intron2-F: ACAGCAGAAGGACTTAGCCA Intron3-R: TCTTGGAATCCTGGGTACCTT	1 μ L Primer Mix 10 μ L KAPAG2 Robust ReadyMix 4 μ L Water 5 μ L Lysis product	95°C 5 mins [95°C 15 s 58°C 30 s 72°C 1 min] x35 72°C 5 mins	838	N/A
Nested_2 for Blastocysts Sanger Sequencing/ TIDER	Intron2-F2: TTGTTTGCAATTGTTTGTGTTTTCC Intron3-R2: TCATAATGCTGGAACCTCTCTCTT	1 μ L Primer Mix 10 μ L KAPAG2 Robust ReadyMix 9 μ L Water 5 μ L Nested 1 Product	95°C 3 mins [95°C 15 s 60°C 30 s 72°C 1 min] x35 72°C 5 mins	376	N/A
Genotype Tissues/ Blood	Intron2-F2: TTGTTTGCAATTGTTTGTGTTTTCC Intron3-R: TCTTGGAATCCTGGGTACCTT	1 μ L F Primer 1 μ L R Primer 12.5 μ L GoTaq Green 10.5 μ L Water 1 μ L DNA	95°C 5 mins [95°C 15 s 60°C 30 s 72°C 45 s] x35 72°C 5 mins	431	N/A
Rare/mosaic allele identification (Lambs 1-7)	Intron2-F2: TTGTTTGCAATTGTTTGTGTTTTCC Intron3-R: TCTTGGAATCCTGGGTACCTT	500 nM ea. primer Q5 High-Fidelity Master Mix	98°C 30 s [98°C 10 s 60°C 30 s 72°C 30 s] x35 72°C 5 mins	431	N/A
Rare/mosaic allele identification (Lamb 8)	Exon3-F: GGTGATCCCCAGGAGTGTC 7DelIntron3_R: TCTTTGGAGAAAGGAATTTTCAGGA	500 nM ea. primer Q5 High-Fidelity Master Mix	98°C 2 mins [98°C 10 s 60°C 30 s 72°C 25 s] x40 72°C 10 mins	201	N/A
Semi-Quantitative RT-PCR of BMPR2 (Lambs 4, 8)	cDNAEx2_F: CGTCTCTGTGCATTTAAAGATCCA cDNAEx4_R: TGTCAGCATCTATATCCAAAGCA	400 nM ea primer Apex Taq RED	95°C 2 mins [95°C 15 s 60°C 30 s 72°C 45 s] x37 72°C 5 mins	435	N/A
Semi-Quantitative RT-PCR of ACTB (Lambs 4, 8)	ACTB_F3: GACACCGCAACCAAGTTCG ACTB_R3: CCCACCATCACGCCCTG	400 nM ea primer Apex Taq RED	95°C 2 mins [95°C 15 s 60°C 30 s 72°C 30 s] x30 72°C 5 mins	157	N/A
Quantitative PCR	ACTB_qPCR_F: TCCTGGGCATGGAATCCTG ACTB_qPCR_R: GGGGCGCGATGATCTTGAT ACTB Ex5Probe: 6-FAM/CGGGACCAC/ZEN/CATGTACCCTGGCATCG/IA BkFQ BMPR2cDNA_F4: TCCCCAGGAGTGTCACTATGAA BMPR2qPCR R3-2: ATGATTATTGTCTCATCTCGGTTAAAT BMPR2_Ex3_WTC_Probe: SUN/CACC+CTC+AAT+TCA+GAATGG/IABkFQ BMPR2_Ex3_WTA_LNA_Probe: SUN/CACCAT+CAA+TTC+AGA+ATGG/IABkFQ	5 μ L TaqMan Fast Advanced (ABI) 900 nM ea. primer 150 nM ea. BMPR2 probe 250 nM ACTB probe 1 μ L cDNA Water to 10 μ L final vol.	50°C 2 mins 95°C 20 s [95°C 5 s 60°C 20 s] x40	ACTB: 201 BMPR2: 189	ACTB: 104 BMPR2: 97
Nested_1 for Germline Transmission	Intron2-F: ACAGCAGAAGGACTTAGCCA Intron3-R: TCTTGGAATCCTGGGTACCTT	0.4 μ L F Primer 0.4 μ L R Primer 10 μ L GoTaq Green 4.2 μ L Water 5 μ L Lysis product	95°C 5 mins [95°C 15 s 55°C 30 s 72°C 90 s] x40 72°C 5 mins	842	N/A
Nested_2 for Germline Transmission	oBPMR2-Ex3-NestR2_F: TGTACTCCATCACATTGTTTGCA oBPMR2-Ex3-NestR2_R: GCCATTAGCTAGATGTACTCTCA	0.4 μ L F Primer 0.4 μ L R Primer 10 μ L GoTaq Green 7.2 μ L Water 2 μ L Nested 1 product	95°C 2 mins [95°C 15 s 62°C 30 s 72°C 1 min] x35 72°C 5 mins	410	N/A