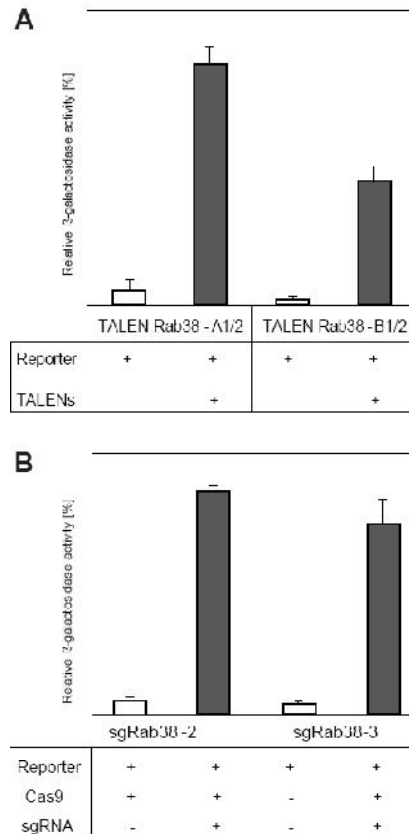


**Supplementary information**

**Supplementary Fig. 1:**

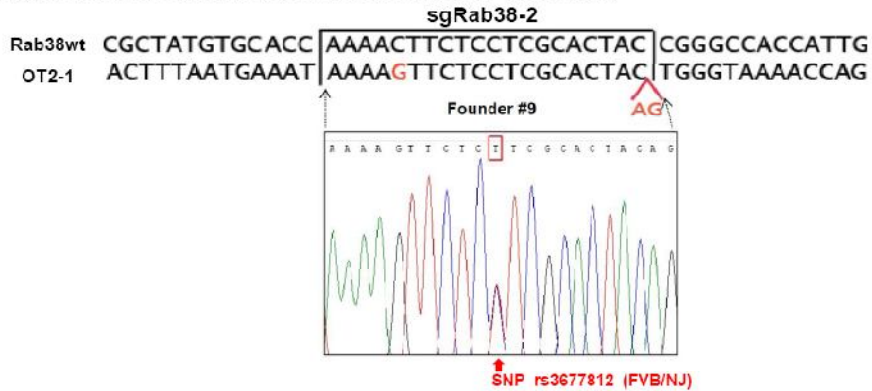


**Supplementary Fig. 1:** Nuclease reporter assay using TALEN or sgRNAs directed against *Rab38* target sequences.

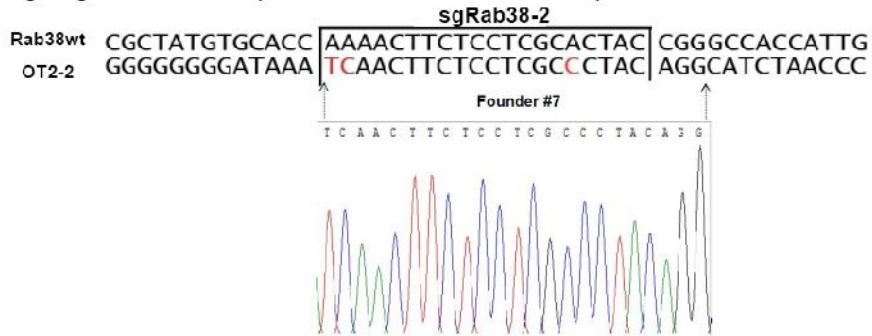
**A:** The target region recognized by TALEN-Rab38-A1/2 or -C1/2 was cloned into the reporter plasmid pTALEN-Rep in between a partially duplicated  $\beta$ -galactosidase gene and transfected without (open bars) or together with TALEN expression vectors (filled bars) into HEK 293 cells. Two days later the nuclease induced repair of the reporter was measured in cell lysates by chemiluminescence. The bars show the results of triplicate samples normalized to a cotransfected luciferase expression vector. **B:** The target region recognized by sgRab38-2 or sgRab38-3 was cloned into the reporter plasmid pTALEN-Rep in between a partially duplicated  $\beta$ -galactosidase gene and transfected without (open bars) or together with the Cas9 expression vector pCAG-Cas9-bpA (filled bars) into HEK 293 cells. Two days later the nuclease induced repair of the reporter was measured in cell lysates by chemiluminescence. The bars show the results of triplicate samples normalized to a cotransfected luciferase expression vector.

## 2. Supplementary Fig. 2

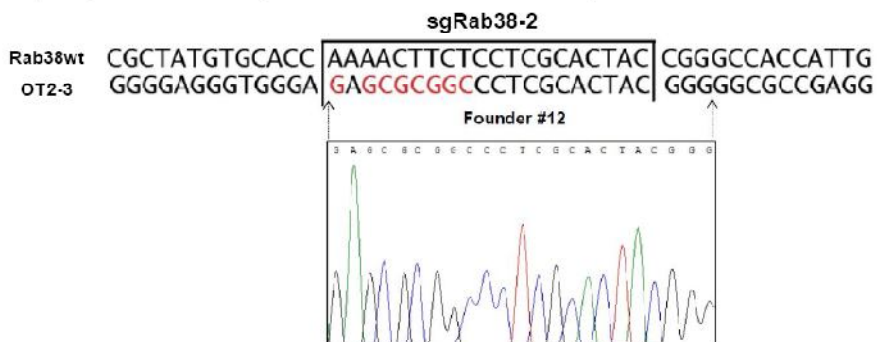
### A Off target sgRab38-2: OT2-1 (GRCm38:6:105178658-105179279)



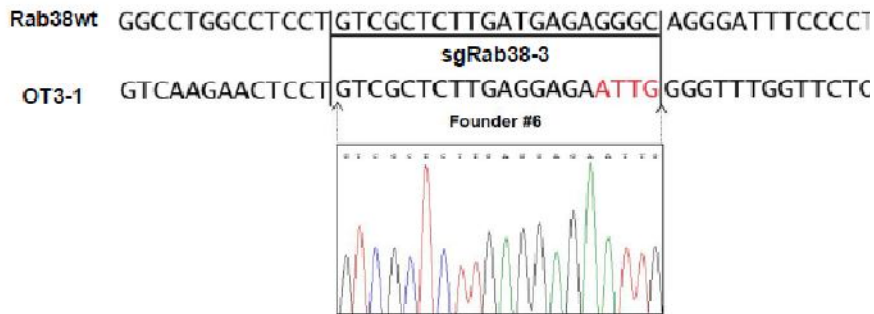
### B Off target sgRab38-2: OT2-2 (GRCm38:17:67629660-67630280)



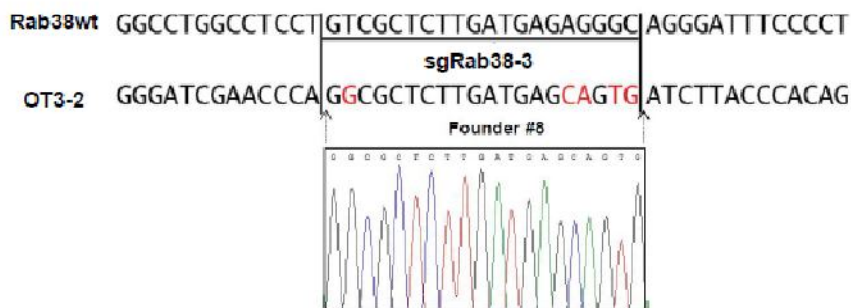
### C Off target sgRab38-2: OT2-3 (GRCm38:5:32135265-32135878)



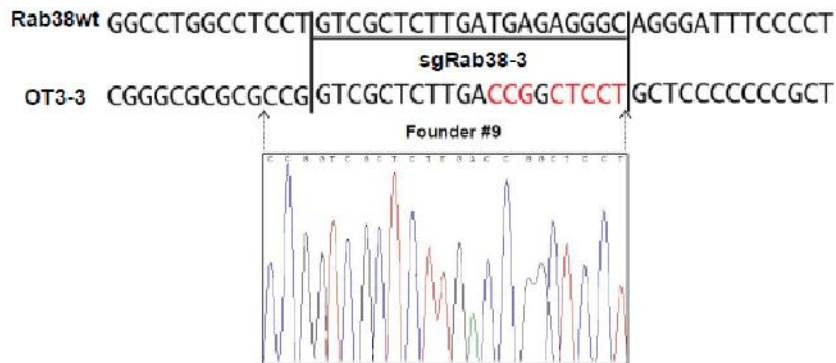
**D** Off target sgRab38-3: OT3-1 (GRCm38:11:68677069-68677687)



**E** Off target sgRab38-2: OT3-2 (GRCm38:4:121990786-121991403)



**F** Off target sgRab38-3: OT3-3 (GRCm38:2:13010722-13011335)



**Supplementary Fig. 2:** Sequencing analysis of potential off target sites in founder mutants.

To assess potential CRISPR off-target activity we amplified and sequenced three genomic regions each related to the sgRab38-2 or sgRab38-3 target sequence using tail DNA of 10 founder mutants (Fig. 4, #6 - #27). For each off-target site, OT2-1 (A), -2 (B), -3 (C), OT3-1 (D), -2 (E), -3 (F) (nucleotides deviating from the on-target shown in red letters), the sequencing result from one representative founder is shown in comparison to the sgRab38-2 or sgRab38-3 on-target site using the C57BL/6 genome sequence as reference. All sequences represent unmodified loci, OT2-1 of founder #9 exhibits a single nucleotide polymorphism (rs 3677812) known to occur in FVB/NJ mice.