

Appendix

Table of contents

Materials and Methods.....	2
<i>Off-target analysis</i>	2
Figures.....	3
Appendix Figure S1. Detection of Trem2 p.T66M mutant protein by ELISA.....	3
Appendix Figure S2. Sequence analysis of eight potential off-target sites	3

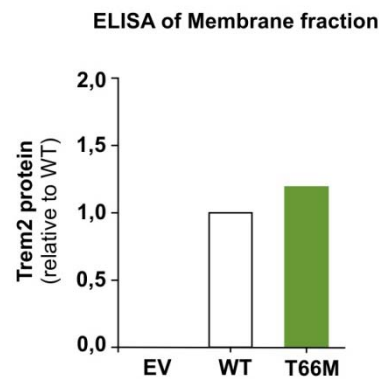
Materials and Methods

Off-target analysis

To exclude incorporation of additional mutations due to off-target effects eight potential off-target sites with the highest scores from three independent *in silico* prediction programs (CROP-IT, COSMID, MIT) were screened by direct Sanger sequencing. Briefly, respective genomic loci were amplified by PCR from genomic DNA isolated from tails of 3 homozygous F1 animals. PCR products were purified using ExoSAP-IT (Affymetrix) and sequences analyzed by direct Sanger sequencing (GATC Biotech). No nucleotide exchanges or Indels were observed in the potential off-target loci (**Appendix Figure S2**). The following primers for off-target analysis of Trem2 p.T66M knock-in mice were used:

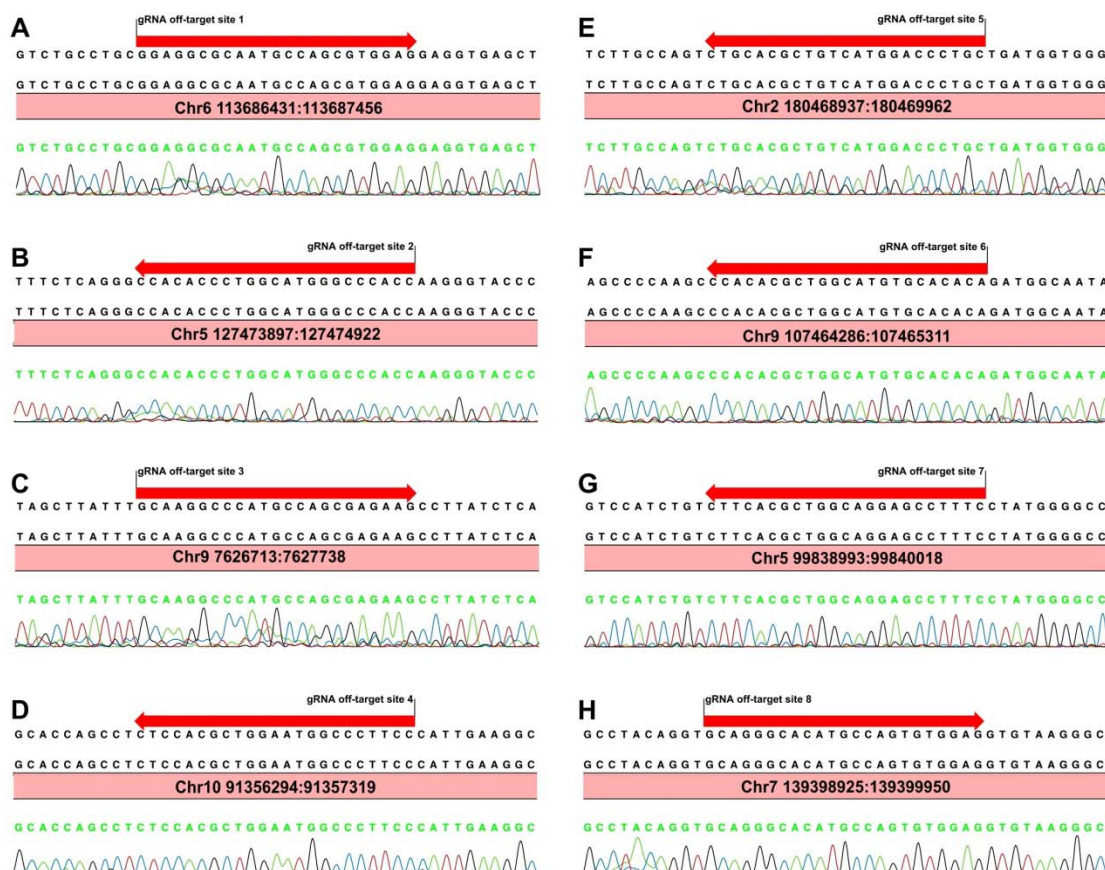
Assembly	Chromosome	Strand	Start	End	Forward primer (5'-3')	Reverse primer (5'-3')
GRC38/mm10	6	+	113686833	113687029	GTGGTGAAAGAGATCTGC	CTAAACTTGGCTTACTGC
GRC38/mm10	5	+	127474294	127474520	ACTGTCTGCCTCTATCC	ACCAACCTTTGAGAAACC
GRC38/mm10	9	+	7627117	7627301	CCAAGCCTCTGAATAAAG	CTCATGTGTGCAGATACA
GRC38/mm10	10	+	91356677	91356909	TGAAGGTGAAGAACAGAG	CTCTAGCTCATGTTTGGT
GRC38/mm10	2	+	180469335	180469582	GTGTACCCTACCTCAGAA	TCTGAGTACCAGTGGAGG
GRC38/mm10	9	+	107464670	107464923	CATGCACATACCCAGAC	GTAGGGAGAATGAATCTG
GRC38/mm10	5	+	99839324	99839628	ATCACTCCAGCCTCTTTC	CAACCACCGAAGCCTAAG
GRC38/mm10	7	+	139399316	139399552	GCTGGTGCATAATGTTC	GAGTAGTAGGCCAATAGG

Figures



Appendix Figure S1. Detection of Trem2 p.T66M mutant protein by ELISA

Membrane fractions of HEK293 FlpIn cells transiently transfected with either empty vector (EV), wild-type murine Trem2 (WT) or p.T66M murine Trem2 (T66M) were analyzed by ELISA demonstrating that the ELISA was capable of detecting mutant Trem2 similarly to the wild-type protein.



Appendix Figure S2. Sequence analysis of eight potential off-target sites

(A-H) Amplification and sequencing of eight loci with potential off-target sites did not show any additional sequence variations.