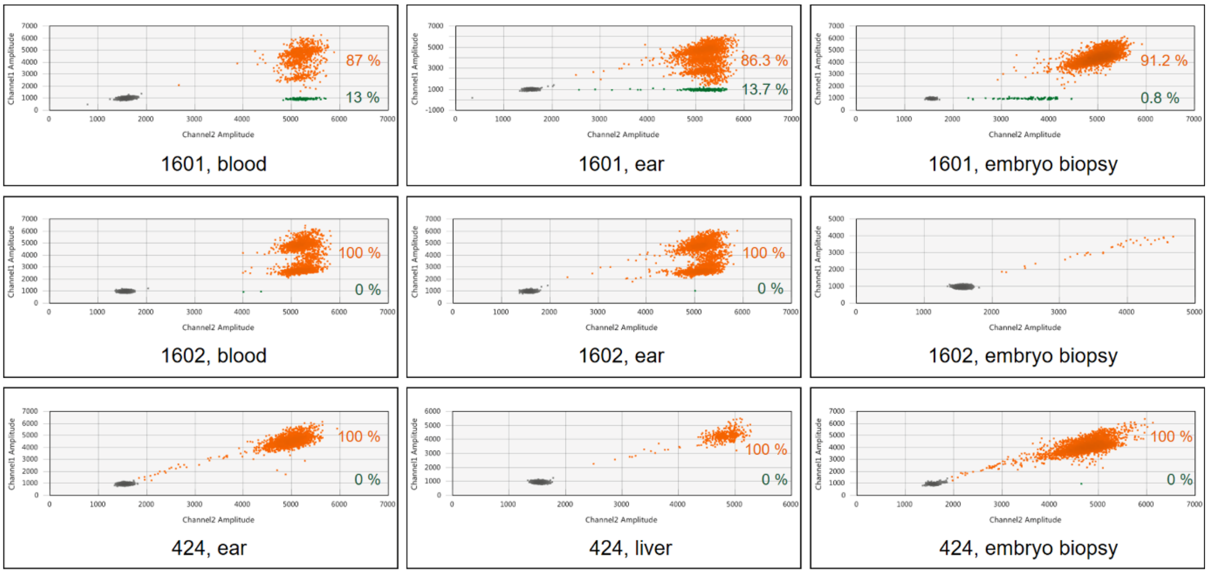
Supplementary Information

Cattle with a precise, zygote-mediated deletion safely eliminate the major milk allergen beta-lactoglobulin

Jingwei Wei, Stefan Wagner, Paul Maclean, Brigid Brophy, Sally Cole, Grant Smolenski, Dan F. Carlson, Scott. C. Fahrenkrug, David N. Wells and Götz Laible

Supplementary Figures



1601, blood

1601, ear

1601, embryo biopsy

1602, blood

1602, ear

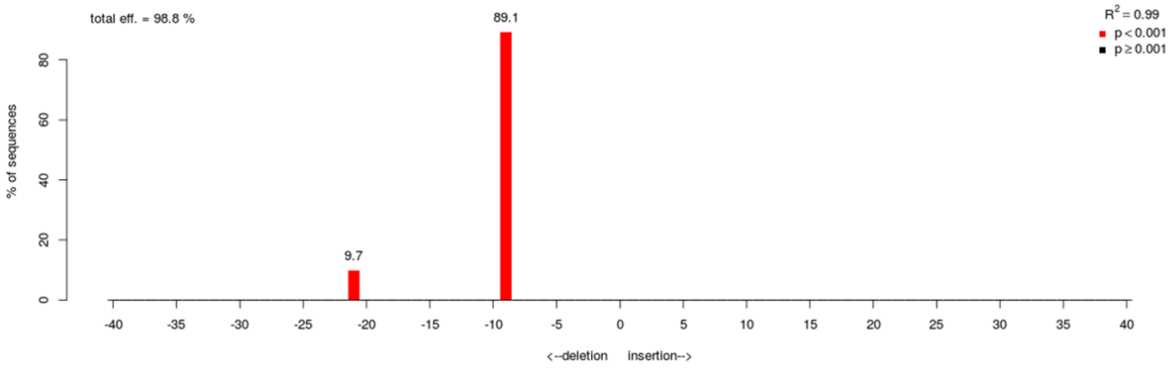
1602, embryo biopsy

424, ear

424, liver

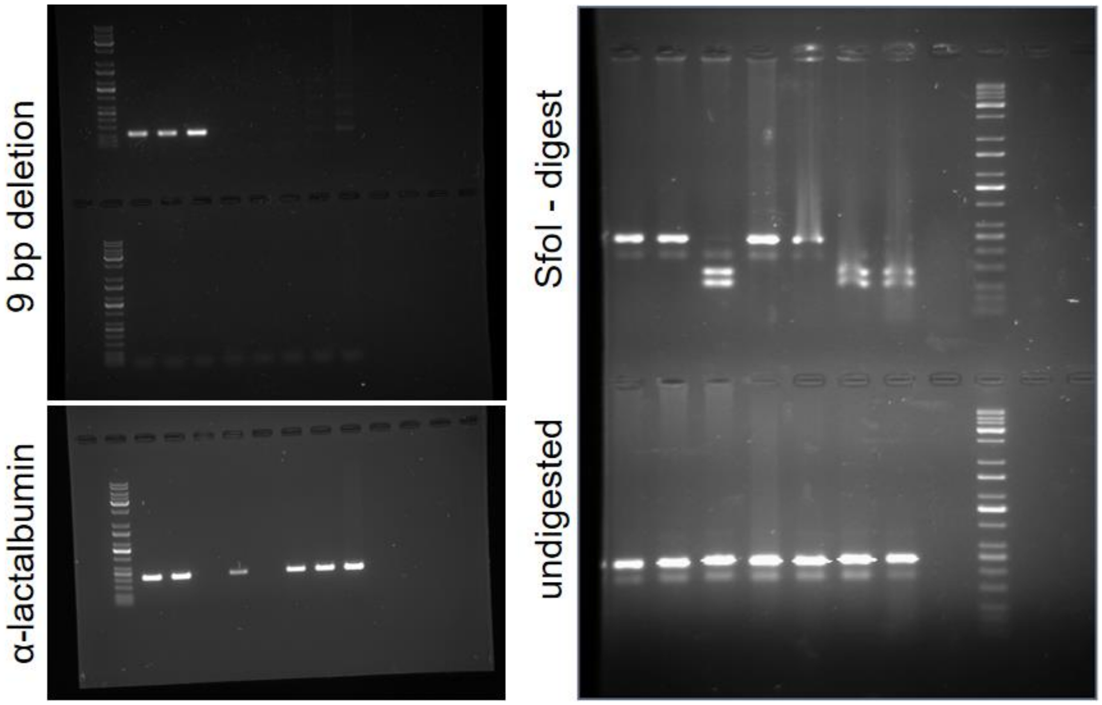
424, embryo biopsy

Supplementary Fig. S1: Assessment of the allelic composition of *LGB* target locus by ddPCR. Shown are 2D amplification plots with the nine bp deletion HDR probe for the indicated 1601, 1602 and 424 samples. Orange dots represent HDR-positive droplets, green droplets represent wild type and non-HDR indel alleles and black dots represent droplets with no amplification. Contributions of the nine bp deletion and wild type/non-HDR indel alleles are given for each plot.

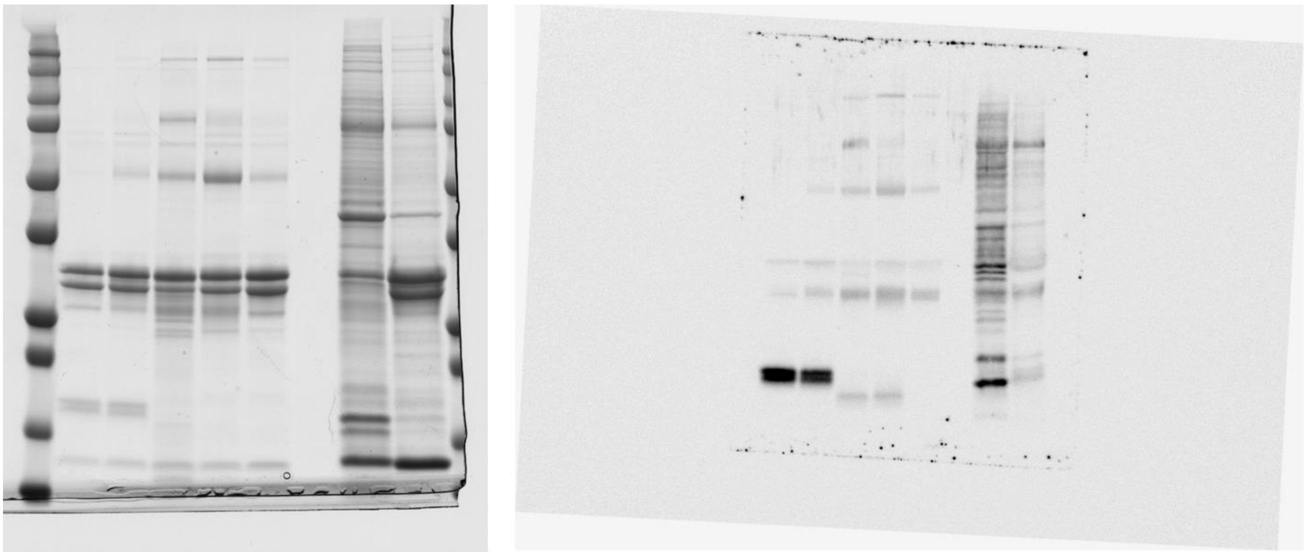


Supplementary Fig. S2: TIDE analysis.

Shown are the two allelic variants and their relative quantities predicted by TIDE analysis to be present in the amplified LGB fragment from 1601 genomic DNA isolated from a blood sample.



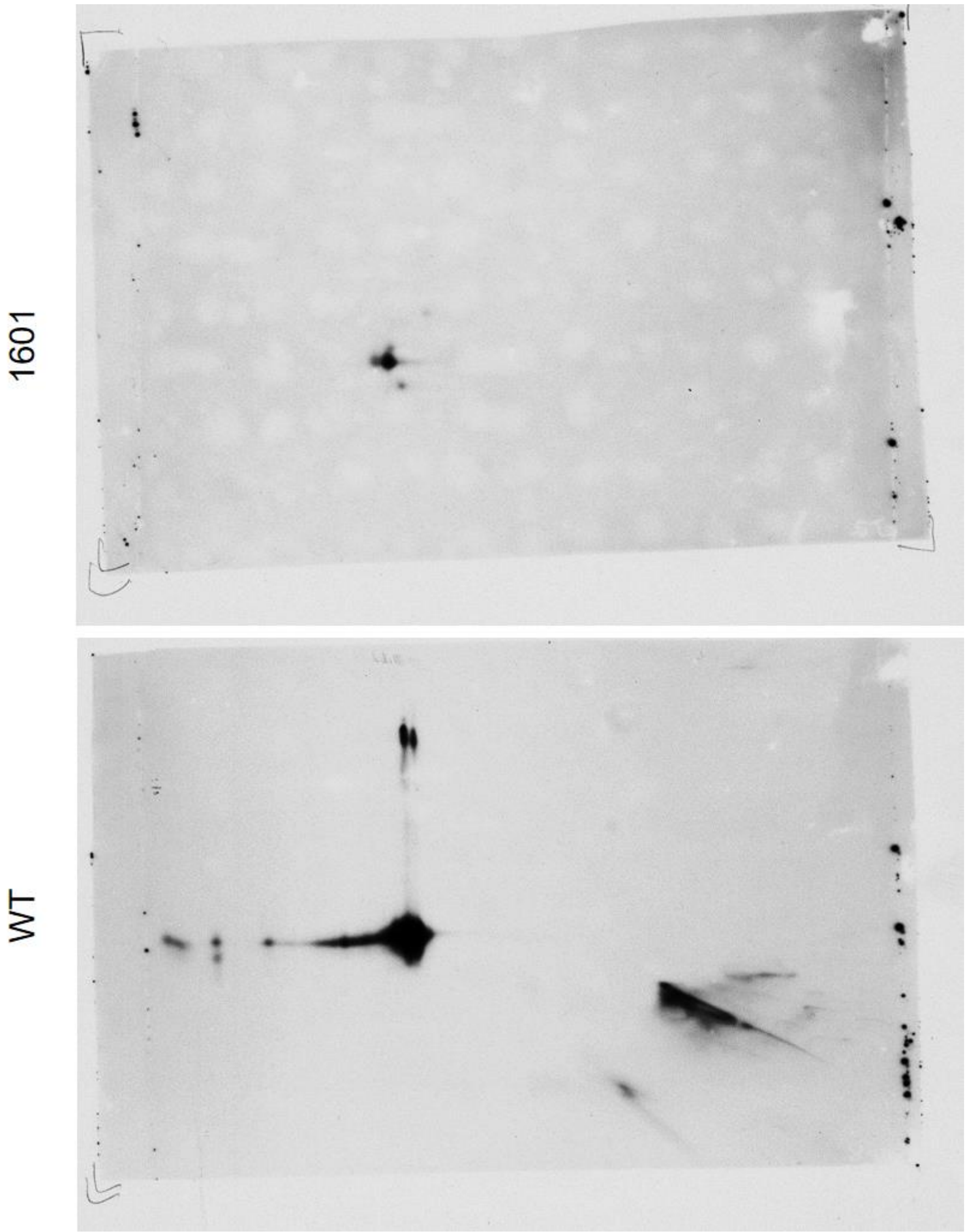
Supplementary Fig. S3: Full size gel photos used for the cropped images shown in Fig. 3.



Coomassie

western

Supplementary Fig. S4: Full size protein gel nd western membrane photos used for the cropped images shown in Fig. 5.



Supplementary Fig. S5: Full size western membrane photos used for the cropped images shown in Fig. 6A.

Supplementary Tables

Table S1: Summary of *in vitro* embryo development of IVF zygotes co-injected with TALENs and ODN HDR template.

IVF Sire	Injection sessions	TALEN plasmids	ssODN	Zygotes injected	Total embryo development	Development to grade1/2	nPCR* +ve	nPCR* +ve & TaqMan +ve
Deacon	20	btBLG 1.2	986	1219	452/1219 (37 %)	193/1219 (16 %)	64/193 (33 %)	43/193 (22 %)
Reeds Chee	4	btBLG 1.2	986	226	83/226 (37 %)	28/226 (12 %)	5/28 (18 %)	1/28 (4 %)
Sirocco	1	btBLG 1.2	986	66	23/66 (35 %)	13/66 (20 %)	6/13 (46 %)	6/13 (46 %)
Total	25			1511	558/1511 (37 %)	234/1511 (15 %)	75/234 (32 %)	50/234 (21 %)

* nPCR: nested PCR

Supplementary Table S2: Quantification of allelic variants present in calves and embryos by deep sequencing.

Sample		Maximum no. of reads per replicate	Wild type allele	9 bp deletion	21 bp deletion	24 bp deletion	66 bp deletion
Calves	1601, blood	85,470	1%	85%	13%	0%	0%
	1601, ear	83,754	2%	81%	17%	0%	0%
	1602, blood	46,928	2%	98%	0%	0%	0%
	424, ear	66,777	1%	99%	0%	0%	0%
	424, muscle	70,751	1%	99%	0%	0%	0%
	424, kidney	56,113	1%	99%	0%	0%	0%
	424, lung	45,747	2%	98%	0%	0%	0%
	424 , testis	49,052	2%	98%	0%	0%	0%
	424, liver	84,551	3%	97%	0%	0%	0%
Embryos	TS26 (1601)	80,196	8%	91%	0%	0%	0%
	TS62 (1602)	82,067	1%	99%	0%	0%	0%
	TS29 (424)	65,709	56%	8%	0%	32%	4%
	WT1	54,025	99%	1%	0%	0%	0%
	WT2	36,568	100%	0%	0%	0%	0%
	WT3	75,636	100%	0%	0%	0%	0%
	WT7	69,058	100%	0%	0%	0%	0%
	WT8	53,413	100%	0%	0%	0%	0%

Supplementary Table S3: Milk composition determined by near infrared analysis.

Sample	Cow age	Type of lactation	Time of lactation	Fat*	Protein*	Lactose*	Total Solid*	Solid Non Fat*
1601	8 months	induced	Day 5	3.6	5.4	3.8	14.4	10.3
KD	9.5 months	induced	Day 3	4.2	5.0	4.5	15.1	10.4
WT	8 months	induced	Day 3	4.7	4.2	4.8	14.6	9.7
WTP	> 2 years	natural	Early lactation	3.7	4.2	4.4	13.0	9.4

* given as percentage (w/w).

Supplementary Table S4: Primer sequences

Primer	Sequence	Application
290	TCCTCTGCCTCTGCTCCAGT	qPCR, CSN2 locus
291	CGGACAGGACCGAGTACAGG	qPCR, CSN2 locus
840	AGGCCTCCTATTGTCCTCGT	Amplification of LGB locus
841	GCAAAGGACACAGGGAGAAG	Amplification of LGB locus
986	GCAGCCATGAAGTGCCTCCTGCTTGCCCTGGCCCTCACYTAGGCCCTCATYGTCACCCAGACCATGAAGGGCCTGGATATCCAGAAGGTTCGAGGGTGSC	Homology repair template
994	CTGGCCCTCACYTAGGCCCT	Primer specific for 9 bp deletion
1015	GCAAGACACTAAACGCCTGG	Amplification of LGB locus
1016	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTGGCCCTCCTCCTGTATA	Illumina sequencing, LGB locus
1017	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAAGACACTAAACGCCTGG	Illumina sequencing, LGB locus
1048	CUCACYUAGGCCCUCA	9 bp deletion TaqMan probe, FAM
1051	TGCAGCCATGAAGTGCCT	TaqMan PCR primer, LGB
1052	CACCCTCGAACCTTCTGGATA	TaqMan PCR primer, LGB
1337	CCTGTATAAGGCCCCGA	ddPCR primer
1338	CCCTCGAACCTTCTGGATA	ddPCR primer
1339	CACTTGTGGCGCCCAGGC	Drop-off probe, FAM

1340	CTCCACTCCCTGCAGAGCTCAGAA	Reference probe, HEX
1341	CCTCACTTAGGCCCTCATTGTCACC	HDR probe, FAM
1342	CCT CAC TTG TGG CGC CCA GGC	Dark probe