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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

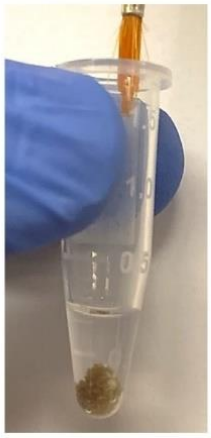
Table S2: Primers used to PCR amplify the respective genes from G_0 larvae, Related to Figures 2, 3, and 5 *primer used for the sequencing.

Figure S1

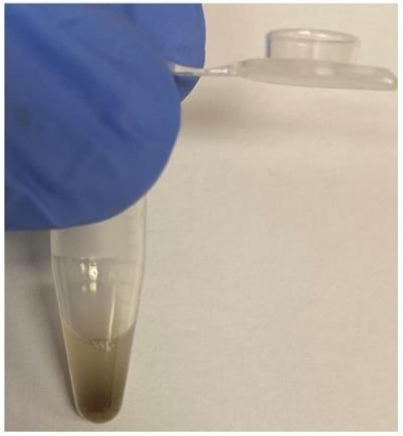
Eggs laid by a Gene`s organ-ablated female

Eggs transferred to micro-centrifuge tube

Added ~200 µl of 5% benzalkonium chloride

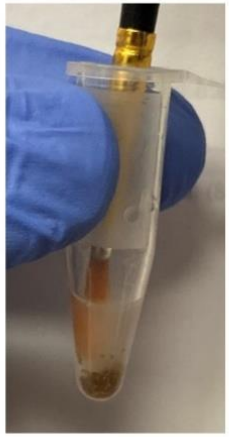


Eggs agitated using a paint brush for 5 min



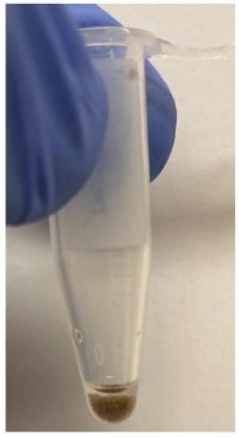
The solutions turns cloudy

2X wash with DI water

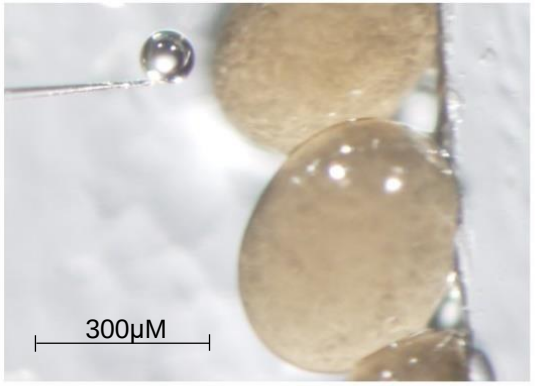


~200 µl of 5% NaCl added and eggs agitated for 5 min.

2X wash with DI water



Eggs kept in 1% NaCl until used for injections.



Eggs aligned and injected.

Figure S2

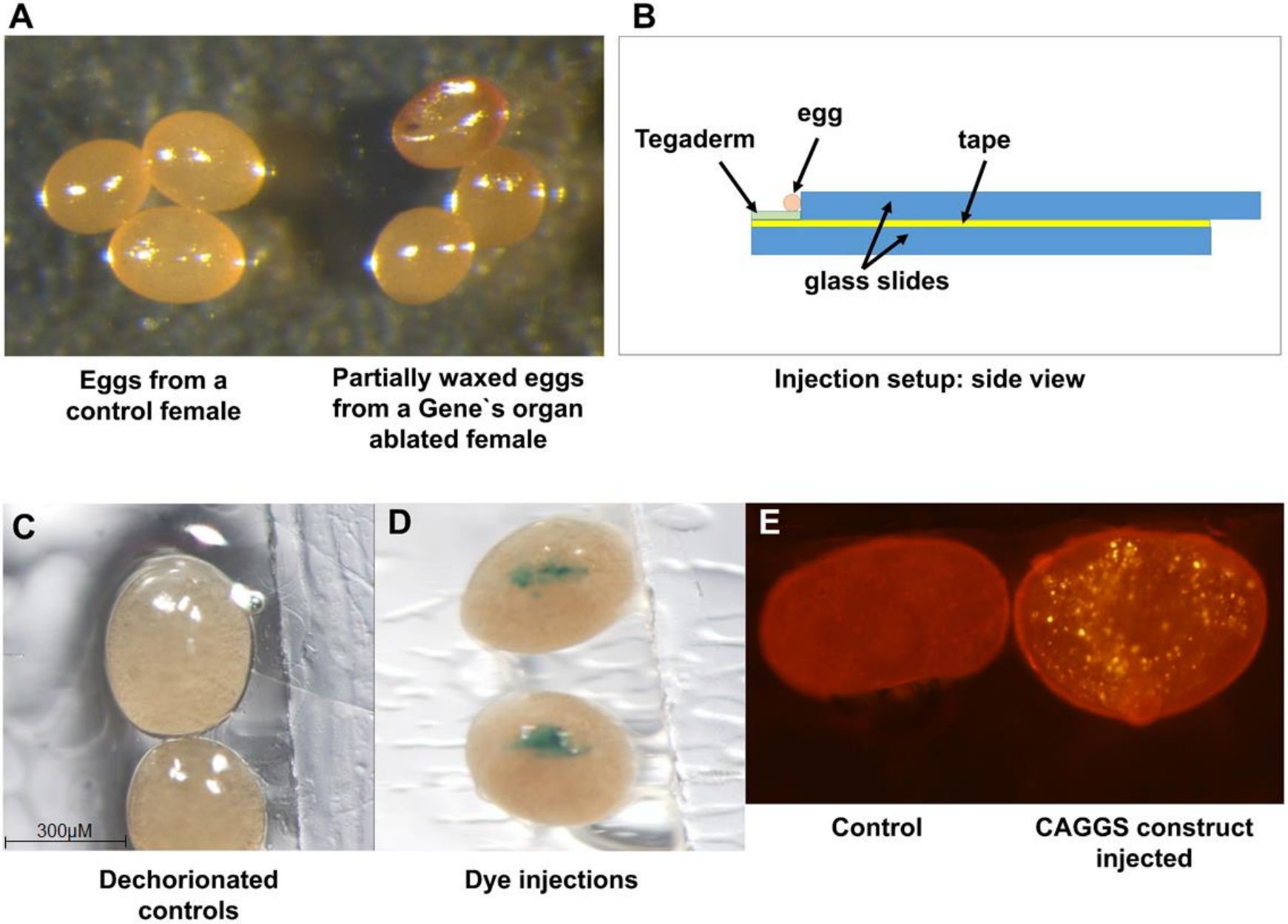
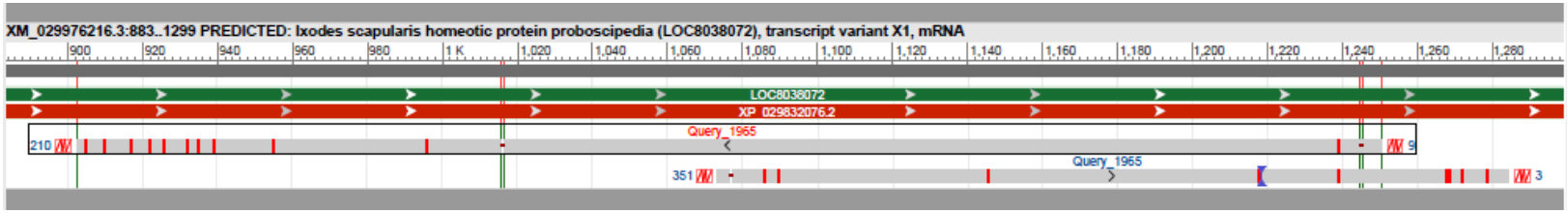
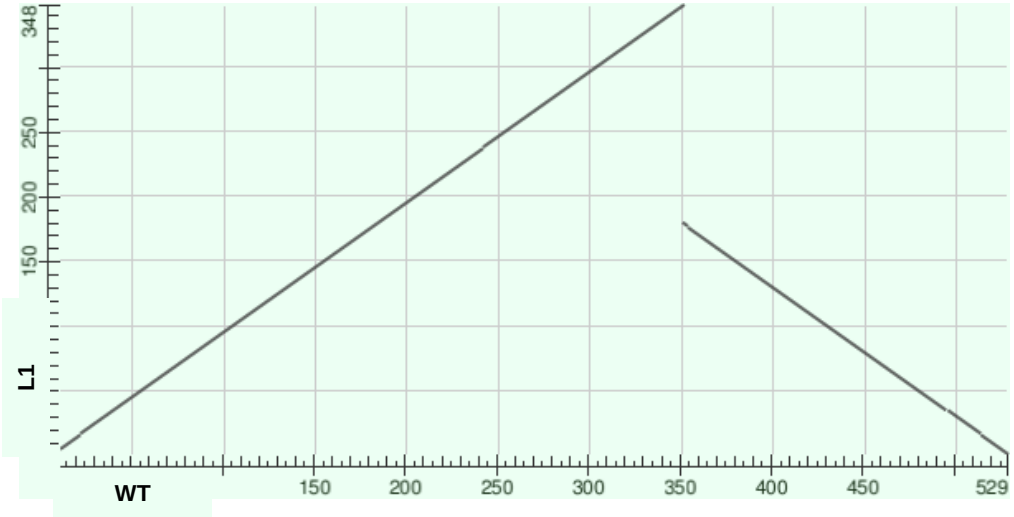

A
B
egg
Tegaderm
tape
glass slides
Eggs from a control female
Partially waxed eggs from a Gene`s organ ablated female
Injection setup: side view
C
D
E
300µM
Dechorionated controls
Dye injections
Control
CAGGS construct injected

Figure S3

A

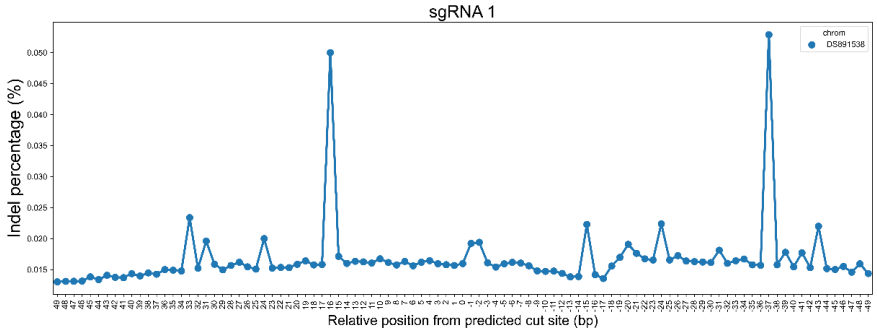


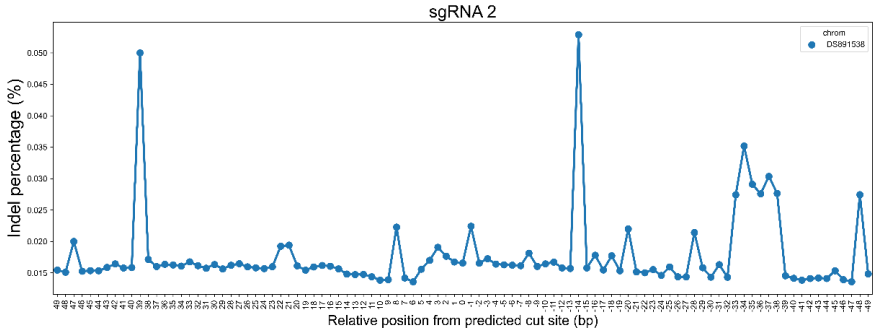
B

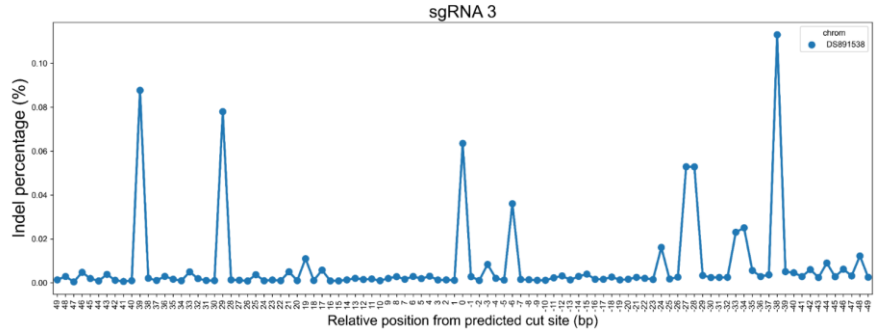


C

Figure S4


sgRNA 1
chrom
DS891538
Indel percentage (%)
Relative position from predicted cut site (bp)


sgRNA 2
chrom
DS891538
Indel percentage (%)
Relative position from predicted cut site (bp)


sgRNA 3
chrom
DS891538
Indel percentage (%)
Relative position from predicted cut site (bp)

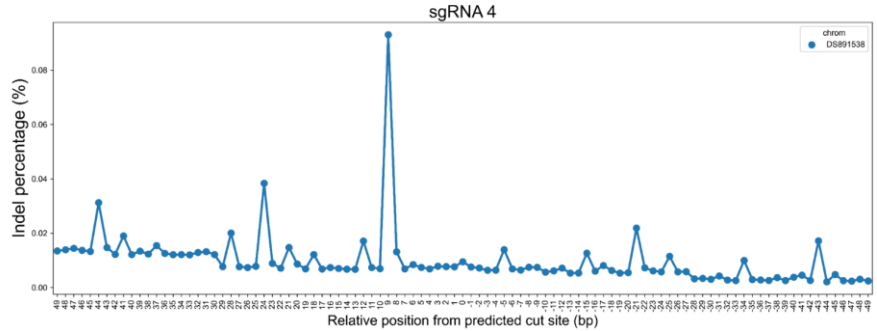

sgRNA 4
chrom
DS891538
Indel percentage (%)
Relative position from predicted cut site (bp)

Figure S5

A

Homozygous In-Frame Deletion

```
WT  TMMSKQDEKGNGDAASEGEASSVEATDRAGASPAGLAPGDEVGKPDPGPCCPARAAAPSPCSSDPDPESKAHLLPGLAGASPARSDKTPTPGGKGADGRAASPAAPRLLSGKAPALCSLD
R4  SMMSKQDEKGNGDAASEGEASSVEATDRAGA----------------------------------------------------------------------SPAAPRLLSGKAPALCSLD
R6  -MMSKQDEKGNGDAASEGEASSVEATDRAGASPAGLAPGDEVGKPDPGPCCPARAAAPSPCSSDPDPESK-------------------------------------APALCSLD
R11 -MMSKQDEKGNGDAASEGEASSVEATDRAGA----------------------------------------------------------------------SPAAPRLLSGKAPALCSLD
     ****************************                                                                                ********
```

B

Homozygous Frameshift Deletion

```
WT  TMMSKQDEKGNGDAASEGEASSVEATDRAGASPAGLAPGDEVGKPDPGPCCPARAAAPSPC----SSDPDPESKAHLL--PGLAG--ASPARSDKTPTPGGKGADGRAASPAAPRLLSGK
R10 TMMSKQDEKGNGDAASEGEASSVEATDRAGASPAGLAPGDEVGKPDPGPCCPARPPLSAPSTVVCARRGSPAPRPHLILVPARVWLLLRTARSSRTCTPTTT----------PRTAQ--
R10*TMMSKQDEKGNGDAASEGEASSVEATDRAGASPAGLAPGDEVGKPDPGPCCPARPPLSAPSTVVCARRGSPAPRPHLILVPARVWLLLRTARSSRTCTPTTT----------PRTAQ--
    ************************************************    :*.   :  .* : **: *. .     ***.:* **  .          **  .

WT  APALCSLDGGLCPQRVASPSAASYPCPSARVASSPNCQVQPHLYANHYAQDRPGGGPMQGYNGQCATAATAVHRGQQSPGVYCAATYRSPPAASAAVGPQVAAATGAQGPPQVGSNSYAG
    AEVPCR------------------------------------------------------------------------------------------------------------
    AEVPCRATTGSAP-------LPPPPCTGDSSR--LESTARPRTVLRRRPAQRSGHR------S----RPPQVPRGLRR*-------------------------------------
    * . *
```

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

sgRNA name	Length (bp)	Target Sequence	PAM
***Proboscipedia* (ISCW021086)**			
sgRNA 1	19	GTGTCTTGTCGGAGCGCGC	AGG
sgRNA 2	19	GCCCACCTTCTGCCGGGAT	TGG
sgRNA 3	19	GTTGGCGTACAGGTGCGGC	TGG
sgRNA 4	19	GCTTTCCCACCTCATCTCC	GGG
sgRNA 5	20	ACAAGTACCTGTGTCGGCCT	CGG
sgRNA 6	20	CCGAGCGCCAAGTCAAGGTG	TGG
sgRNA 7	20	GGGGCTGGCGCCGGCCCTGT	CGG
***Chitinase* (ISCW003950)**			
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* (ISCW021086)**	
IscprobF1	CAATTTCTGCCGTTTTCTTGAT
IscprobF2	CTGCCGTTTTCTTGATTAACAC
IscprobR 1	ATAGCTGTTGCTGCCTACCTG
IscprobR 2*****	GTAGACTCCAGGCGACTGCT
***Proboscipedia* (ISCW021086)**	
IscprobF1	CAATTTCTGCCGTTTTCTTGAT
IscprobR 1	ATAGCTGTTGCTGCCTACCTG
Iscprob F2	GCGACAGACGATGATGAGCA
Iscprob R2*****	GTAGACTCCAGGCGACTGCT
***Chitinase* (ISCW003950)**	
IschtF*****	AGACATGCTTAGCCGCTTTC
IschtR	TGTGGGCTTGAAAAATCTAGG