SUPPLEMENTAL INFORMATION TO DURDEVIC ET AL.

Supplemental methods

Fly strains

w^{1118} (Bloomington), w^{1118}; $Dnmt2^{99}$ (Schaefer et al, 2010), w^{1118}/Y-HA1925 and w^{1118}/Y-HA1925; $Dnmt2^{149}$ (Phalke et al, 2009), D2-TG (w^{1118}; pGeno>>*Dnmt2*-EGFP; Schaefer et al., 2008), D2-OE (w^{1118}; pUbq>>*Dnmt2*-EGFP),D2-catΔ (w^{1118}; $Dnmt2^{99}$; pUbq>>*Dnmt2*-cat^{mutant}-FLAG), Act5C-GAL4/CyO Ubi-GFP (cross of Bloomington 4888 and 4414), y^{d2} w^{1118}; $Dcr\text{-}2^{L811fsX}$ (Bloomington). All flies were kept at 25°C on standard *Drosophila* medium.

Heat shock experiments

Heat shock treatments of 2nd to third instar larvae were performed at 37°C for 2 times 45 minutes (with an interval of 1 hour recovery time) in a water bath. After the heat shock larvae were kept at 25 °C on standard *Drosophila* medium until hatching.

RNA extraction, reverse transcription (RT) and q-PCR

Total RNA was extracted using Trizol (Invitrogen). The RNA was isolated from whole flies. For first strand cDNA synthesis, RNA was either reverse-transcribed using the QuantiTect Reverse Transcription Kit (Qiagen) or was treated with TurboDNAse (Ambion), before reverse transcription using SuperScript III (Invitrogen). qPCR analyses (primers, Table S4) were performedd on a LightCycler 480 Real Time PCR System (Roche) using the ABsolute QPCR SYBR Green Mix (Thermo Scientific)

Diagnostic PCR

Genomic DNA from whole flies was extracted using the DNeasy Blood and Tissue Kit (Qiagen). Diagnostic q-PCR analyses were performed on 100 ng of genomic DNA per reaction on a LightCycler 480 Real Time PCR System (Roche) using the ABsolute QPCR SYBR Green Mix (Thermo Scientific).

Northern blotting

RNA was separated on Urea-PAGE or Formamid-Gels, transferred to Nylon membranes (Roche) and hybridized overnight at 40ºC with DIG-labeled PCR probes (primers, Table S4). For probe labeling, 1 nmol DIG-dUTP (Roche) was used in a standard PCR reaction using genomic DNA as a template. Pre-hybridization, washing steps, incubation with antibodies and detection were performed according to the manufacturer's recommendations (DIG Northern Starter Kit, Roche).

Immunostainings of gut tissues

After inoculation by feeding, animals were incubated for 24 hours on normal food. Gut tissues were dissected in PBS, fixed for 15 minutes in 4% paraformaldehyde in PBS. Tissues were washed with PBS/0.05% Triton X-100, blocked in blocking solution (3% BSA, 0.05% Triton X-100, 0.05% sodium azide in PBS). Incubation with primary antibodies (anti-EGFP, Abcam, 1:1000; anti-DCV capsid, 1:5000) was performed in the cold (over night). Secondary antibodies (Alexa Fluor-conjugated, Invitrogen) were applied for 2 hours at room temperature. Samples were analyzed by confocal laser scanning microscopy (TCS SP2, Leica Microsystems, Germany).

RNA immunoprecipitations

Inoculation of D2-TG recipents was performed as described above but continuously for 72 hours. Adult flies were homogenized in Empigen buffer (HEPES-KOH pH 7.4, 30 mM; NaCl 150 mM; MgOAc 2 mM; Empigen 0.5%, 0.01 U/μl RNAsIn; DTT 5 mM, PMSF 1mM, 1x Protease Inhibitors). Tissue was disrupted by 20 strokes in a douncer, followed by centrifugation for 30 minutes at 16.000g. 1.2 mg of total protein extracts were incubated with EGFP nanobodies (ChromoTek) for 1 hour at 4°C. Paramagnetic beads were washed in Empigen buffer, followed by proteinase K digestion (0.2 mg/ml) for 30 minutes at 37°C and Phenol/Chloroform extraction of bound RNA.

Fly infection by feeding

Inoculation experiments were performed with crude extracts from 200 µg of w^{1118}, $Dnmt2^{99}$, D2-

TG flies. After homogenization in 500 µl H_2O, carcasses were mixed with 500 µl yeast paste. This mix (donors) was used for feeding of freshly hatched flies (recipients). After 24 hours flies were transferred to standard *Drosophila* medium and RNA was isolated at specified time points.

Supplemental references

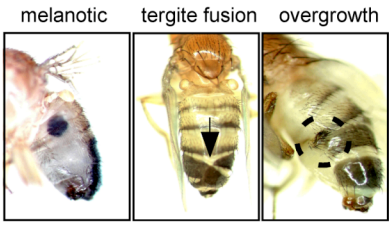
Schaefer M, Pollex T, Hanna K, Tuorto F, Meusburger M, Helm M, Lyko F (2010) RNA methylation by Dnmt2 protects transfer RNAs against stress-induced cleavage. *Genes & Development* **24**(15)**:** 1590-1595

Phalke S, Nickel O, Walluscheck D, Hortig F, Onorati MC, Reuter G (2009) Retrotransposon silencing and telomere integrity in somatic cells of *Drosophila* depends on the cytosine 5 methyltransferase *DNMT2*. *Nat. Genet*. **41**(6): 696-702

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(1)**: e1414

Durdevic_Figure S1

A



B

geno / pheno	D2$^{+/+}$		*D2*$^{-/-}$	
	F	M	F	M
melanotic	1.9%	0	37.1%	30.0%
leg malformed	5.7%	0	3.0%	2.3%
tergite fusion	0	0	1.4%	9.8%
appendage overgrowth	0	0	0	3.9%

Fig S1. Stress-Induced phenotypic changes in *Dnmt2* mutant adults

(A) Melanotic lesions can be induced by heat shock treatment. Examples of melanotic lesion (left), incomplete tergite fusion (middle, arrow) and appendage overgrowth (right, dashed circle) in flies, originating from heat-shocked larvae of the genotypes: w^{1118}/Y-HA1925 (D2$^{+/+}$) and w^{1118}/Y-HA1925; *Dnmt2*^{149} (D2$^{-/-}$).

(B) Quantification of phenotypic abnormalities in wildtype (D2$^{+/+}$, n=100) and *Dnmt2* mutant (D2$^{-/-}$, n=126) flies from experiments as described in **(A)**.

Durdevic_Figure S2

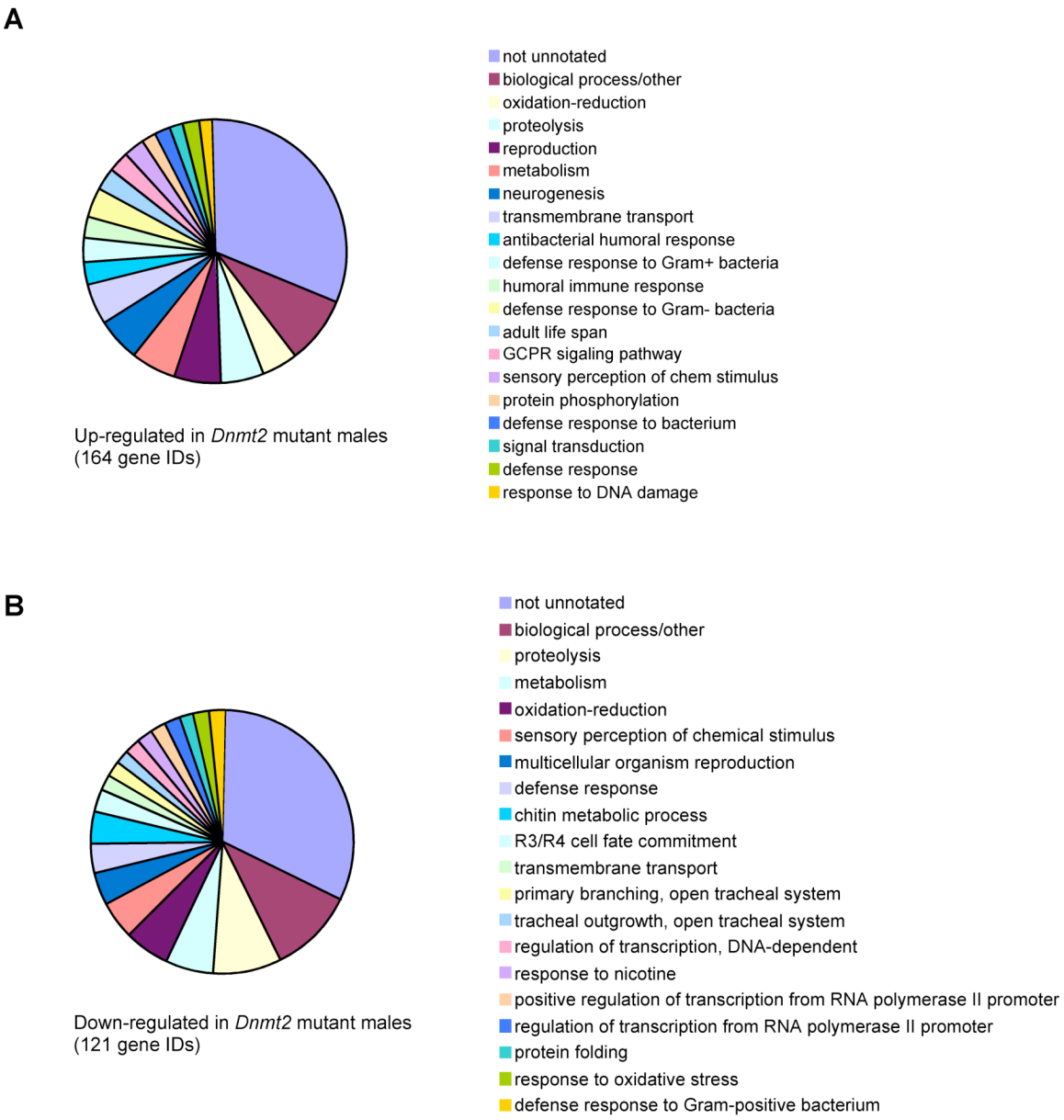


Fig S2. Mis-Regulation of gene expression in adult *Dnmt2* mutants

(A) *Dnmt2* mutants up-regulate genes across a wide range of biological processes. Genes up-regulated at least 2-fold (see **Table S1**) were assigned functional categories using the GO annotation of the Flybase consortium (http://flybase.org/)

(B) *Dnmt2* mutants down-regulate genes across a wide range of biological processes. Genes down-regulated at least 2-fold (see **Table S2**) were assigned functional categories using the GO annotation of the Flybase consortium (http://flybase.org/)

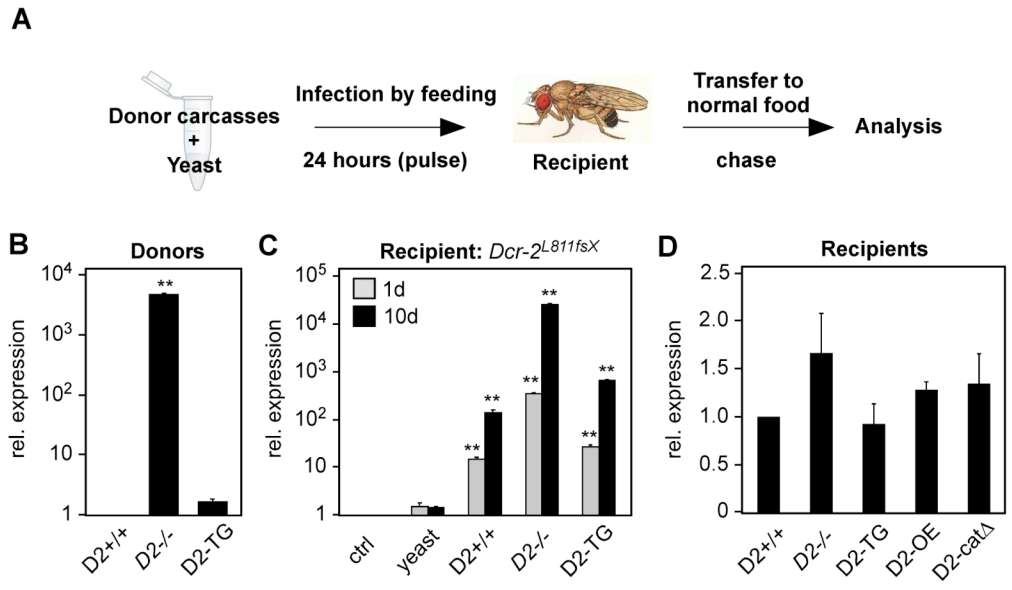


Fig S3. Infection-by-feeding experiment

(A) Design of infection experiments using adult donors and recipients. Feeding of recipients with donors was carried for 24 hours (pulse) before flies were moved to standard food (chase) until analysis.

(B) Q-PCR analysis for DCV in 30 days old wildtype ($D2^{+/+}$), *Dnmt2* mutant ($D2^{-/-}$) and transgenic rescue (D2-TG) donors.

(C) Q-PCR analysis for DCV in *Dicer-2* mutant recipients ($Dcr\text{-}2^{L811fsX}$) after feeding with donors as described in **(B)**. Two time points after infection (1 day, grey bars; 10 days, black bars) were analyzed.

(D) Q-PCR analysis for DCV in freshly hatched recipients.

RNA expression of virus was set to 1 in non-infected (ctrl) and normalized to *rp49* mRNA in individual experiments. Error bars represent standard deviations from three biological replicates. Student's t-test p-values are indicated (* $p<0.05$; ** $p<0.01$).

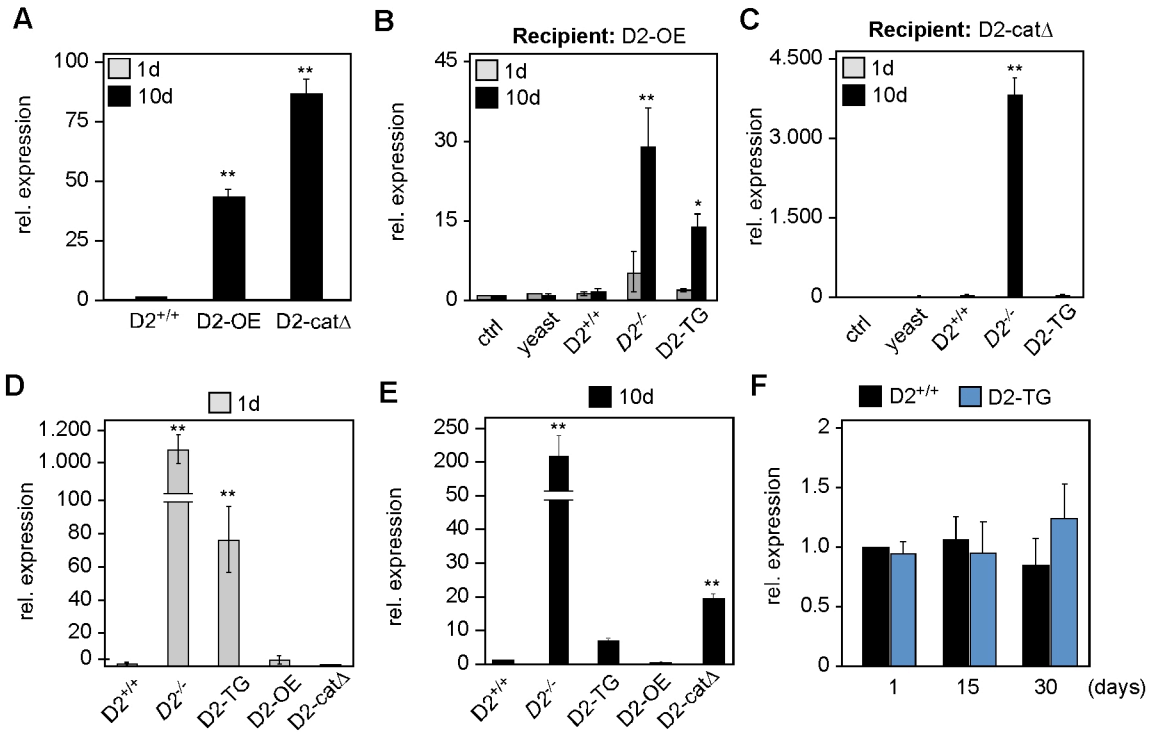


Fig S4. The catalytic activity of Dnmt2 contributes to DCV suppression.

(A) Q-PCR analysis for Dnmt2 mRNA expression in wild-type flies ($D2^{+/+}$), wild-type flies that over-express Dnmt2 (w^{1118}; pUbq>>*Dnmt2*; D2-OE) and *Dnmt2* mutant flies expressing a catalytically inactive *Dnmt2* ($D2^{-/-}$; pUbq>>*Dnmt2*-catΔ-FLAG).

RNA expression of Dnmt2 was normalized to *rp49* mRNA and set to 1 in wild-type flies ($D2^{+/+}$). Error bars represent standard deviations from three biological replicates. Student's t-test p-values are indicated (**p<0.01).

(B) Q-PCR analysis for DCV expression in recipient flies that over-express Dnmt2 (w^{1118}; pUbq>>*Dnmt2*; D2-OE) after feeding with donors as described in **Fig 3A** and incubation periods of 1 or 10 days.

(C) Q-PCR analysis for DCV expression in recipient *Dnmt2* mutant flies expressing a catalytically inactive *Dnmt2* ($D2^{-/-}$; pUbq>>*Dnmt2*-catΔ-FLAG) after feeding with donors as in **(B)**. RNA expression was set to 1 in controls at day 1 and normalized to *rp49* in individual experiments. Error bars represent standard deviations from 3 biological replicates.

RNA expression of virus was set to 1 in non-infected (ctrl) and normalized to *rp49* mRNA in individual experiments. Error bars represent standard deviations from three biological replicates. Student's t-test p-values are indicated (* $p<0.05$; ** $p<0.01$).

(D) Comparison of the DCV levels of various recipients that were fed with *Dnmt2* mutant donors at day 1. Data were extracted from **(B-C)** and **Fig 3A**. DCV levels were set to 1 in wild-type recipients at day 1.

(E) Comparison of the DCV levels of various recipients that were fed with *Dnmt2* mutant donors at day 10. Data were extracted as described in **(D)**. DCV levels were set to 1 in wild-type recipients at day 10.

Error bars represent standard deviations from three biological replicates. Student's t-test p-values are indicated (** $p<0.01$).

(F) Q-PCR analysis for Dnmt2 mRNA expression in wildtype ($D2^{+/+}$) and transgenic rescue (D2-TG) flies at three different ages (1, 15, 30 days after hatching).

RNA expression of Dnmt2 was normalized to *rp49* mRNA and set to 1 in wild-type flies ($D2^{+/+}$). Error bars represent standard deviations from three biological replicates.