sequences could be unambiguously mapped to the *Invader4* long terminal repeats (LTR), but this experiment failed to provide any evidence for DNA methylation (**Fig. 1b**). Our results are thus fundamentally different from the data reported by Phalke *et al.*[1], which had indicated that *Invader4* LTR sequences are methylated at the majority of their cytosine residues in every sequence context. Lastly, we also extended our analysis to *Invader4* LTR-containing subtelomeric repeats at chromosome 3R (previously associated with Dnmt2-dependent silencing[1]). More than 1,000 reads from 0–2-hour-old wild-type and Dnmt2 mutant embryos could be unambiguously mapped to subtelomeric chromosome 3R repeats, and these reads failed to reveal any evidence for DNA methylation (**Fig. 1c,d**). In conclusion, our results suggest that Dnmt2 does not methylate *Invader4* LTR sequences in *Drosophila* genomic DNA.

It is noteworthy that Dnmt2-dependent methylation of $tRNA^{Asp}$ and other tRNAs can be readily detected by bisulfite sequencing[7,8], although it has been notoriously difficult to establish robust and quantitative assays for locus-specific Dnmt2-mediated DNA methylation. Whereas the recently described use of a DNA methyltransferase mechanism for the methylation of RNA substrates[9] might provide an explanation for the spurious DNA methylation patterns observed in *Drosophila* genomic DNA[4] and in cell-free Dnmt2-dependent DNA methylation assays[3], these seemingly random methylation marks are unlikely to have a biological function. However, various lines of evidence[1,10], including those in our own unpublished data, suggest that Dnmt2 is required for efficient transposon silencing, and it will be interesting to investigate the role of RNA methylation in this pathway.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS
This work was supported by grants from the Deutsche Forschungsgemeinschaft to M.S and F.L. (Priority Programme Epigenetics, FOR1082).

AUTHOR CONTRIBUTIONS
M.S. performed the DNA methylation analysis. M.S. and F.L. conceived the study and wrote the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

Matthias Schaefer & Frank Lyko

Division of Epigenetics, DKFZ-ZMBH Alliance, German Cancer Research Center, Heidelberg, Germany. Correspondence should be addressed to F.L. (f.lyko@dkfz.de).

1. Phalke, S. *et al. Nat. Genet.* **41**, 696–702 (2009).
2. Schaefer, M. & Lyko, F. *Chromosoma* **119**, 35–40 (2010).
3. Hermann, A., Schmitt, S. & Jeltsch, A. *J. Biol. Chem.* **278**, 31717–31721 (2003).
4. Lyko, F., Ramsahoye, B.H. & Jaenisch, R. *Nature* **408**, 538–540 (2000).
5. Goll, M.G. *et al. Science* **311**, 395–398 (2006).
6. Zemach, A., McDaniel, I.E., Silva, P. & Zilberman, D. *Science* **328**, 916–919 (2010).
7. Schaefer, M., Pollex, T., Hanna, K. & Lyko, F. *Nucleic Acids Res.* **37**, e12 (2009).
8. Schaefer, M. *et al. Genes Dev.* **24**, 1590–1595 (2010).
9. Jurkowski, T.P. *et al. RNA* **14**, 1663–1670 (2008).
10. Kuhlmann, M. *et al. Nucleic Acids Res.* **33**, 6405–6417 (2005).

Phalke *et al.* reply:

In their Correspondence regarding our work published in *Nature Genetics*[1], Schaefer and Lyko argue that the processive DNA methyltransferase activity in every dinucleotide context at *Invader4* retroelements that we observed is difficult to reconcile with other reports about the activity of DNMT2 in *Drosophila*. However, until now, no other studies specifically analyzed DNA methylation at *Invader4* retroelements. Using high through-put bisulfite sequencing of *Invader4* long terminal repeats (LTRs), Schaefer and Lyko could not detect significant DNA methylation in a wild-type w^{1118} strain, which contradicts our published data showing early embryonic, DNMT2-dependent DNA methylation at *Invader4* LTRs. Schaefer and Lyko ultimately conclude that both the primers we used and the bisulfite treatment we performed in our analysis caused this discrepancy. However, methodological differences do not explain why our experiments generated more complete conversion of cytosines in the *Dnmt2*-null mutant control and in older embryos as compared to all the experiments reported by Schaefer and Lyko.

Schaefer and Lyko did not exactly repeat our experiments; instead, they modified the primers we used. Whereas our primers preferentially amplified fully methylated (and potentially unconverted) clones, their primer sets were designed for unbiased amplification or for preferential amplification of clones with fully converted cytosines and with no methylation in the primer binding sequence. Also, Schaefer and Lyko did not test the *Dnmt2*-null mutant for DNA methylation at functional *Invader4* retroelements.

In our studies, we used a newly established w^{1118} isogenic strain developed for genome-wide construction of molecularly mapped deletions[2]. The strain was selected from approximately 40 newly established isogenic lines on the basis of normal learning and memory behavior. In this background, we isolated variegating $P\{w^{+}\}$ insertions in order to identify DNMT2 targets and resolve their role in control of retrotransposon silencing in *Drosophila* somatic cells. This strain showed significant *Dnmt2* expression in 0–3-hour-old embryos[1]. We now have preliminary evidence that *Dnmt2* expression is highly variable in different strains of *Drosophila melanogaster* (unpublished data). Consequently, the differences found between the data presented by Schaefer and Lyko and our data might be explained by strain-specific differences in the early embryonic activity of *Dnmt2*. Further studies are needed to resolve the possible correlation between DNA methylation at retrotransposon LTRs and early embryonic *Dnmt2* expression.

Furthermore, it should be recognized that annotated *Invader4* LTR elements are structurally heterogeneous and are differentially distributed within the *Drosophila* genome, as demonstrated by FISH analysis using different probes[1]. Large clusters of *Invader4* LTRs are found at the subtelomeric regions of chromosome arms 2R and 3R. The 3R subtelomeric *Invader4* cluster contains six tandemly organized 984-bp repeats consisting each of a 381-bp telomere-specific repeat and of 603 bp of three rearranged crippled copies of *Invader4* LTRs[1]. Two of the three sequence datasets (including the only dataset from a *Dnmt2*-null mutant) presented by Schaefer and Lyko are exclusively derived from these defective subtelomeric elements. We previously concluded that subtelomeric-defective *Invader4* elements are silenced by a DNMT2-dependent and SUV4-20–dependent pathway, but we did not provide evidence of DNA methylation at these elements[1].

AUTHOR CONTRIBUTIONS
S.P. and O.N. analyzed the data. G.R. conceived the study and wrote the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

Sameer Phalke, Olaf Nickel & Gunter Reuter

Institute of Biology and Genetics, Martin Luther University Halle-Wittenberg, Halle, Germany. Present address: Institute of Molecular and Cell Biology, 61 Biopolis Way, Singapore (S.P.). Correspondence should be addressed to G.R. (reuter@genetik.uni-halle.de).

1. Phalke, S. *et al. Nat. Genet.* **41**, 696–702 (2009).
2. Ryder, E. *et al. Genetics* **177**, 615–629 (2007).

© 2010 Nature America, Inc. All rights reserved.