Supplemental information

Cas9-mediated gene editing
in the black-legged tick, *Ixodes scapularis*,
by embryo injection and ReMOT Control

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Supplemental Materials

Results; Materials and Methods

Figure S1. Schematic of the operating procedure developed for *Ixodes scapularis* embryo injections, Related to STAR Methods and Figure 1.

Figure S2. Embryo injection protocol and successful embryo injections, Related to STAR Methods and Figure 1.

Optional legend: (A) Eggs deposited from a control (left) and from a female with a dissected Gene`s organ (right). (B) Slide setup used for securing eggs for embryo injections. (C) *I. scapularis* dechorionated control embryos (no injection) and (D) embryos injected with green food color. (E) Expression of the CAGGS promoter-reporter construct in injected embryos. Embryos injected with green food color (without promoter construct) have no visible fluorescence whereas CAGGS injected embryos show reporter expression in multiple cells.

Figure S3. Alignment of **ProbP** mutant sequences with possible inversion, Related to STAR Methods and Figure 2.

Optional legend: (A) Sequence alignment in graphic format in NCBI with BLAST tool shows near-perfect match with Ixodes scapularis homeotic protein **Proboscipedia** (LOC8038072). The sequence matches two parts with plus/plus and plus/minus. (B) Dot plot of **ProbP** sequence also showing sequence match with possibly inverted sequence. (C) Chromatogram of L1 Sanger sequencing showing mixed bp in the region of potential inversion.

Figure S4. Summary of **Proboscipedia** Illumina deep sequencing data, Related to STAR Methods and Figure 2.

Optional legend: Animals were injected with sgRNAs 1-4. Indel percentage is on the Y-axis. The X-axis depicts predicted Cas9 cut site (position 0) and sequences up- and down-stream of the cut site.

Figure S5. Amino acid sequences of **Proboscipedia** showing deletions in ReMOT Control edited larvae, Related to STAR Methods and Figures 2 and 5.

Optional legend: A) Multiple sequence alignment of amino acid sequences of representative homozygous mutants (R4, R6, and R11) depicting in-frame deletion. B) Multiple sequence alignment of amino acid sequences of a representative homozygous mutant depicting frameshift deletion. R10 is translated amino acid sequence from DNA sequencing, R10* is the predicted amino acid sequence with the frame shift. Alignments were generated using Clustal Omega (Sievers et al., 2011).

Table S1: Single guide RNAs designed to target genes in *Ixodes scapularis*, Related to Figure 1

Materials and Methods

Table S2: Primers used to PCR amplify the respective genes from G0 larvae, Related to Figures 2, 3, and 5 *primer used for the sequencing.
Figure S1

Eggs laid by a gene's organ-ablated female

Eggs transferred to micro-centrifuge tube

Added ~200 μl of 5% benzalkonium chloride

Eggs agitated using a paint brush for 5 min

Eggs aligned and injected.

Eggs kept in 1% NaCl until used for injections.

~200 μl of 5% NaCl added and eggs agitated for 5 min.

The solutions turns cloudy
Figure S2

A

Eggs from a control female

Partially waxed eggs from a Gene's organ ablated female

B

Injection setup: side view

Tegaderm

egg

tape

glass slides

C

Dechorionated controls

D

Dye injections

E

Control

CAGGS construct injected
Figure S3

A

XM_029676216.1.3199 PREDICTED: bodes seapulans homeotic protein proboscipedia (LOC503887/2), transcript variant X1, mRNA

B

C

WT

L1
Figure S4

- sgRNA 1
- sgRNA 2
- sgRNA 3
- sgRNA 4
Figure S5

A  

Homozygous In-Frame Deletion

WT  TMWSKQDEKNGDAASEGEASAEESSVEATDRAASPAPLAPDEVGKDPDPGCCPPARAAAPSPCSSDDPDPSKAIHL--PGLAG--ASPARSDDTTPPGKGAAGRAASPAAPRLLSGLKAPALCSLD
R4  SMWSKQDEKNGDAASEGEASAEESSVEATDRAAGA-----------PGLAG--ASPARSDDTTPPGKGAAGRAASPAAPRLLSGLKAPALCSLD
R6  IMWSKQDEKNGDAASEGEASAEESSVEATDRAASPAPLAPDEVGKDPDPGCCPPARAAAPSPCSSDDPDPSK--APALCSLD
R11 IMWSKQDEKNGDAASEGEASAEESSVEATDRAAGA-----------PGLAG--ASPARSDDTTPPGKGAAGRAASPAAPRLLSGLKAPALCSLD

B  

Homozygous Frameshift Deletion

WT  TMWSKQDEKNGDAASEGEASAEESSVEATDRAASPAPLAPDEVGKDPDPGCCPPARAAAPSPCSSDDPDPSKAIHL--PGLAG--ASPARSDDTTPPGKGAAGRAASPAAPRLLSGL
R10 TMWSKQDEKNGDAASEGEASAEESSVEATDRAASPAPLAPDEVGKDPDPGCCPPARPLASPTWCCARRGSPAPRPHILVPARWILLRTARRSSRTCTPTTT--------PRTAQ--
R10* TMWSKQDEKNGDAASEGEASAEESSVEATDRAASPAPLAPDEVGKDPDPGCCPPARPLASPTWCCARRGSPAPRPHILVPARWILLRTARRSSRTCTPTTT--------PRTAQ--

WT  APALCSLDGLCPQRVASPASAYPCPSARVASSPNNQCVQPHLYAIIHYAQORPGQIPQ6PMQ6NGOCATAATAVHRGQQPQYCAARYRSPPAAASAVGQPAAATGACQUPQVGSNSYAG
AEVPCR------------------------------------------------------------------------------------------
AEVPCRATTGSAP-------LPPPCTGDSSR--LESTARPTRVLRRPAPRSGHR-------S---RPPQVRGLRR*-------------------------------------------------

*
Table S1 (Related to Figure 1): Single guide RNAs designed to target genes in *Ixodes scapularis*.

| sgRNA name       | Length (bp) | Target Sequence       | PAM  |
|------------------|-------------|-----------------------|------|
| **Proboscipedia (IS CW021086)**     |             |                       |      |
| sgRNA 1          | 19          | GTGTCTTTGTCGGAGCGCGC  | AGG  |
| sgRNA 2          | 19          | GCCCACCTTCTGCCGGGAT   | TGG  |
| sgRNA 3          | 19          | GTTGGCCGTACAGGTGCCGC  | TGG  |
| sgRNA 4          | 19          | GCTTTCCACCTCATCTCC    | GGG  |
| sgRNA 5          | 20          | ACAAGTACCTGTGTCGGCCT  | CGG  |
| sgRNA 6          | 20          | CCGAGCGCAGAAGTCAAGGTG | TGG  |
| sgRNA 7          | 20          | GGGGCTGGCGGCCCGCCCTGT | CGG  |
| **Chitinase (IS CW003950)**          |             |                       |      |
| sgRNA 1          | 20          | GTATCGAGACGTGTCTCCAT  | CGG  |
| sgRNA 2          | 20          | GACCCGGTAGTAGGACCAGC  | TGG  |

Table S2 (Related to Figures 2, 3, and 5): Primers used to PCR amplify the respective genes from *G*₀ larvae.

* primer used for the sequencing

| Primer name        | Sequence (5’-3’) |
|--------------------|-----------------|
| **Proboscipedia (IS CW021086)** |                 |
| IscprobF1          | CAATTTCCTGCCGTTTCTTGAT |
| IscprobF2          | CTGCCGTTTCTTGATTAACAC |
| IscprobR 1         | ATAGCTGTGGCTGCTACCTG |
| Iscprob R2*        | GTAGACTCCAGCGACTGCT |
| **Proboscipedia (IS CW021086)** |                 |
| IscprobF1          | CAATTTCCTGCCGTTTCTTGAT |
| IscprobR 1         | ATAGCTGTGGCTGCTACCTG |
| Iscprob F2         | GCCACAGACGATGAGCA   |
| Iscprob R2*        | GTAGACTCCAGCGACTGCT |
| **Chitinase (IS CW003950)** |               |
| IschtF*            | AGACATGCTTAGCGCTTTC |
| IschtR             | TGTGGGCTTGAATCTAGG  |