SUPPLEMENTARY INFORMATION FOR:

Genetic engineering a large animal model of human hypophosphatasia in sheep

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Supplementary Information

Human	1	MISPFLVLAIGTCLTNSLVPEKEKDPKYWRDQAQETLKYALELQKLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHN	80
Sheep	1	MISPFLVLAIGTCLASSLVPEKEKDPKYWRDQAQQTLKNALRLQTLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHN	80
Mouse	1	MISPFLVLAIGTCLTNSFVPEKERDPSYWRQQAQETLKNALKLQKLNTNVAKNVIMFLGDGMGVSTVTAARILKGQLHHN	80
Human	81	PGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERSRCNTTQGNEVTSILRWAKDAGKS	160
Sheep	81	PGEETKLEMDKFPYVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATQRSQCNTTQGNEVTSILRWAKDAGKS	160
Mouse	81	TGEETRLEMDKFPFVALSKTYNTNAQVPDSAGTATAYLCGVKANEGTVGVSAATERTRCNTTQGNEVTSILRWAKDAGKS	160
Human	161	VGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNIRDIDVIMGGGRKYMYPKNKTDVEYESDE	240
Sheep	161	VGIVTTTRVNHATPSASYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNVKDIEVIMGGGRKYMFPKNRTDVEYELDE	240
Mouse	161	VGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNIKDIDVIMGGGRKYMYPKNRTDVEYELDE	240
Human	241	KARGTRLDGLDLVDTWKSFKPRHKHSHFIWNRTELLTLDPHNVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVVVAIQIL	320
Sheep	241	KARGTRLDGLNLVDIWKSFKPKHKHSHYVWNRTDLLALDPHTVDYLLGLFEPGDMQYELNRNNVTDPSLSEMVEMAIRIL	320
Mouse	241	KARGTRLDGLDLISIWKSFKPRHKHSHYVWNRTELLALDPSRVDYLLGLFEPGDMQYELNRNNLTDPSLSEMVEVALRIL	320
Human	321	RKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDRAI**S**QAGSLTSSEDTLTVVTADHSHVFTFGGYTPRGNSIFGLAP	400
Sheep	321	NKNPKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDQAI**G**QAGAMTSVEDTLTVVTADHSHVFTFGGYTPRGNSIFGLAP	400
Mouse	321	TKNLKGFFLLVEGGRIDHGHHEGKAKQALHEAVEMDQAI**G**KAGAMTSQKDTLTVVTADHSHVFTFGGYTPRGNSIFGLAP	400

<p style="text-align:center">Ile>Met</p>

Human	401	MLSDTDKKPFTAILYGNGPGYKVVGGERENVSMVDYAHNNYQA**QSAVPLRHETHGGED**VAVFSKGPMAHLLHGVHEQNYV	480
Sheep	401	MVSDTDKKPFTAILYGNGPGYKVVGGERENVSMVDYAHNNYQA---------------VAVFAKGPMAHLLHGVHEQNYI	465
Mouse	401	MVSDTDKKPFTAILYGNGPGYKVVDGERENVSMVDYAHNNYQA**QSAVPLRHETHGGED**VAVFAKGPMAHLLHGVHEQNYI	480
Human	481	PHVMAYAACIGANLGHCA**PAS**SAGSLAA---------------GPLLLALALYPLSVLF-------------	524
Sheep	466	PHVMAYAACIGANRDHCA---SASSPLP**argprkpksachppg**SPLESSTAARSVAGLQ**pppppaapfwptg**	534
Mouse	481	PHVMAYASCIGANLDHCA**WAG**SGSAPSP---------------GALLLPLAVLSLRTLF-------------	524

Supplemental Fig. 1. Amino Acid sequence alignment of human, sheep and mouse TNSALP protein sequences. Conserved Exon 10 Ile342 > Met for targeted mutation is shown.
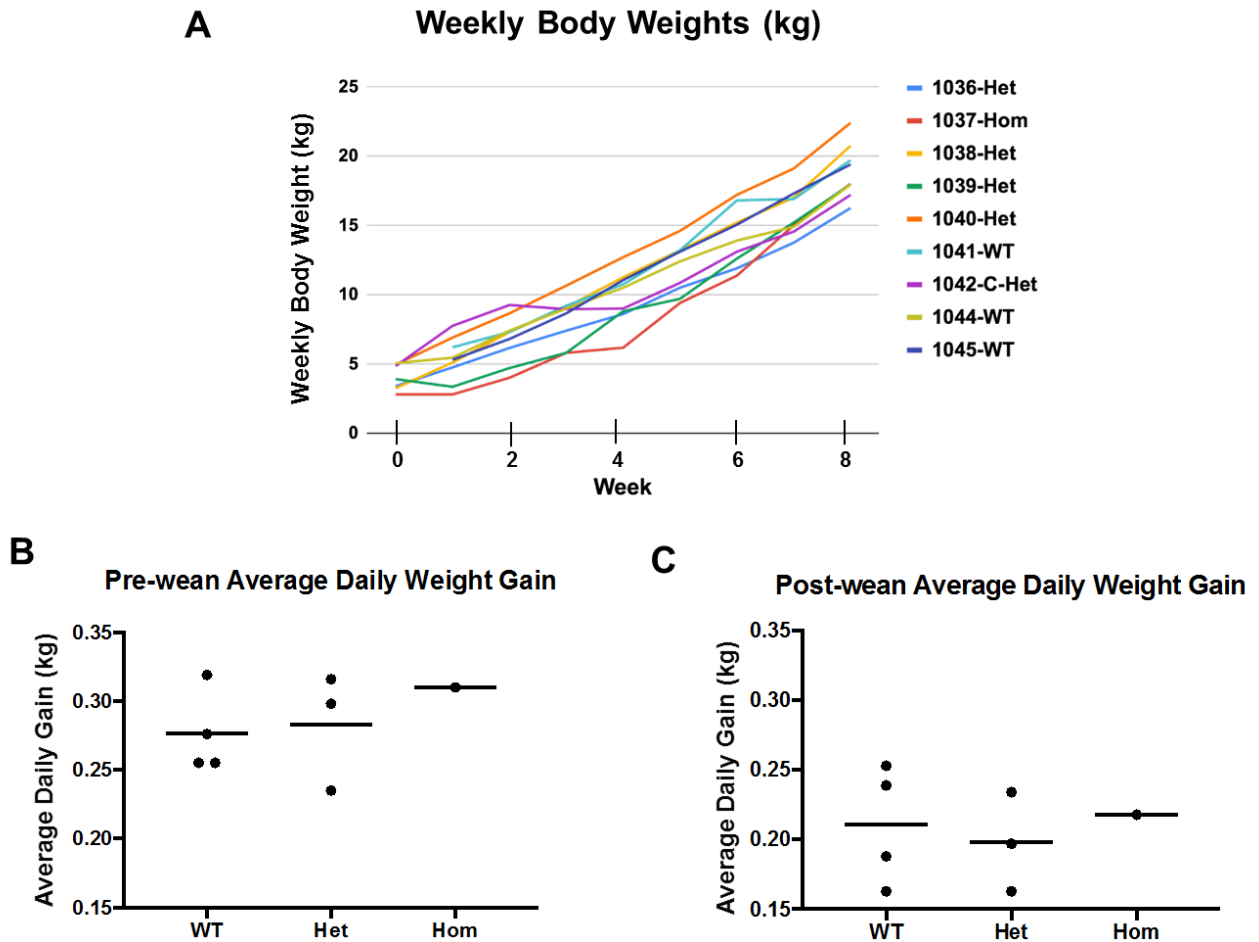
Supplemental Fig. 2 Longitudinal weight gain was similar across all genotypes. Weights were measured at birth and weekly during life. (**A**) Individual preweaning weekly body weights are plotted for all 9 animals, showing similar growth rates over the first 8 weeks of life. Average daily weight gain for each animal is plotted by genotype during (**B**) pre-weaning and (**C**) post-weaning to 6 months of age. No significant difference in weight gain by genotype is observed.
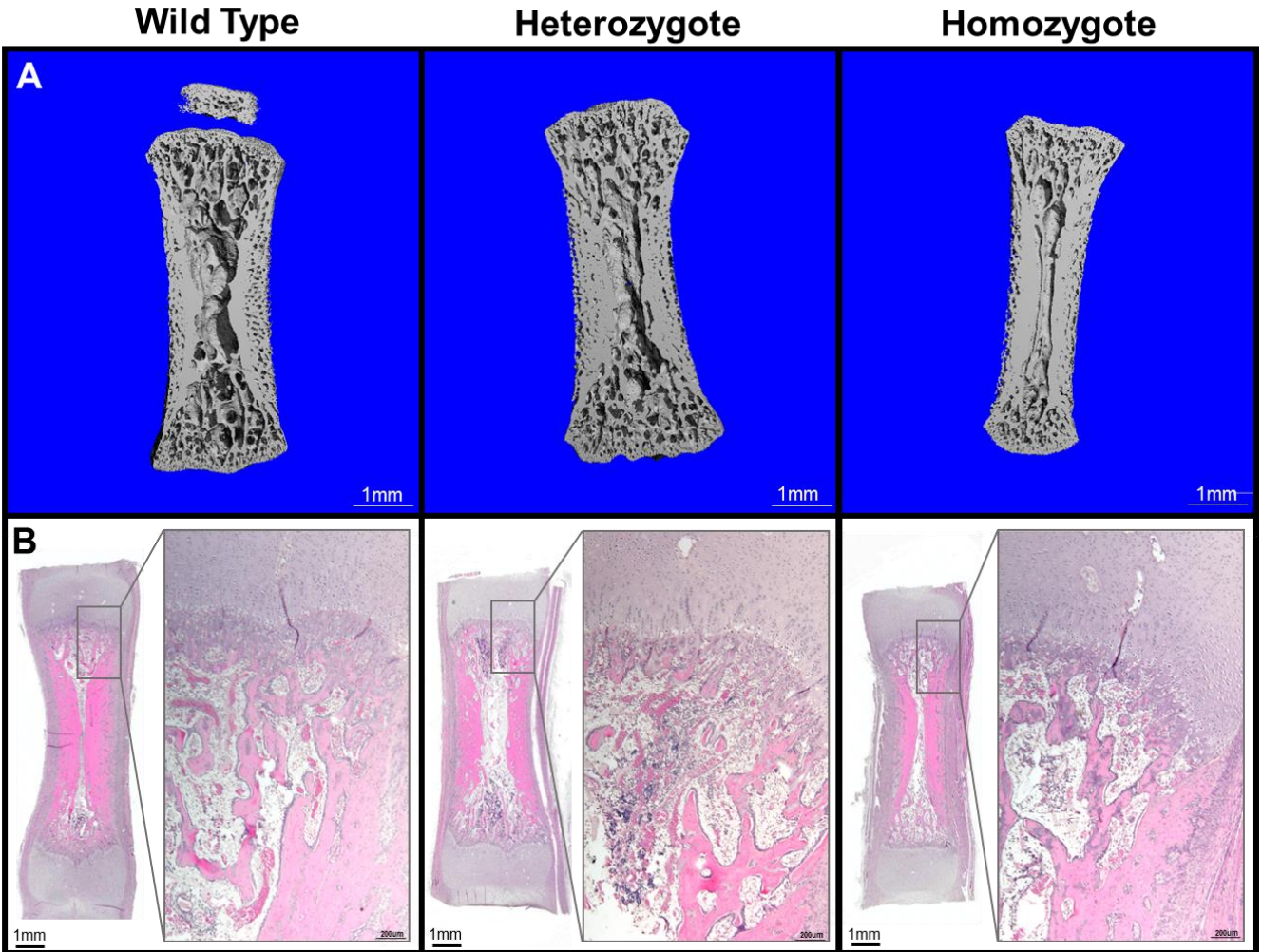
Supplemental Fig. 3 MicroCT reconstructions and histology of *ALPL* **c.1077C>G targeted sheep vertebrae** (**A**). 3D renderings of individual caudal vertebrae from wild type, heterozygous and homozygote lambs. Images are sagitally cut to show the extent of mid-shaft cortical and trabecular bone in each vertebral body. (**B**) H&E stained histologic sections from the same wild type, heterozygous and homozygote vertebrae shows a similar sagittal cross-sectional view of the vertebrae representative of each genotype. Insets show developing bone formation, teams of osteoblasts lining trabecular spicules and osteons in the developing sheep cortical bone.

Supplemental Table 1. Minimal potential off-target sites using the ALPL c1077C>G guide #1 sequence.

Sequence	PAM	Score (%)	Gene	Chromosome	Strand	Position	bp Mismatch
GATGGACCAGGCCATCGGGC	AGG	100	ENSOARG00000008507	chr2	-1	244078281	0
GCTGGACCAGGCCATCGGGG	AGG	8.611956522		chr11	-1	33997742	2
GAAGAAGCAAGCCATCGGGC	AAG	0.924515551		chr1	-1	246604745	4
CAGGGACCACGCCATCGGGT	CGG	0.859266382		chr24	-1	41705814	4
CTGGGACCAGGACATCGGGC	AGG	0.796410829		chr11	1	13803103	4
CTGGGACCAGGACATCGGGC	AGG	0.796410829		chr11	1	13798153	4
GTTGGCGGAGGCCATCGGGC	TGG	0.595983173	ENSOARG00000001576	chr26	-1	32197030	4
TAGGGACCCGGCCATCGGGG	CGG	0.56287496		chr3	-1	219963922	4
GCTGGGCGAGCCCATCGGGC	AGG	0.526959664		JH924580.1	1	31944	4
GCTGGGCCCCGCCATCGGGC	CAG	0.525048121	ENSOARG00000018751	chr24	-1	24785660	4
GCTGAGCCAGCCCATCGGGC	AAG	0.522357396		chr11	-1	18680004	4

ENSOARG00000008507= *ALPL* gene