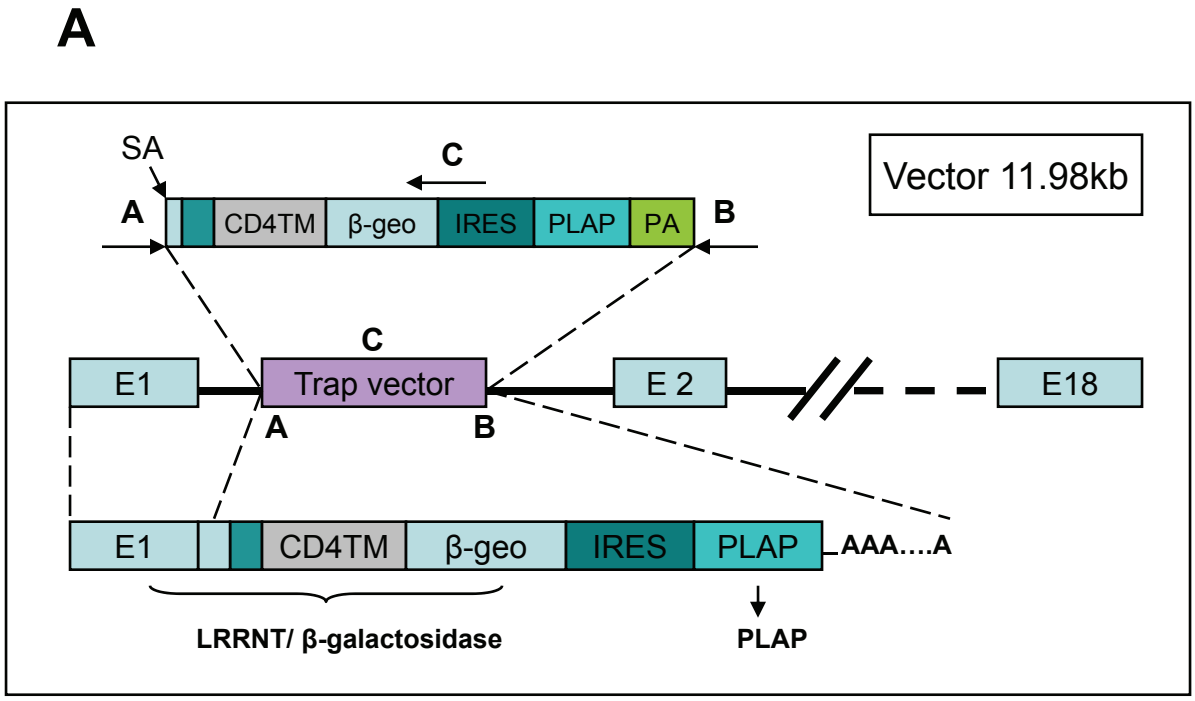
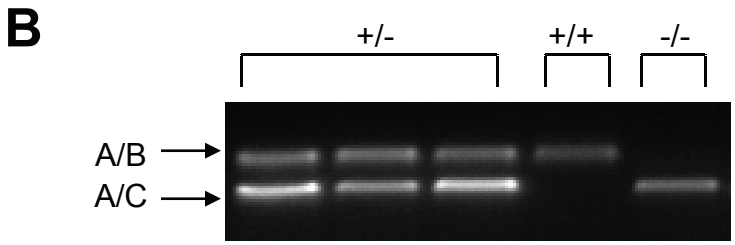
Supplementary Data

Suppl. Figure 1. Gene targeting and genotyping of Gpr48 knockout mice

Genomic structure of the mouse Gpr48 gene and mutant model by way of gene trap approch. Gpr48 gene contains 18 exons and 17 introns. The secretory-trap vector (11.98 kb), which reconstructs a frame fused with En-2 SA sequence, the CD4 transmembrane domain, β-geo, IRES, PLAP and simian virus 40 polyadenylation signal, was inserted at the 5'-end of intron 1 of the Gpr48 gene. This random insertion leads to the knock-out of the Gpr48 gene and expression of a chimeric mRNA which encodes two proteins, one protein is composed of LRRNT domain and β-galactosidase, the other is PLAP.

PCR amplification of a Gpr48 fragment and the transgene in wild-type (+/+), heterozygous (+/–), and homozygous (–/–) mice. PCR was performed using genomic DNA as the template together with four primers representing A/B for wild type and A/C for mutant respectively. Perimer A and B allowed the amplication of a Gpr48 fragment (A/B, 450 bp) in the wild-type allele whereas primers A and C amplified a transgene fragment (A/C, 750 bp) in the mutant allele. In heterozygous mice, both A/B and A/C fragments were generated.


A
SA
C
A
CD4TM
β-geo
IRES
PLAP
PA
B
Vector 11.98kb
C
E1
Trap vector
E 2
E18
A
B
E1
CD4TM
β-geo
IRES
PLAP
AAA....A
LRRNT/ β-galactosidase
PLAP


B
+/-
+/+
-/-
A/B
A/C