SUPPLEMENTARY DATA

Supplementary Table 1. Primers used for PCRs screening of the miniBAC clone collection.

Primer name	Primer sequence 5'-3'	Size of the probe
10867rTg-KMA1	AAGACTAGGCACACCATCTCATCACTGG	435 bp
10868rTg-KMA1	GTCCCATCGTAGTCCAAATCTCTAAATGC	
10869rTg-KMA1	CATTGGTGCCATGTGGGATGTGC	540 bp
10870rTg-KMA1	ACGGCGCTGATGAATGGCTTCC	

Supplementary Table 2. The primer sets used for establishing the 5' PCR screening.

Primer pair	Primer name	Primer sequence 5'-3'	PCR product size
1	10906sa-KMA1	5'-CTGAGTATCTGAAGTCACTGGCTATTGATGG-3'	2523 bp
	01-NEO-GX2003	5'-TGACTAGGGGAGGAGTAGAAGGTGGC-3'	
2	10907sa-KMA1	5'-CAGTGGTGATTATCTTTGTCGCCTGAGTATC-3'	2602 bp
	02-NEO-GX1406	5'-CTACTTCCATTTGTCACGTCCTGCACG-3'	
3	10908sa-KMA1	5'-GTGAAGACTGAATGAGTTAGAGGTGGTGGTAG-3'	3053 bp
	05-NEO-GX1790	5'-CATCAGAGCAGCCGATTGTCTGTTG-3'	

SUPPLEMENTARY DATA

Supplementary Table 3. Standard conditions used for 5'PCR screening.

Reaction Mix			Reaction conditions			
KMA1-SA-C+ DNA	0.1- 10	Copies	Step	Temp.	Time	Cycles
+/- genomic DNA	+/ - 10	ng	Denaturing	94° C	120s	1 x
Primers	each 15	Pmol	Denaturing	94° C	30s	
dNTPs	0.5	mM	Annealing	65° C	30s	35 x
Reaction Buffer 3	0.1	Vol	Extension	68° C	300s	
Expand High Fidelity Polymerase (Roche)	2.6	U	Completion	68° C	480s	1 x
Reaction Volume	50.0	µl				

Supplementary Table 4. Prime pair used for establishing the 3' PCR screening.

Primer name	Primer sequence 5'-3'	PCR product size	
		In WT allele	In recombined allele
11610lox-KMA1	5'-TAGTGCTGACTCCACTCCTGACGTAAACC-3'	5413 bp	5474 bp, product was sequenced
11611lox-KMA1	5'-GGCAACAGAGCAAACAGTGAGTCGG-3'		

Supplementary Table 5. Standard conditions used for the 3' PCR screening.

Reaction Mix			Reaction conditions			
genomic DNA	10	ng	Step	Temp.	Time	Cycles
11610lox-/11611lox-KMA1 primers	each 15	pmol	Denaturing	94° C	120s	1 x
dNTPs	0.5	mM	Denaturing	94° C	30s	
Reaction Buffer 2	0.1	Vol	Annealing	65° C	30s	35 x
Expand High Fidelity Polymerase (Roche)	2.6	U	Extension	68° C	420s	
Reaction Volume	50.0	µl	Completion	68° C	480s	1 x

SUPPLEMENTARY DATA

Supplementary Table 6. Primer pair used to generate the external 5’ probe SA-E-A.

Primer name	Primer sequence 5’-3’	Size of the probe
11604PRO-KMA1	5'-TACATTTCATCCTTCAGTATTGGC-3'	512 bp
11605PRO-KMA1	5'-TACCTCCTGCCTCAATCTTATTTAC-3'	

Supplementary Table 7. Primer pair used to generate the internal 3’ probe LA-I-B.

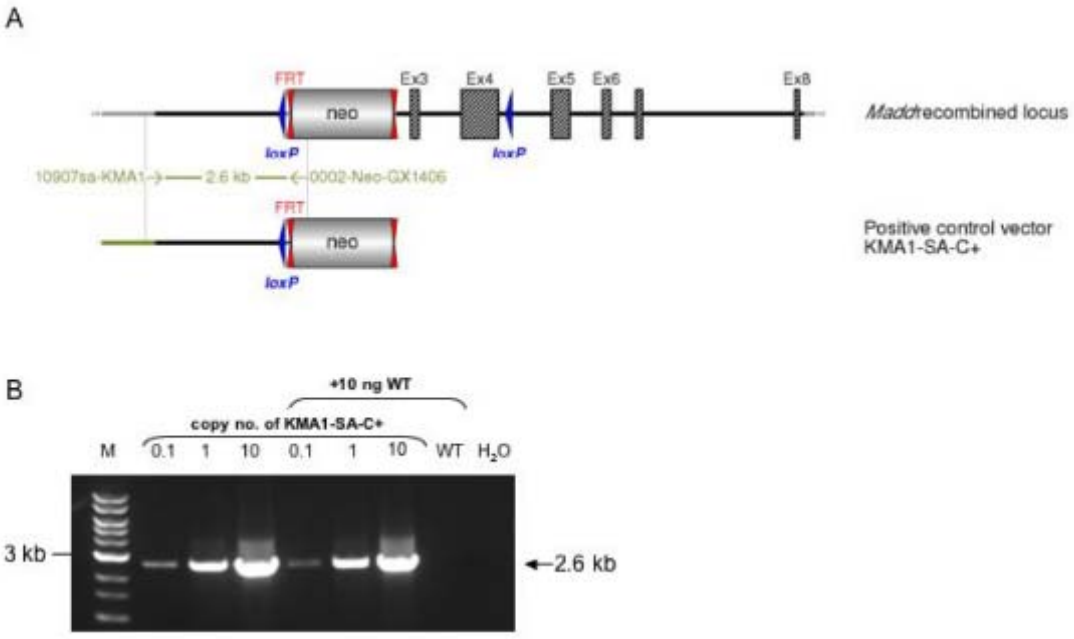
Primer name	Primer sequence 5’-3’	Size of the probe
11606PRO-KMA1	5'-TATCAGCTCTCCTTCAGTGTGTAGTC-3'	528 bp
11607PRO-KMA1	5'-CAGTGCAAAGCCATCCAAAA-3'	

Supplementary Table 8. Primers used for the genotyping The Flox and Cre Allele.

Primer name	Primer sequence	PCR product size in allele of			
		Wild type	Floxed	Cre	Internal Control
KMA1-Flp-F	5'-GACTGAAACAGAGTCTCAGAAACTCGGCT-3'	245bp	363bp		
KMA1-flp-R	5'-TGAACTCACGATAGAATCTGACGCTTGC-3'				
Cre-F	5'- AACCTGGATAGTGAAACAGGGGC-3'			410bp	
Cre-R	5'- TTCCATGGAGCGAACGACGAGACC-3'				
Internal Control-F	5'- CAAATGTTGCTTGTCTGGTG-3'				200bp
Internal Control-R	5'- GTCAGTCGAGTGCACAGTTT-3'				

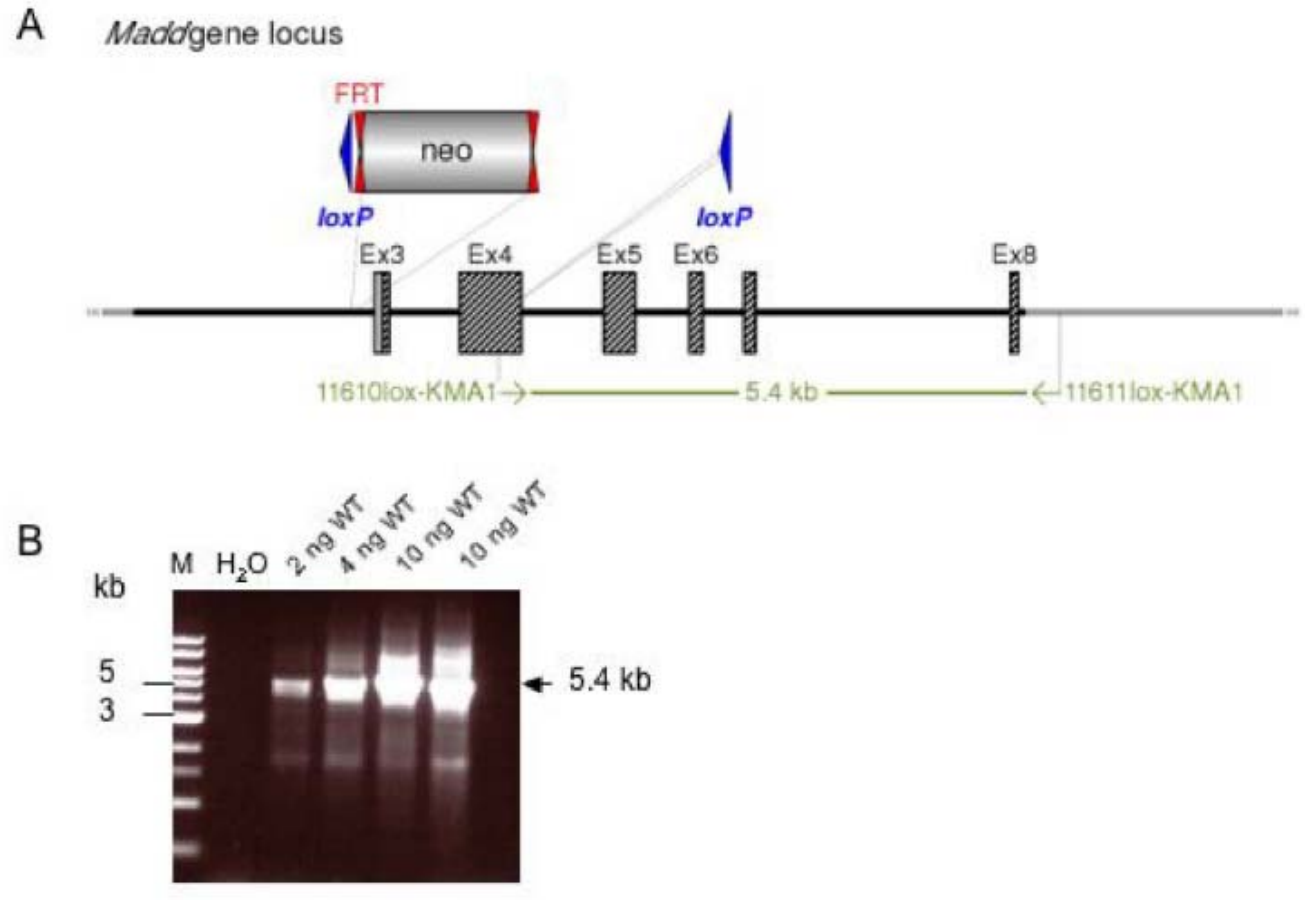
SUPPLEMENTARY DATA

Supplementary Figure 1. PCR identification of the homologous recombination event at the 5' end. (a) The figure indicates the PCR screening strategy for the 5' homologous recombination event. Green arrows illustrate the locations of primers. (b) Testing the sensitivity of the screening PCR using the primers shown on Table S2 with the PCR conditions shown on Supplementary Table 3. The optimised PCR screen was tested on 0.1, 1 and 10 copies of the KMA1-SA-C+ vector, alone or in the presence of 10 ng genomic C57BL/6J DNA as template. PCR amplification buffer was used as a negative control. M: 1 kb DNA Ladder (NEB).

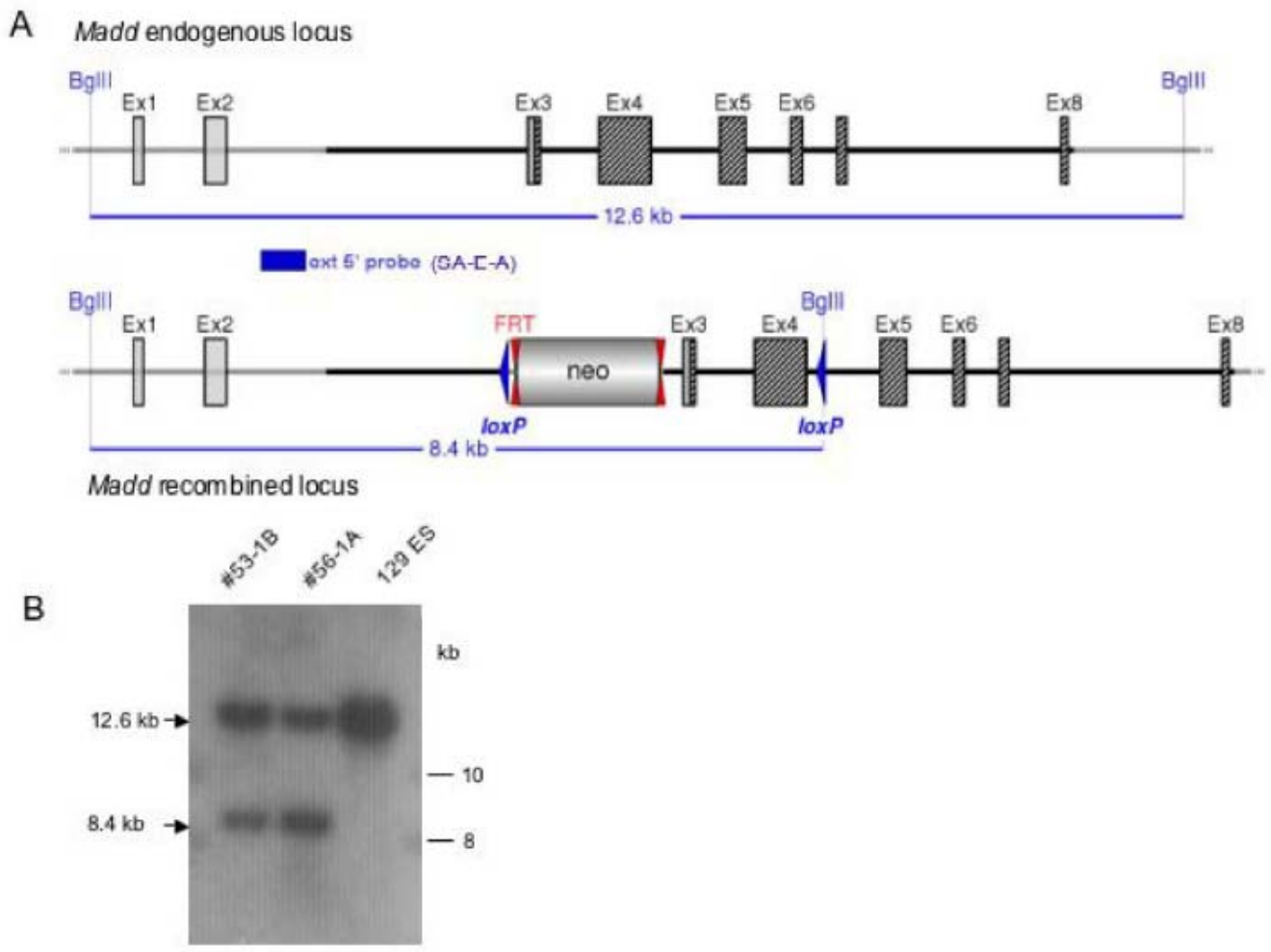


SUPPLEMENTARY DATA

Supplementary Figure 2. PCR identification of the homologous recombination event at the 3' end, including the distal loxP integration. (a) The figure indicates the PCR screening strategy for the 3' homologous recombination event. Green arrows illustrate the primer localisations. (b) Testing the sensitivity of the screening PCR using the primers shown on Supplementary Table 4 with the PCR conditions shown on Supplementary Table 5. The optimised PCR screen was conducted using 10 ng genomic 129Sv DNA from ES cell as template. PCR amplification buffer was used as a negative control. M: 1 kb DNA-Ladder (NEB).

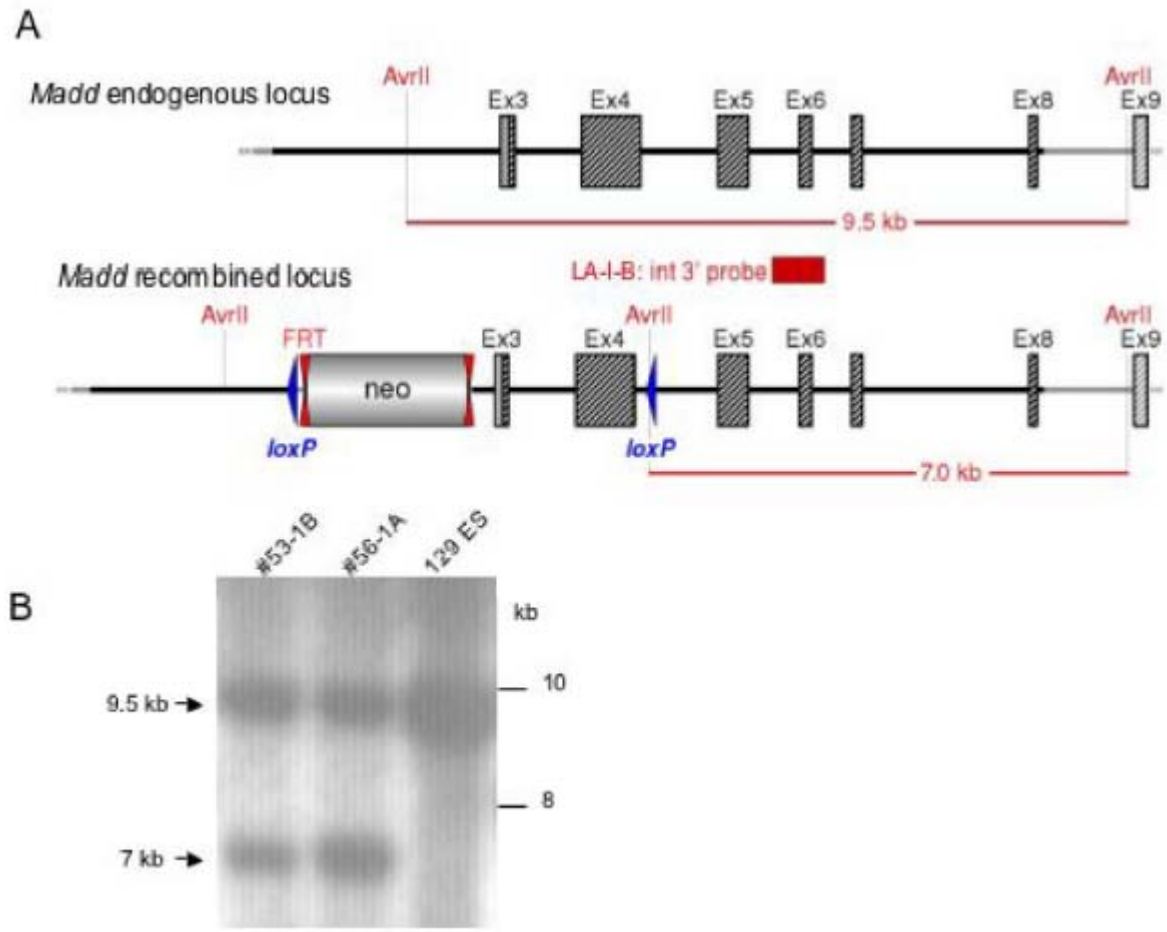


Supplementary Figure 3. Southern blot analysis for 5' homologous recombination in ES cells. (a) Schematic representation of the wild-type MADD allele and the recombined allele with the relevant restriction sites for the Southern blot analysis is shown. The strategy for the 5' Southern blot analysis is indicated in blue. (b) The genomic DNA of the tested ES cell clones were compared with wild-type DNA (129ES). The digested DNAs were blotted on nylon membrane and hybridized with the external 5'-probe SA-E-A to screen for 5' homologous recombination events. The primer set for generation 5'-probe SA-E-A was listed in Supplementary Table 6.



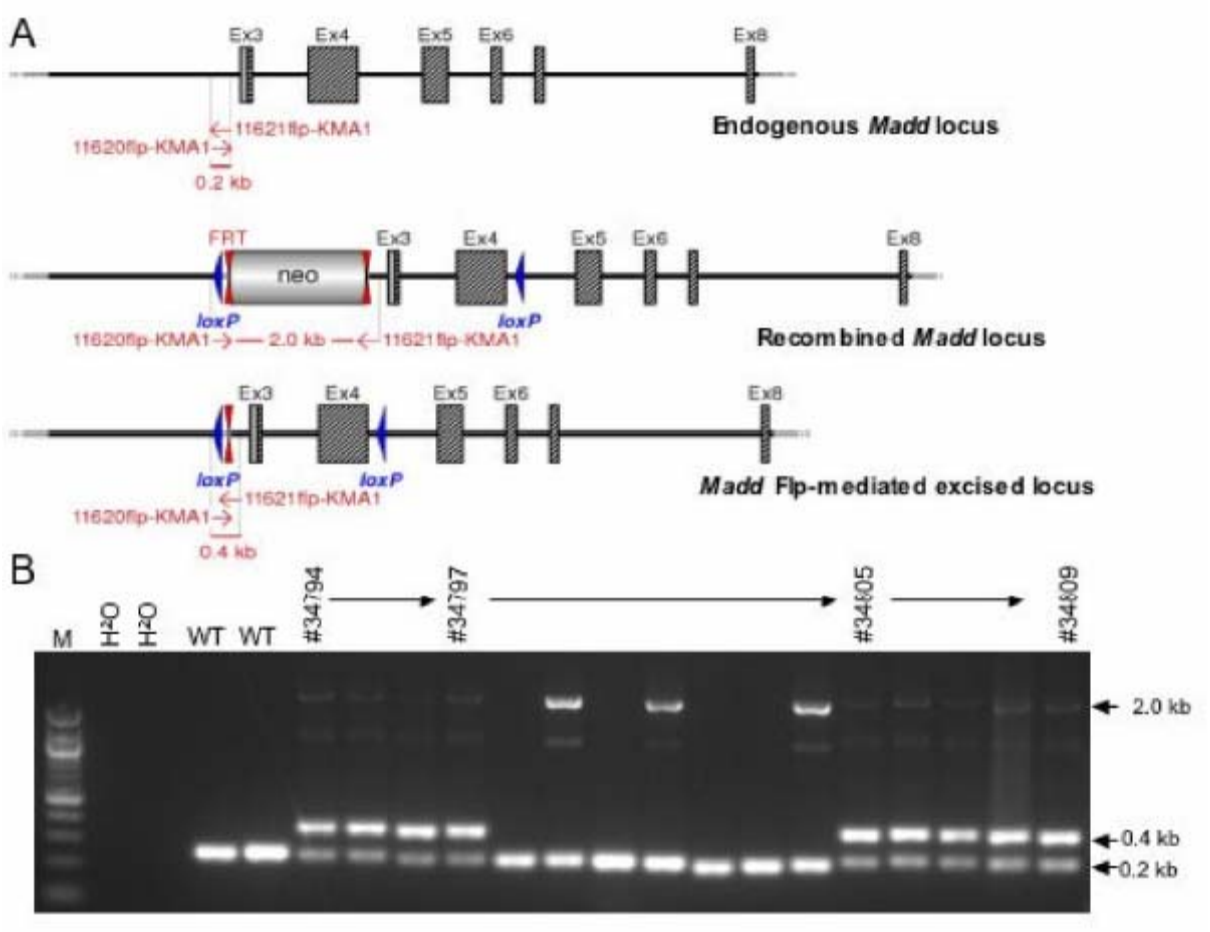
SUPPLEMENTARY DATA

Supplementary Figure 4. Southern blot analysis for 3' homologous recombination in ES cells. (a) Schematic representation of the wild-type Madd allele and the recombined allele with the relevant restriction sites for the Southern blot analysis. The strategy for the Southern blot detection of the 3' recombination event is indicated in red. (b) The genomic DNA of the tested ES cell clones was compared with wild-type DNA (129ES). The digested DNAs were blotted on nylon membrane and hybridized with the internal 3'-probe LA-I-B to screen for 3' homologous recombination events. The primer set for generation 3'-probe LA-I-B was listed in Supplementary Table 7.



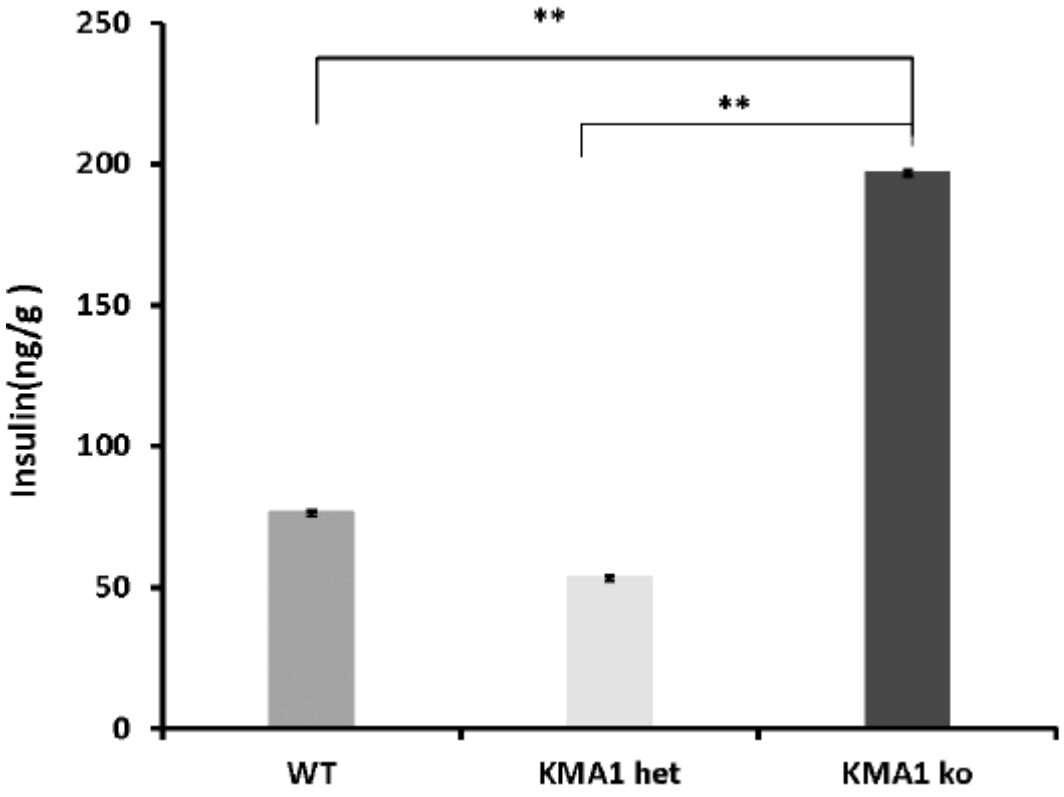
SUPPLEMENTARY DATA

Supplementary Figure 5. Detection of the Flp-mediated excision event by PCR. The genotypes of the agouti pups derived from the F1 breeding with Flp deleter mice were tested by PCR using the primer combination KMA1-Flp-F/ KMA1-Flp-R (listed in Supplementary Table 8) (A) to analyze the excision status of the MADD allele (B). PCR using DNA wild-type ES cells (WT) was used as positive control. PCR without template served as a negative control. M: 100 bp DNA-Ladder (NEB).



SUPPLEMENTARY DATA

Supplementary Figure 6. Insulin content in the total pancreas. Pancreas from WT, KMA1het and KMA1ko mice were collected after 5 days of Tamoxifen treatment and homogenized in a cold acid/ethanol mixture containing 75% ethanol, 23.5% distilled water, 1.5% 2N hydrochloric acid to extract insulin. The level of insulin in the extract was measured using an Ultrasensitive Insulin ELISA kit (Crystal Chem, Downers Groove, IL). Data are expressed as means ± SE, n = 2 mice per group.



SUPPLEMENTARY DATA

Supplementary Figure 7. pancreatic β-cells from KMA1ko mice show defective glucose induced insulin secretion. Mice were treated with Tamoxifen consecutively for 5 days, and sacrificed thirty days later. (A). Shows results from the perifusion assay. Islets isolated from WT or KMA1ko mice were used in perifusion assays. Every twenty minutes the islets were stimulated with 14 mM of glucose and perifusates were collected to determine the levels of insulin. Representative results from 4 mice per group are shown (B). and (C) show area under the curve for peak 1 and peak 2 respectively, (indicated in panel A) for each of the groups. *p < 0.05, **p<0.01, n = 4 mice per group.