

Insights into RNAi-based antiviral immunity in Lepidoptera: acute and persistent infections in *Bombyx mori* and *Trichoplusia ni* cell lines

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Supplementary material

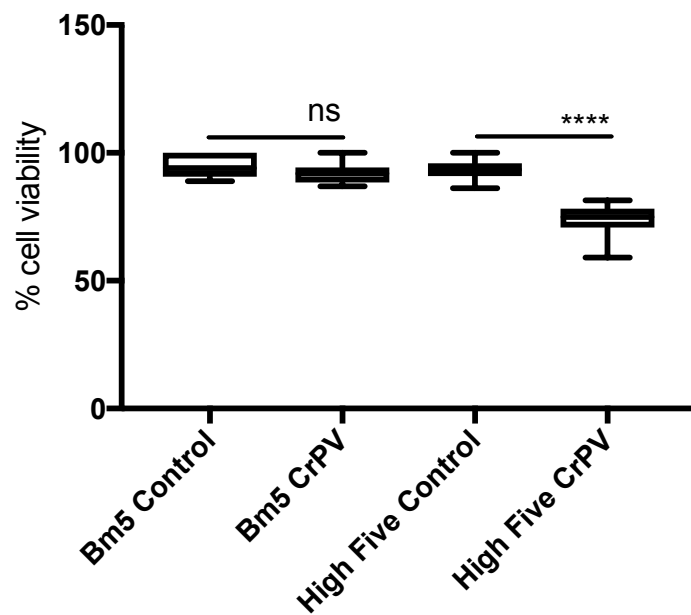


Figure S1: High Five cells are more sensitive to CrPV infection than Bm5 cells. Bm5 and High Five cells were infected with CrPV, MOI25, or treated with PBS (Control). Mortality was assessed after 24h. The graph depicts box-plots of the cell viability of each group, in percentage. Statistical analysis (Shapiro-Wilk normality test and Unpaired T-tests) was performed in GraphPad Prism 7 (**** $P < 0.0001$; $n = 12$).

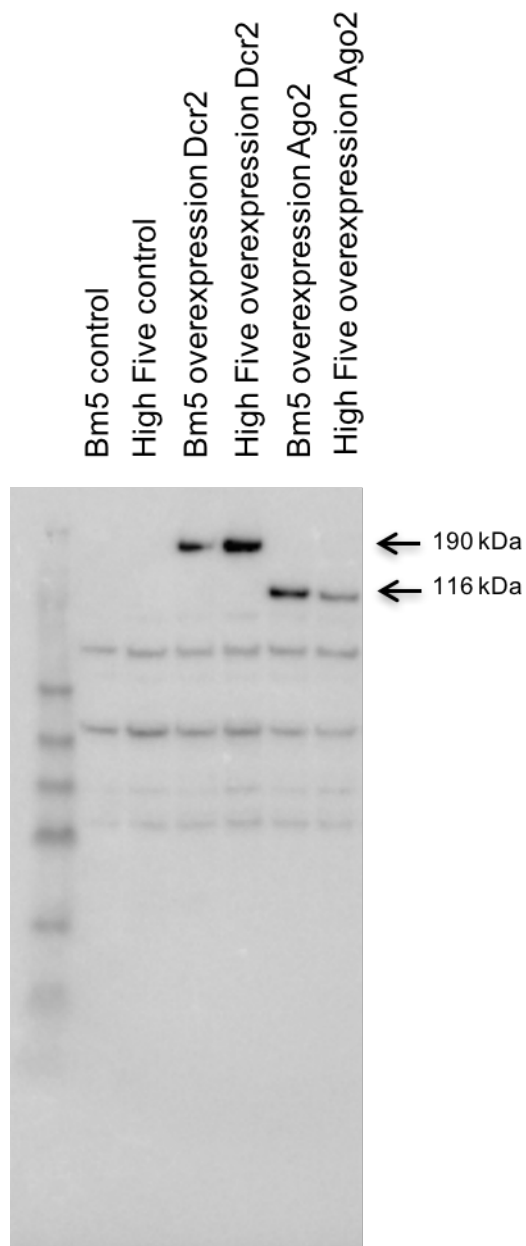


Figure S2: Overexpression of *B. mori* RNAi factors in Bm5 and High Five cells. A western blot analysis performed with an antibody specific to the Myc tag of the overexpressed proteins confirmed the overexpression of *Bm-Dcr2* (190 kDa) and *Bm-Ago2* (116 kDa) in Bm5 and High Five cells transfected with with the pEA-pac control vector. Lane 1: SeeBlue Plus2 Pre-Stained Protein Standard ladder (Novex).

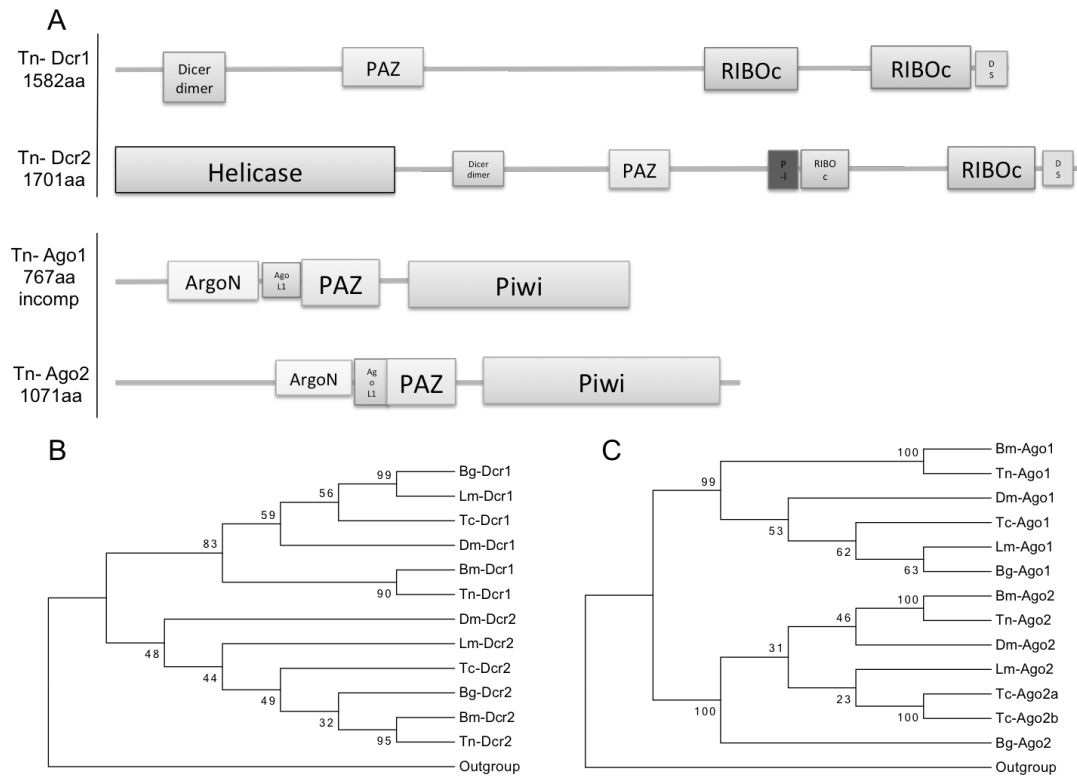


Figure S3: Identification of *T. ni* Dicer and Argonaute proteins. **A:** Protein domain prediction of *Tn-Dcr1*, *Tn-Dcr2*, *Tn-Ago1* and *Tn-Ago2*. **B:** Maximum likelihood phylogenetic tree (100 bootstraps) with the amino acid sequence of the PAZ domain of Dicer proteins of *B. mori* (*Bm-Dcr1* and *Bm-Dcr2*), *Blattella germanica* (*Bg-Dcr1* and *Bg-Dcr2*), *D. melanogaster* (*Dm-Dcr1* and *Dm-Dcr2*), *Locusta migratoria* (*Lm-Dcr1* and *Lm-Dcr2*), *Tribolium castaneum* (*Tc-Dcr1* and *Tc-Dcr2*), and *T. ni* (*Tn-Dcr1* and *Tn-Dcr2*). For the outgroup, the PAZ domain a Dicer sequence of a phylogenetically distant organism, namely *Arabidopsis thaliana*, was used. **C:** Maximum likelihood phylogenetic tree (100 bootstraps) with the amino acid sequence of the PIWI domain of Argonaute proteins of *B. mori* (*Bm-Ago1* and *Bm-Ago2*), *B. germanica* (*Bg-Ago1* and *Bg-Ago2*), *D. melanogaster* (*Dm-Ago1* and *Dm-Ago2*), *L. migratoria* (*Lm-Ago1* and *Lm-Ago2*), *T. castaneum* (*Tc-Ago1*, *Tc-Ago2A* and *Tc-Ago2B*), and *T. ni* (*Tn-Ago1* and *Tn-Ago2*). For the outgroup, the PIWI domain an Argonaute sequence of a phylogenetically distant organism, namely *Schizosaccharomyces pombe*, was used. (Helicase: helicase insert domain; Dicer dimer: dicer dimerization domain; PAZ: PAZ domain; RIBOc: Ribonuclease III C terminal domain; DS: Double-stranded RNA binding motif; ArgoN: N-terminal domain of Argonaute; P-I: P-loop NTPase superfamily domain; incomp: incomplete sequence).

[illegible]

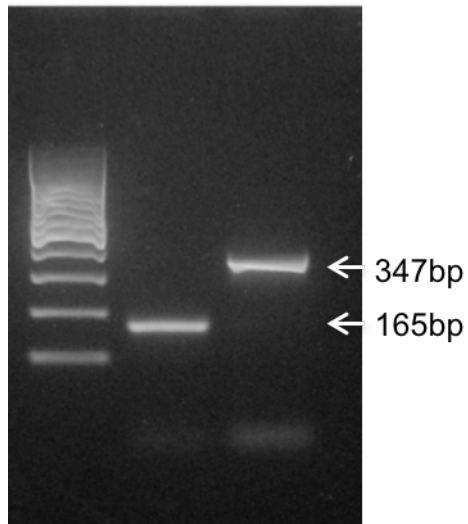


Figure S5: *Bm*-MLV is persistently present in the available stock of High Five cells. The figure depicts an agarose gel electrophoresis of two *mlv*-specific fragments, amplified from High Five cells cDNA. Lane 1: 100bp ladder. Lane 2: fragment of *Bm-mlv* amplified with the primers *mlv*-Fw1/Rv1 (165bp). Lane 3: fragment of *Bm-mlv* amplified with the primers *mlv*-Fw2/Rv2 (347bp).

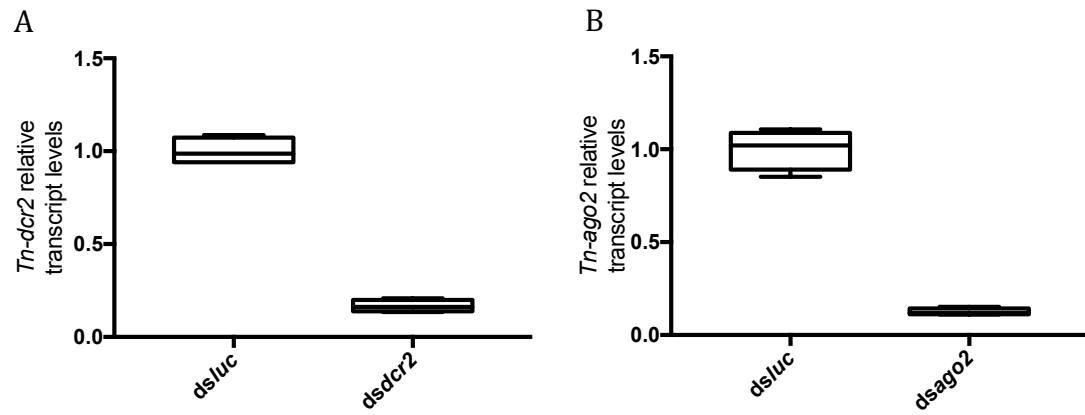


Figure S6: Efficient transcript knockdowns of *dcr2* and *ago2* in High Five cells. Cells were transfected with *dsLuc* (control), *dsdcr2* and *dsago2*. The relative transcript levels of *Tn-dcr2* (A) and *Tn-ago2* (B) were measured after 3 days. The analysis was performed in GraphPad Prism 7 (n=4).

Table S1: Primer sequences used for the production of *Tn-dcr2* and *Tn-ago2* dsRNA.

The T7 promoter sequence is shown in bold.

<i>Tn-dcr2</i> _T7_Fw	TAATACGACTCACTATAGGGG CAGACCAGACGTCGAGAAA
<i>Tn-dcr2</i> _T7_Rv	TAATACGACTCACTATAGGGG CGCTGTCCAGGAATATCGCT
<i>Tn-ago2</i> _T7_Fw	TAATACGACTCACTATAGGGG AAAGATGCATTTACTTCCCAATGGA
<i>Tn-ago2</i> _T7_Rv	TAATACGACTCACTATAGGGG CCAAGATTAACGAAC TTTGGTTTGG

Table S2: Primer sequences used for qRT-PCR.

<i>Tn-rps18</i> _qPCR_Fw	GCTCTGTATCGCCGTAACCA
<i>Tn-rps18</i> _qPCR_Rv	TGCCGTTTCGAACACAAGAC
<i>Tn-ef1a</i> _qPCR_Fw	GTCCACAACCACTGGTCACT
<i>Tn-ef1a</i> _qPCR_Rv	TCACGCTCAGCCTTCAGTTT
<i>Tn-actin</i> _qPCR_Fw	CGCACACGGTGCCCATCTA
<i>Tn-actin</i> _qPCR_Rv	CTCGGTGAGGATCTTCATCA
<i>Tn-tubulin</i> _qPCR_Fw	CGCAACCTCGACATTGAAC
<i>Tn-tubulin</i> _qPCR_Rv	TCGGTAAGGTCGACGTTGA
<i>Tn-elf4a</i> _qPCR_Fw	GTGAGCGCGAAGTTATTATGC
<i>Tn-elf4a</i> _qPCR_Rv	AGAAACTTGCTGCACGTCAAT
<i>Tn-dcr2</i> _qPCR_Fw	CGGTGACTTGCAGACTGTCT
<i>Tn-dcr2</i> _qPCR_Rv	TTCCTGGCTGTTGCGGTAT
<i>Tn-ago2</i> _qPCR_Fw	GACTGCGCGTCGTATAAACC
<i>Tn-ago2</i> _qPCR_Rv	TGACATTGCCGTCCCTCAAA
<i>mlv</i> _qPCR_Fw	TGAGTTGCGTTCCGAATGGA
<i>mlv</i> _qPCR_Rv	ACCAAGACGGGTGCTGATTT

Table S3: Primer sequences used for detection of *Bm*-MLV in High Five cells.

<i>mlv</i> -Fw1	CGTCCTCCCAACTTGTTTGT
<i>mlv</i> -Rv1	CTCCATTTTCTTCCGTTCCA
<i>mlv</i> -Fw2	ATTCTTCGGCGTCTCGACTA
<i>mlv</i> -Rv2	AGTTGAAGGGTTGGTCGTTG