

scientific report

Efficient RNA virus control in *Drosophila* requires the RNA methyltransferase Dnmt2Zeljko Durdevic¹, Katharina Hanna¹, Beth Gold², Tim Pollex^{1†}, Sara Cherry², Frank Lyko¹
& Matthias Schaefer¹⁺¹Division of Epigenetics, DKFZ-ZMBH Alliance, German Cancer Research Center, Heidelberg, Germany, and ²Department of Microbiology, Penn Genome Frontiers Institute, University of Pennsylvania, Philadelphia, Pennsylvania, USA

***Drosophila* use small-interfering RNA mechanisms to limit the amplification of viral genomes. However, it is unclear how small RNA interference components recognize and separate viral from cellular RNA. Dnmt2 enzymes are highly conserved RNA methyltransferases with substrate specificity towards cellular tRNAs. We report here that Dnmt2 is required for efficient innate immune responses in *Drosophila*. Dnmt2 mutant flies accumulate increasing levels of *Drosophila* C virus and show activated innate immune responses. Binding of Dnmt2 to DCV RNA suggests that Dnmt2 contributes to virus control directly, possibly by RNA methylation. These observations demonstrate a role for Dnmt2 in antiviral defence.**

Keywords: innate immunity; DCV; Dnmt2; RNA; methylation
EMBO reports (2013) 14, 269–275. doi:10.1038/embor.2013.3

INTRODUCTION

Efficient immune responses are essential for the control and elimination of infections with viruses and the survival of the infected host. Recent work in *Drosophila* has revealed the importance of small RNA processing machineries for virus control [1,2]. In particular, the activity of Dicer-2 (Dcr-2) is required for the production of virus-derived small-interfering RNAs (vsiRNAs), which are loaded into Argonaute-2 to target and cleave viral RNA genomes [3,4]. While Dcr-2 accepts long double-stranded RNA substrates and dices these into vsiRNAs, it is unclear where and how Dcr-2 detects and sorts its substrate RNAs. Membrane compartments that are crucially important for viral entry and replication [5] have also been linked to the activation of innate immune responses [6,7] and small-interfering RNA

pathway activity [8]. These observations suggest that siRNA-mediated antiviral defence mechanisms evolve at the intersection of cellular and endosomal membrane systems.

Dnmt2 genes have been strongly conserved during evolution, suggesting a biological function that would justify long-term evolutionary selection [9,10]. Although being annotated as DNA methyltransferases, recent findings have established that *Dnmt2* enzymes are transfer RNA methyltransferases [11] and that this methylation becomes important under stress conditions [12,13]. To analyse the biological function of *Dnmt2* in adult flies, transcriptional profiling was performed. Several stress response genes, including components of the *Drosophila* innate immune system, were upregulated in *Dnmt2* mutant flies. Accumulation of (+) RNA viruses was associated with increased immune responses in *Dnmt2* mutants. Infection studies suggested that Dnmt2 has a role in the acute immune response to *Drosophila* C virus (DCV) infection by binding to and possibly methylation of viral RNA. Our data indicate that *Dnmt2* proteins are required to control specific RNA viruses in *Drosophila*.

RESULTS

Adult flies with a null mutation in *Dnmt2* are sensitive to heat and oxidative stress [12]. To better understand this increased sensitivity to heat stress, larvae were heat-shocked and the resulting adults were scored for phenotypes. A significant increase of black spots was detected in *Dnmt2* null mutant flies (supplementary Fig S1A,B online). These spots resembled melanotic lesions, which have been associated with upregulated immune responses in flies [14,15]. Also, when *Dnmt2* mutants were cultured under sub-optimal conditions (for example, crowded population), they regularly developed melanotic lesions (Fig 1A). These observations indicate that stress-induced signals caused increased immune responses in *Dnmt2* mutant animals.

To analyse the molecular effects of the *Dnmt2* mutation, adult wild-type and *Dnmt2* mutant flies were compared using gene expression micro-arrays. This analysis showed that 164 genes displayed a robust and significant (fold change ≥ 2 , $P < 0.05$) upregulation, whereas 121 genes showed a robust and significant downregulation in *Dnmt2* mutant flies (supplementary Table S1, S2 online). Enrichment analysis revealed clusters of genes

¹Division of Epigenetics, DKFZ-ZMBH Alliance, German Cancer Research Center, Im Neuenheimer Feld 580, Heidelberg 69120, Germany²Department of Microbiology, Penn Genome Frontiers Institute, University of Pennsylvania, Philadelphia, Pennsylvania 19146, USA[†]Present address: Mammalian Developmental Epigenetics Group, Institut Curie, CNRS UMR3215, INSERM U934, 26, rue d'Ulm, F-75005 Paris, France⁺Corresponding author. Tel: +49 6221 423804; Fax: +49 6221 423802; E-mail: m.schaefer@dkfz.de

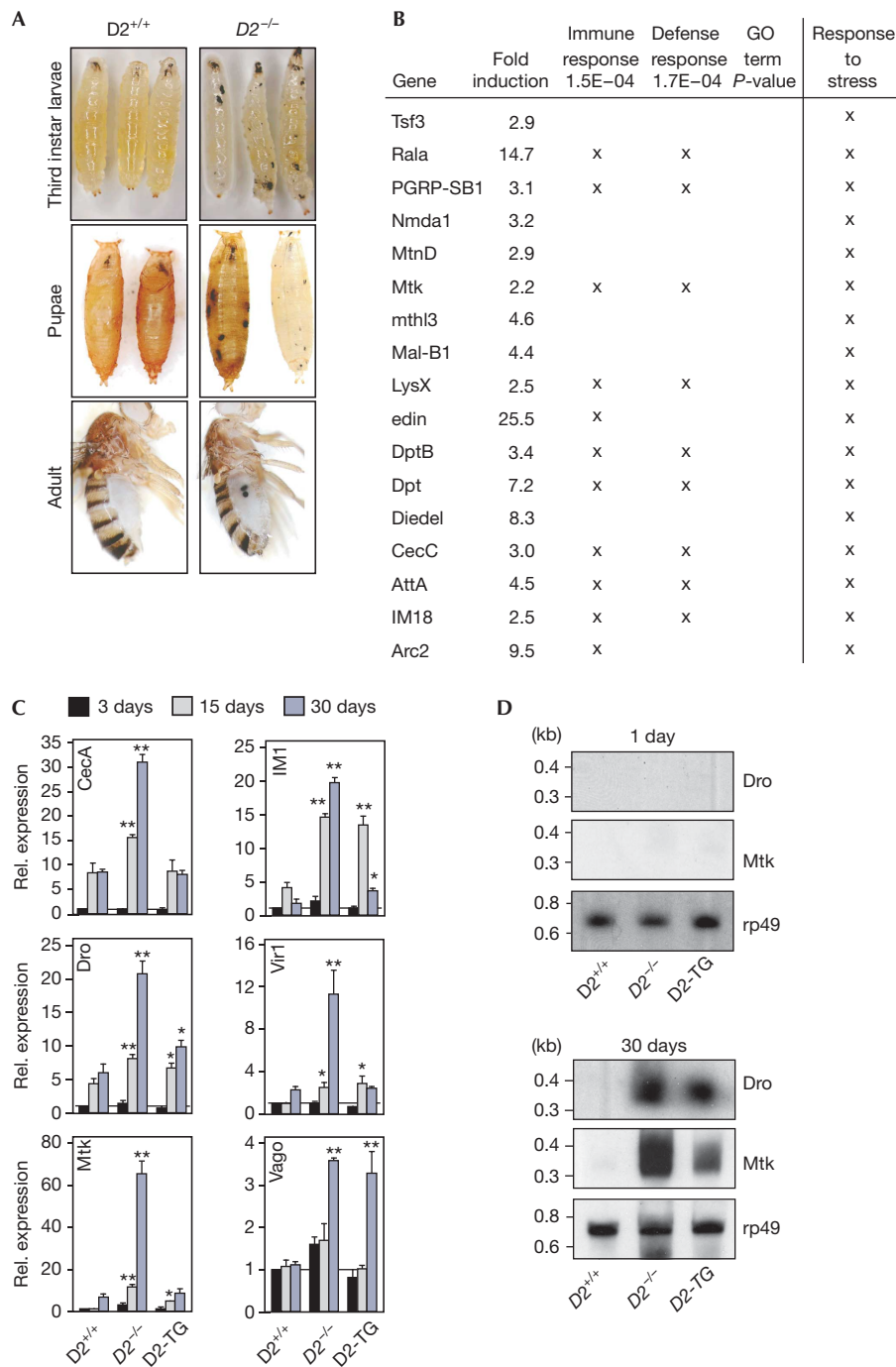


Fig 1 | Increased stress and immune responses in *Dnmt2* mutant flies. (A) Melanotic spots after keeping fly stocks under crowded growth conditions. Third instar larvae, pupae and adults of wild-type ($D2^{+/+}$) and *Dnmt2* mutants ($D2^{-/-}$) flies are shown. (B) Enrichment analysis for ≥ 2 -fold upregulated genes in *Dnmt2* mutants relative to w^{1118} males that can be linked to stress responses. Benjamini-Hochberg multiple hypothesis correction *P*-value is displayed for two stress-related GO classifications and (X) marks genes that are associated with these terms. Last column includes genes that are not associated with GO terms but can be linked to stress responses using annotation of the Flybase consortium (<http://flybase.org/>). (C) Q-PCR analysis for immune response genes in wild-type ($D2^{+/+}$), *Dnmt2* mutant ($D2^{-/-}$) and transgenic rescue ($D2$ -TG) flies at various time points after hatching (3, 15 and 30 days). (D) Northern blotting for *Drosocin* and *Metchnikowin* from young (1 day) and aged (30 days) flies of the genotypes as in (C). *Rp49* was probed as loading control. RNA expression of genes in wild-type was set to 1 and normalized to *rp49* messenger RNA in individual experiments. Error bars represent s.d.'s from three biological replicates. *P*-values were determined by Student's *t*-test (* $P < 0.05$; ** $P < 0.01$). GO, gene ontology; Q-PCR, quantitative PCR; rel., relative.

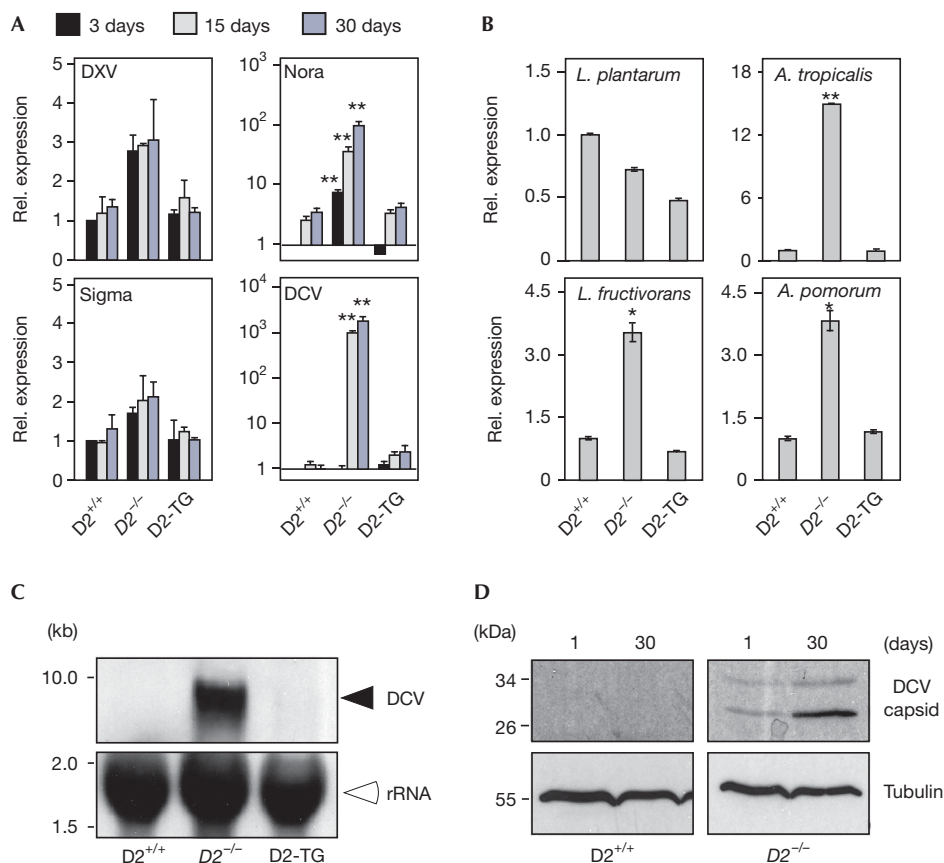


Fig 2 | Increase of (+) RNA viruses and bacteria in *Dnmt2* mutant flies. (A) Q-PCR analysis for dsRNA *Drosophila X* virus, (-) RNA (Sigma) virus and (+) RNA (Nora and DCV) viruses in wild-type ($D2^{+/+}$), *Dnmt2* mutant ($D2^{-/-}$) and transgenic rescue ($D2\text{-TG}$) and at various time points after hatching (3, 15, 30 days). (B) Q-PCR analysis for *Lactobacillus plantarum*, *Acetobacter tropicalis*, *Lactobacillus fructivorans* and *Acetobacter pomorum* in genotypes as in (A) flies 30 days after hatching. DNA content of bacteria in wild-type was set to 1 and normalized to *rp49* content in individual experiments. Error bars represent s.d.'s from three biological replicates. (C) Northern blotting for DCV genomes from aged (30 days) flies of the genotypes as in (A). Cross-hybridization of the DCV probe with ribosomal RNA was used as loading control. (D) Western blotting for DCV capsid from young (1 day) and aged (30 days) wild-type ($D2^{+/+}$) and *Dnmt2* mutant ($D2^{-/-}$) flies. Tubulin was probed as loading control. RNA expression of genes in wild-type was set to 1 and normalized to *rp49* mRNA in individual experiments. Error bars represent s.d.'s from three biological replicates. Student's *t*-test *P*-values are indicated (* $P < 0.05$; ** $P < 0.01$). DCV, *Drosophila C* virus; dsRNA, double-stranded RNA; Q-PCR, quantitative PCR; rel., relative.

associated with general stress response (for example, cytochrome and glutathione *S*-transferase genes, Table S3) as well as with immune response pathways (Fig 1B). Gene ontology (GO) term analysis indicated that various processes that were associated with the innate immune response and related stress pathways were misregulated in *Dnmt2* mutants (supplementary Fig S2A,B online).

Quantitative PCR analysis was used to validate the micro-array data. *Dnmt2* mutant flies showed significantly increased expression of genes in response pathways to microbes, fungi and viruses that became more pronounced with age (Fig 1C). The expression of anti-microbial peptide RNA in *Dnmt2* mutants was also confirmed by northern blotting (Fig 1D). Importantly, a fly line that carries a transgenic *Dnmt2*-EGFP construct in the *Dnmt2* mutant background ($D2\text{-TG}$, [16]) showed gene expression levels, which were similar to wild-type controls (Fig 1C, D) indicating the rescue of the *Dnmt2* mutant phenotype. These results confirm the induction of innate immune responses in *Dnmt2* mutants.

Elevated expression of virus-induced genes (Fig 1B, C) indicated that viruses were present in *Dnmt2* mutant flies. As DNA viruses have not been detected in *Drosophila* laboratory strains and *Dnmt2* is an RNA methyltransferase, we determined the levels of naturally occurring RNA viruses in *Dnmt2* mutant flies. While the RNA levels of double-stranded (ds) *Drosophila X* virus and single-stranded (-) Sigma virus were low and not affected by the *Dnmt2* mutation and age of the flies (Fig 2A), *Dnmt2* mutants contained high RNA levels of single-stranded (+) RNA viruses. Compared with wild-type flies, Nora virus and DCV levels were increased between 100 and 3,000-fold, respectively, particularly in older *Dnmt2* mutant animals (Fig 2A). Transgenic rescue flies ($D2\text{-TG}$) carried viral RNA levels that were similar to wild-type controls (Fig 2A). We also determined the bacterial load of adult *Drosophila* using diagnostic quantitative PCR. *Wolbachia* was not present in *Dnmt2* mutant flies (data not shown). Further analysis showed comparably moderate differences for three out

of four dominant gut bacteria [17] between the tested genotypes (Fig 2B). These results indicate that highly increased levels of (+) RNA viruses rather than gut bacteria levels caused the upregulation of innate immunity responses in *Dnmt2* mutant flies.

DCV infection has been extensively studied in *Drosophila* [18]. Northern blotting confirmed full-length DCV RNA genomes in 30-day-old *Dnmt2* mutant flies (Fig 2C), and western blotting revealed significant amounts of DCV capsid proteins in *Dnmt2* mutants of this age (Fig 2D). These results indicate that *Dnmt2* mutants were unable to control DCV levels and suggested a role for Dnmt2 in antiviral defence mechanisms.

The natural route for DCV infections is the digestive system. To establish a physiological infection system, fly carcasses of 30-day-old flies (donors) were fed for 24 h to freshly hatched (recipient) flies (supplementary Fig S3A online). In a first set of experiments, *Dicer-2* mutants (*Dcr-2^{L811fsX}*) served as recipients, because *Dcr-2* has been shown to be important for the restriction of viruses in *Drosophila* [4]. Infection with DCV supplied by donors (supplementary Fig S3B online) was monitored using quantitative PCR at two time points (1 and 10 days after feeding). *Dcr-2* mutant recipients showed slightly increased DCV RNA levels at day 1 after infection with wild-type or D2-TG donors (supplementary Fig S3C online). In contrast, *Dnmt2* mutant donors caused a 250-fold increase of DCV RNA in *Dcr-2* mutants (supplementary Fig S3C online). Importantly, at day 10 after infection with *Dnmt2* mutant donors, *Dcr-2* mutant recipients had accumulated 12,000 times more DCV RNA than control recipients (Fig S3C online). These results confirm that infection-by-feeding (oral infection) can be used to propagate DCV in flies with compromised antiviral defence mechanisms.

To analyse the function of Dnmt2 in DCV suppression, wild-type, *Dnmt2* mutant and D2-TG recipients were fed with *Dnmt2* mutant donors (supplementary Fig S3B online). DCV levels in recipient flies were comparable at the time of hatching (supplementary Fig S3D online). Wild-type recipients suppressed DCV levels efficiently with only residual virus detectable after feeding with *Dnmt2* mutant donors (Fig 3A, left). In contrast, *Dnmt2* mutant recipients showed significant amplification of DCV RNA (10,000 to 35,000-fold, Fig 3A, middle). DCV levels in D2-TG recipients were similar to wild-type controls, thus confirming that DCV suppression was Dnmt2-dependent (Fig 3A, right). Northern blotting (10 days after infection) showed full-length DCV RNA genomes in *Dnmt2* mutant but not in wild-type recipients (Fig 3B). Western blotting also confirmed that infected *Dnmt2* mutant recipients contained more viral capsid protein if compared with wild-type and D2-TG recipients (Fig 3C). To further demonstrate a role for Dnmt2 in the control of DCV infection, we used purified DCV to challenge young animals by intra-thoracic injection. Using this approach, we found that *Dnmt2* mutants succumbed more rapidly to the infection than transgenic rescue controls (D2-TG, Fig 3D). These results clearly show that Dnmt2 is required for the efficient control of DCV in *Drosophila*.

To test whether Dnmt2 directly affects the accumulation of DCV, recipients that overexpressed Dnmt2 (supplementary Fig S4A online) were used in oral infection experiments. Expression of Dnmt2 under the ubiquitin promoter in the wild-type background (*w¹¹¹⁸*; pUbq>>*Dnmt2*-EGFP; D2-OE) suppressed DCV RNA levels during the infection with *Dnmt2*

mutant donors (supplementary Fig S4B online). Importantly, recipients that expressed a catalytically inactive *Dnmt2* (point mutation in motif IV) under the same promoter but in the *Dnmt2* mutant background (*Dnmt2⁹⁹*; pUbq>>*Dnmt2*-cat^{mutant}-FLAG; D2-catΔ) also suppressed DCV but not as efficiently as catalytically active Dnmt2 (supplementary Fig S4C–E online). To further analyse the contribution of the catalytic activity of Dnmt2 to virus control, we challenged these transgenic flies with purified DCV by intra-thoracic injection. We found that D2-catΔ flies succumbed more rapidly to the infection than D2-OE flies (Fig 3E). These results show that Dnmt2 is necessary and sufficient to suppress DCV levels and indicate that the cytosine-5 methyltransferase activity of *Dnmt2* contributes to Dnmt2-mediated DCV control.

Gene expression analysis for anti-microbial genes and the DCV-induced gene, *vir-1*, after oral infection with *Dnmt2* mutant donors showed rapid upregulation of immune response genes in control, but not in *Dnmt2* mutant recipients (Fig 4A). Northern blotting also confirmed that wild-type but not *Dnmt2* mutant recipients expressed high RNA levels of the immune-inducible peptide *Metchnikowin* at day 1 after infection (Fig 4B). In contrast, *Dnmt2* mutant recipients showed significantly increased immune responses only at day 10 after oral infection (Fig 4A). Importantly, at this time, wild-type and D2-TG recipients had efficiently downregulated the initial immune response (Fig 4A). These results indicate that Dnmt2 function is important during the acute phase of immune system activation.

To further explore the function of Dnmt2 during acute infection, Dnmt2 localization was analysed in infected gut tissues. To this end, transgenic rescue flies (D2-TG) were used, which express Dnmt2-EGFP at comparable levels to wild-type flies (supplementary Fig S4E online). While Dnmt2-EGFP was ubiquitous in controls, feeding *Dnmt2* mutant donors caused the accumulation of Dnmt2-EGFP into distinct cytoplasmic macromolecular structures (Fig 4C). This observation indicates that relocalization of Dnmt2 is important for its function during the response to infection.

Finally, the interaction of Dnmt2 with DCV was tested using RNA immunoprecipitation (RIP) with EGFP nanobodies after infection of D2-TG recipients with *Dnmt2* mutant donors. Control RIP was performed on infected flies, which expressed EGFP from the ubiquitin promoter (Ubq::EGFP). The specificity of RIP was confirmed by analysing the precipitate for tRNA-Asp^{GTC}, which is a substrate of Dnmt2. The analysis showed that Dnmt2-EGFP complexes specifically interacted with tRNA-Asp^{GTC} and with at least three regions of the DCV RNA genome (Fig 4D). These results indicate that Dnmt2 is associated with DCV genomic RNA and suggest direct interactions of Dnmt2 with viral RNA.

DISCUSSION

Eukaryotic cells induce the innate immune system after detecting parts of a pathogen such as RNA, DNA or specific surface molecules of microbial and viral stressors. Vertebrates have developed an intricate system of membrane-based receptors, which can distinguish and bind various polynucleotides on the basis of their termini and structure [19–21]. Invertebrates share such receptor-based detection but additionally use RNA-mediated interference-based mechanisms, which limit the amplification of viral RNA genomes and RNA-based intermediates of replicating

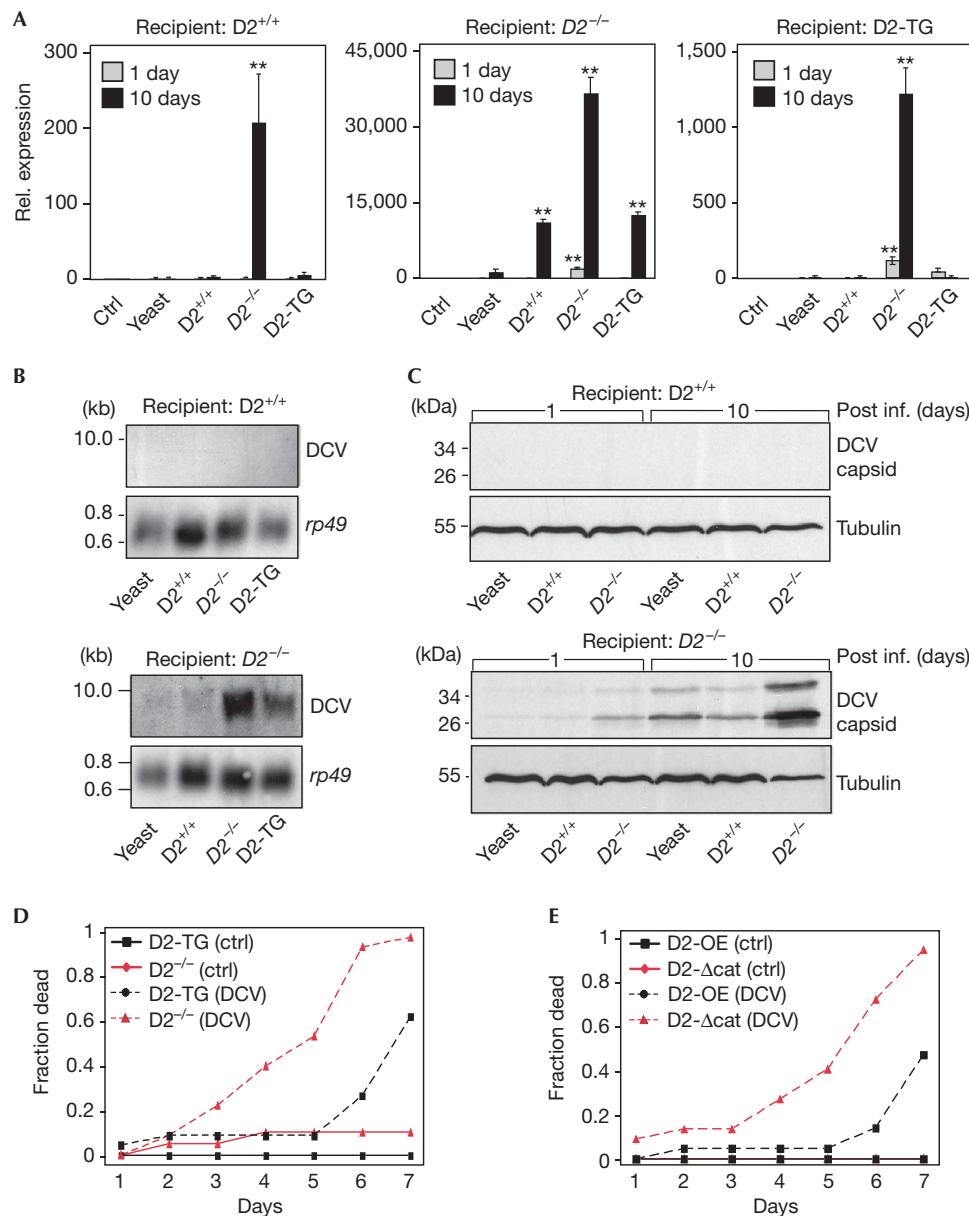


Fig 3 | *Dnmt2* is required to suppress DCV accumulation. (A) Q-PCR analysis for DCV in wild-type ($D2^{+/+}$), *Dnmt2* mutant ($D2^{-/-}$) and transgenic rescue ($D2-TG$) recipients after feeding with yeast or donors (x axis) and two incubation periods (1 or 10 days). (B) Northern blotting for DCV in wild-type ($D2^{+/+}$) and *Dnmt2* mutant ($D2^{-/-}$) recipients after feeding with yeast or donors as described in (A). *Rp49* was probed as loading control. (C) Western blotting for DCV capsid from wild-type ($D2^{+/+}$) and *Dnmt2* mutant ($D2^{-/-}$) recipients after feeding with donors and incubation periods as described in (A). Tubulin was probed as loading control. RNA expression of virus was set to 1 in non-infected (ctrl) and normalized to *rp49* mRNA in individual experiments. Error bars represent s.d.'s from three biological replicates. *P*-values were determined by Student's *t*-test (** $P < 0.01$). (D) Lethality of wild-type ($D2^{+/+}$) and *Dnmt2* mutant ($D2^{-/-}$) flies challenged with purified DCV (2×10^4 virus particles per μ l) by intra-thoracic injection. A representative of three experiments is shown; $D2^{-/-}$ and $D2-TG$ (ctrl) $P = 0.533$; $D2^{-/-}$ and $D2-TG$ (DCV) $P = 0.000034$ by log-rank test. (E) Lethality of flies that overexpress *Dnmt2* ($D2-OE$) and flies overexpressing a catalytically inactive *Dnmt2* ($D2-cat\Delta$) challenged with purified DCV (2×10^4 virus particles per μ l) by intra-thoracic injection. A representative of three experiments is shown; $D2-OE$ and $D2-cat\Delta$ (ctrl) $P = 1$; $D2-OE$ and $D2-cat\Delta$ (DCV) $P = 0.005$ by log-rank test. DCV, *Drosophila C* virus; Q-PCR, quantitative PCR; rel., relative.

virus. As pattern recognition receptors can also detect the modification status of nucleic acids [21–23], it is conceivable that host-induced modification of viral RNA genomes facilitates their detection and contributes to efficient antiviral mechanisms.

Our findings support this notion and implicate the RNA methyltransferase *Dnmt2* in the activation of the innate immune system during (+) RNA virus infections. This conclusion is based upon the observation that immune peptides are induced in

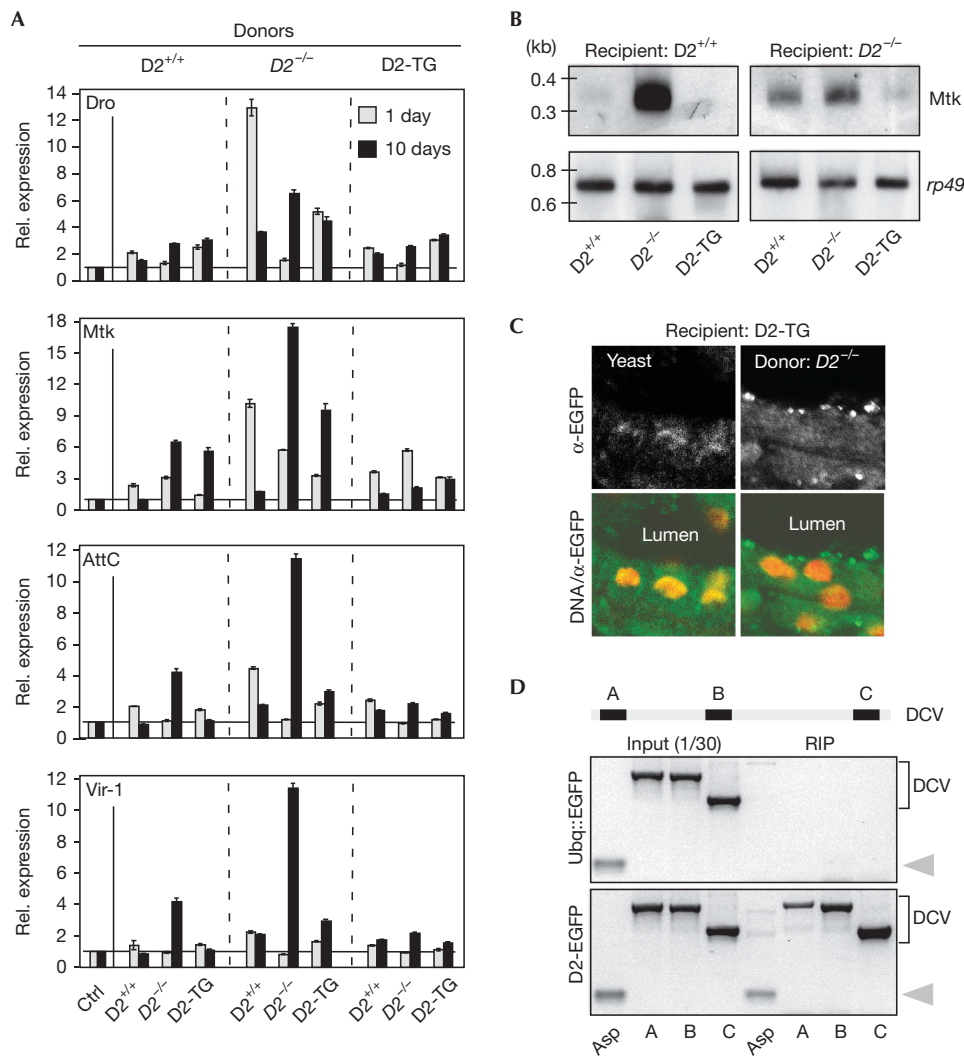


Fig 4 | *Dnmt2* mutants show delayed immune responses and Dnmt2-EGFP relocalizes and interacts with DCV RNA during infection. (A) Q-PCR analysis for immune response genes in wild-type ($D2^{+/+}$), *Dnmt2* mutant ($D2^{-/-}$) and transgenic rescue ($D2$ -TG) recipient flies after feeding with donors and incubation periods as described in Fig 3A. (B) Northern blotting for *Metchnikowin* RNA in wild-type ($D2^{+/+}$) and *Dnmt2* mutant ($D2^{-/-}$) recipients after feeding with donors as described in Fig 3A and an incubation period of 1 day. *Rp49* was probed as loading control. (C) Immunofluorescence images of fixed *Drosophila* hindgut tissue from $D2$ -TG recipients after feeding with yeast alone (yeast) or *Dnmt2* mutant ($D2^{-/-}$) donors followed by an incubation period of 1 day. The border between lumen and gut cells is shown. *Dnmt2*-EGFP fusion protein was visualized using antibodies against EGFP (white in upper panels, green in lower panels). DNA is shown in red. (D) Schematic illustration of the DCV RNA genome with three primer sets used for the analysis of RIPs. Agarose gels show the results of RIP from control (Ubq::EGFP) and $D2$ -TG ($D2$ -EGFP) recipients after continuous feeding (72 h) with *Dnmt2* mutant donors. Both samples contained tRNA-Asp^{GTC} (grey arrowhead) and DCV in the input fraction. After RIP, only *Dnmt2*-EGFP complexes contained tRNA-Asp^{GTC} (grey arrowhead) and DCV. RNA expression was set to 1 in non-infected recipients (ctrl) and normalized to *rp49* mRNA in individual experiments. Error bars represent s.d.'s from three biological replicates. DCV, *Drosophila* C virus; Q-PCR, quantitative PCR; RIP, RNA immunoprecipitation; rel., relative.

wild-type but not in *Dnmt2* mutants after oral infection. Additionally, the relocalization of Dnmt2 into distinct macromolecular foci in gut cells suggests compartmentalized activity of Dnmt2 during this phase of infection. The observation that high levels of non-functional Dnmt2 are unable to efficiently suppress DCV after oral infection and that these flies are more sensitive to intra-thoracic infections using pure DCV suggests a role for RNA methylation in

the process. An analysis of the RNA methylation status of the DCV genome is complicated by the suppression of viral genomes through Dnmt2 activity and needs to be addressed in future studies. It has also been reported that Dnmt2 can bind tightly to DNA without methylating it [24]. It is therefore also conceivable that Dnmt2 presents viral RNA to innate immunity receptors or RNA processing enzymes to control specific RNA viruses in *Drosophila*.

METHODS

Fly husbandry and phenotypic analysis. Flies were usually maintained on standard medium at 25°C, 60% humidity, under a 12-h light–dark cycle. For culturing flies under crowded conditions, flies were allowed to hatch but were not removed from the vials. These flies reproduced and finally succumbed to the crowded conditions. Developing larvae were feeding not only on medium but also on carcasses of the parental generation. Larvae, pupae and adults arising from this generation were scored for melanotic inclusions.

Gene expression analysis. RNA was isolated from two biological replicates (50 male flies per replicate, non-stressed, 15 days after hatching) per genotype (*w¹¹¹⁸* and *Dnmt2⁹⁹* [12]) and analysed for gene expression using the *Drosophila* Gene Expression Microarray 4 × 44K (Agilent Technologies). Sample preparation and hybridization were performed according to the manufacturer's instructions. Gene expression array raw data were preprocessed and analysed using Chipster v1.4. Data were filtered for significance (Student's *t*-test) and analysed using DAVID 6.7 (<http://david.abcc.ncifcrf.gov/>) and tools provided by the GO consortium (<http://www.geneontology.org/>).

Protein extraction and western blotting. Twenty adult flies were homogenized in 1 volume of RIPA buffer and incubated at 4°C for 10 min. Cells were disrupted by 20 strokes in a douncer, followed by passing the extract 10 × through a syringe needle (G20), followed by centrifugation. Fifty micrograms of total protein extracts were analysed by 10% SDS–PAGE. Western blotting was performed using antibodies against DCV capsid (chicken; 1:25,000) and Tubulin (mouse DM1A; 1:10,000; Sigma).

Fly infection by feeding. Inoculation experiments were performed with crude extracts from 200 mg of *w¹¹¹⁸*, *Dnmt2⁹⁹*, D2-TG flies. After homogenization in 500 μl H₂O, carcasses were mixed with 500 μl yeast paste. This mix (donors) was used for feeding of freshly hatched flies (recipients). After 24 h, flies were transferred to standard *Drosophila* medium and RNA was isolated at specified time points.

Intra-thoracic injections. DCV was purified and used for intra-thoracic challenge of groups of 20 flies (4–7-day-old) as previously described [25]. Mortality was monitored as a function of time. A log-rank test was used to determine significance.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We thank Nevcin Senturk for the establishment of RIPs. This work was supported by grants from the Deutsche Forschungsgemeinschaft to M.S. and F.L. (FOR1082). Z.D. is a fellow of the HBIGS graduate programme at the University of Heidelberg.

Author contributions: M.S. conceived the study. Z.D. and T.P. performed molecular work and phenotypic characterization of *Drosophila*. T.P. performed microarray expression analysis. Z.D. and K.H. performed validation of RNA expression analysis and northern blotting. Z.D. performed oral infection experiments. B.G. and S.C. performed intra-thoracic infections and Z.D. performed RIP assays. Z.D., S.C., F.L. and M.S. designed the experiments and interpreted the results. MS wrote the manuscript with contributions from Z.D., S.C. and F.L. as well as input from the other co-authors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- van Rij RP, Saleh MC, Berry B, Foo C, Houk A, Antoniewski C, Andino R (2006) The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in *Drosophila melanogaster*. *Genes Dev* **20**: 2985–2995
- Wang XH, Aliyari R, Li WX, Li HW, Kim K, Carthew R, Atkinson P, Ding SW (2006) RNA interference directs innate immunity against viruses in adult *Drosophila*. *Science* **312**: 452–454
- Galiana-Arnoux D, Dostert C, Schneemann A, Hoffmann JA, Imler JL (2006) Essential function *in vivo* for Dicer-2 in host defense against RNA viruses in *Drosophila*. *Nat Immunol* **7**: 590–597
- Deddouche S, Matt N, Budd A, Mueller S, Kemp C, Galiana-Arnoux D, Dostert C, Antoniewski C, Hoffmann JA, Imler JL (2008) The DEXD/H-box helicase Dicer-2 mediates the induction of antiviral activity in *Drosophila*. *Nat Immunol* **9**: 1425–1432
- Cherry S, Silverman N (2006) Host-pathogen interactions in *Drosophila*: new tricks from an old friend. *Nat Immunol* **7**: 911–917
- Dalpke A, Helm M (2012) RNA mediated Toll-like receptor stimulation in health and disease. *RNA Biol* **9**: 828–842
- Huang HR, Chen ZJ, Kunes S, Chang GD, Maniatis T (2010) Endocytic pathway is required for *Drosophila* Toll innate immune signaling. *Proc Natl Acad Sci USA* **107**: 8322–8327
- Lee YS et al (2009) Silencing by small RNAs is linked to endosomal trafficking. *Nat Cell Biol* **11**: 1150–1156
- Goll MG, Bestor TH (2005) Eukaryotic cytosine methyltransferases. *Annu Rev Biochem* **74**: 481–514
- Schaefer M, Lyko F (2010) Solving the Dnmt2 enigma. *Chromosoma* **119**: 35–40
- Goll MG, Kirpekar F, Maggert KA, Yoder JA, Hsieh CL, Zhang X, Golic KG, Jacobsen SE, Bestor TH (2006) Methylation of tRNAsp by the DNA methyltransferase homolog Dnmt2. *Science* **311**: 395–398
- Schaefer M, Pollex T, Hanna K, Tuorto F, Meusburger M, Helm M, Lyko F (2010) RNA methylation by Dnmt2 protects transfer RNAs against stress-induced cleavage. *Genes Dev* **24**: 1590–1595
- Thiagarajan D, Dev RR, Khosla S (2011) The DNA methyltransferase Dnmt2 participates in RNA processing during cellular stress. *Epigenetics* **6**: 103–113
- Rodriguez AJ, Zhou Z, Tang ML, Meller S, Chen J, Bellen H, Kimbrell DA (1996) Identification of immune system and response genes, and novel mutations causing melanotic tumor formation in *Drosophila melanogaster*. *Genetics* **143**: 929–940
- Watson KL, Johnson TK, Denell RE (1991) Lethal(1) aberrant immune response mutations leading to melanotic tumor formation in *Drosophila melanogaster*. *Dev Genet* **12**: 173–187
- Schaefer M, Steringer JP, Lyko F (2008) The *Drosophila* cytosine-5 methyltransferase Dnmt2 is associated with the nuclear matrix and can access DNA during mitosis. *PLoS ONE* **3**: e1414
- Wong ZS, Hedges LM, Brownlie JC, Johnson KN (2011) Wolbachia-mediated antibacterial protection and immune gene regulation in *Drosophila*. *PLoS ONE* **6**: e25430
- Dostert C, Jouanguy E, Irving P, Troxler L, Galiana-Arnoux D, Hetru C, Hoffmann JA, Imler JL (2005) The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of *Drosophila*. *Nat Immunol* **6**: 946–953
- Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* **124**: 783–801
- Pétrilli V, Dostert C, Muruve DA, Tschopp J (2007) The inflammasome: a danger sensing complex triggering innate immunity. *Curr Opin Immunol* **19**: 615–622
- Rehwinkel J, Reis e Sousa C (2010) RIGorous detection: exposing virus through RNA sensing. *Science* **327**: 284–286
- Gehrig S et al (2012) Identification of modifications in microbial, native tRNA that suppress immunostimulatory activity. *J Exp Med* **209**: 225–233
- Ishii KJ, Akira S (2005) TLR ignores methylated RNA? *Immunity* **23**: 111–113
- Dong A, Yoder JA, Zhang X, Zhou L, Bestor TH, Cheng X (2001) Structure of human DNMT2, an enigmatic DNA methyltransferase homolog that displays denaturant-resistant binding to DNA. *Nucleic Acids Res* **29**: 439–448
- Cherry S, Perrimon N (2004) Entry is a rate-limiting step for viral infection in a *Drosophila melanogaster* model of pathogenesis. *Nat Immunol* **5**: 81–87