A *Drosophila* Smyd4 Homologue Is a Muscle-Specific Transcriptional Modulator Involved in Development

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**Abstract**

**Background:** SET and MYND domain (Smyd) proteins are involved in the transcriptional regulation of cellular proliferation and development in vertebrates. However, the *in vivo* functions and mechanisms by which these proteins act are poorly understood.

**Methodology/Principal Findings:** We have used biochemical and genetic approaches to study the role of a Smyd protein in *Drosophila*. We identified eleven *Drosophila* genes that encode Smyd proteins. CG14122 encodes a Smyd4 homologue that we have named dSmyd4. dSmyd4 repressed transcription and recruited class I histone deacetylases (HDACs). A region of dSmyd4 including the MYND domain interacted directly with ~150 amino acids at the N-termini of dHDAC1 and dHDAC3. dSmyd4 interacts selectively with Ebi, a component of the dHDAC3/SMRTER co-repressor complex. During embryogenesis dSmyd4 was expressed throughout the mesoderm, with highest levels in the somatic musculature. Muscle-specific RNAi against dSmyd4 resulted in depletion of the protein and lead to severe lethality. Eclosion is the final moulting stage of *Drosophila* development when adult flies escape from the pupal case. 80% of dSmyd4 knockdown flies were not able to eclose, resulting in late pupal lethality. However, many aspects of eclosion were still able to occur normally, indicating that dSmyd4 is likely to be involved in the development or function of adult muscle.

**Conclusions/Significance:** Repression of transcription by dSmyd4 and the involvement of this protein in development suggests that aspects of Smyd protein function are conserved between vertebrates and invertebrates.

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**Introduction**

Development requires the establishment and maintenance of patterns of gene expression. The activity of a gene is dependent on both the available repertoire of transcription factors and the local packaging of DNA into chromatin. Proteins involved in regulation of chromatin structure can therefore act as important determinants of developmental processes. SET and MYND domain (Smyd) proteins are conserved from yeast to vertebrates and the functions and mechanisms by which these proteins act are poorly understood.

SET and MYND domain (Smyd) proteins are involved in the transcriptional regulation of cellular proliferation and differentiation processes [7,16]. Smyd3 is over-expressed in most hepatocellular and colorectal carcinomas and a number of gene targets have been identified [7]. Smyd1 is expressed specifically in muscle tissue and loss-of-function studies in vertebrates identified an important role for Smyd1 in muscle development [8,16], but it is unclear how it fulfils this role *in vivo*. *Drosophila* provides a highly tractable system for *in vivo* studies of novel genes. We have identified eleven genes encoding Smyd homologues in the *Drosophila* genome. Data from gene expression databases indicate that six of these genes are expressed predominantly in the mesoderm, which develops to become muscle. Mesoderm is specified early in *Drosophila* embryogenesis [17]. Patterns of gene expression established within the mesoderm define regions of cardiac, visceral and somatic muscle [18,19]. The somatic musculature is formed by the fusion and migration of groups of cells to form a stereotypical pattern of larval musculature.
[20,21]. During the pupal stage these larval muscles are broken down and adult muscles are formed from pools of adult myoblasts specified during embryogenesis [22]. This study provides the first characterisation of a Drosophila Smyd protein. CG14122 (FlyBase accession number: FBgn0036282), named Drosophila Smyd4 (dSmyd4) is homologous to human Smyd4. Like vertebrate Smyd1 and Smyd2, Drosophila Smyd4 is able to repress transcription. dSmyd4 interacts directly with Drosophila class I HDACs via their N-termini. dSmyd4 is expressed throughout the mesoderm of Drosophila embryos and knockdown of dSmyd4 by RNAi results in lethality, predominantly at the late pupal stage. dSmyd4 loss-of-function results in a defect in eclosion of adult flies from the pupal case, suggesting an important role for dSmyd4 in development.

**Results**

**Identification of Drosophila Smyd proteins**

We performed basic local alignment search tool (BLAST) searches using each of the human Smyd homologues to comprehensively identify putative Smyd proteins encoded in the Drosophila genome. We identified eleven genes that contained both SET and MYND domains. Other BLAST hits were discarded. CG11160, CG12119, CG14122, CG14590, CG1868, CG18136, CG7759, CG8378, CG8503, CG29642 and MSTA contained well-conserved MYND and SET domains compared to human Smyd homologues (Fig S1). Gene expression data from FlyAtlas and the Berkeley Drosophila Genome Project in situ project suggest that most Drosophila Smyd proteins are expressed specifically in muscle, brain or sex specific tissues (Table S1) [Supplementary Table 1; 23,24]. The subcellular localisation of six of these proteins was analysed. CG14122, CG1868, CG11160 and CG8378 exhibited predominantly cytoplasmic over-expression patterns, whilst CG12119 was predominantly nuclear and CG8503 was exclusively cytoplasmic (Fig S2).

**CG14122 is homologous to human Smyd4**

The domain structures of CG14122 and human Smyd4 are shown in figure 1A. CG14122 shares 34% and 40% identity with the SET and MYND domains of human Smyd4 respectively (Fig 1B and C). CG14122 contains a split SET domain common to Smyd proteins. CG14122 also contains tetratricopeptide repeat motifs that are a feature of human Smyd4, but not other human Smyd proteins. We have named this protein Drosophila Smyd4 (dSmyd4).

dSmyd4 directly recruits histone deacetylases

Since Smyd proteins are able to modulate transcription, we analysed the activity of a dSmyd4-LexA fusion at a LexA dependent promoter (Fig 2A). In a reporter assay in S2 cells dSmyd4 consistently repressed transcription between two and four-fold compared to LexA alone. Vertebrate Smyd1 and Smyd2 repress transcription by recruiting HDACs, therefore we tested whether the mechanism of repression is conserved [9,16]. Both Drosophila class I HDACs, dHDAC1 and dHDAC3, co-immunoprecipitated with dSmyd4 (Fig 2B). The MYND domain containing protein MTG8 is implicated in the recruitment of

![Figure 1. CG14122 is homologous to human Smyd4.](doi:10.1371/journal.pone.0003008.g001)
dSmyd4 interacts with Ebi

dHDAC1 and dHDAC3 exist in four independent co-repressor complexes in Drosophila. dHDAC1 is found in the NuRD, Sin3A and CoREST complexes, whereas dHDAC3 is a component of SMRTER, the Drosophila homologue of NCoR/SMRT complex [15]. We tested whether dSmyd4 co-immunoprecipitated with additional components of these complexes. dSmyd4 interacted with Ebi, a component of the dHDAC3/SMRTER complex [25], but not dMi2 a component of the NuRD complex (Fig 2E), or Sin3A or CoREST (data not shown). This indicates that dSmyd4 can be specifically engaged with the SMRTER co-repressor complex rather than participating in general interactions with all Drosophila HDAC complexes.

dSmyd4 is expressed in the mesoderm

To gain insight into the in vivo function of dSmyd4 we determined its expression pattern. Over-expressed dSmyd4 was predominantly cytoplasmic in S2 cells (Fig 4A). We generated an antibody against dSmyd4 that specifically recognised dSmyd4 in western blots and immunofluorescence (Fig S3). In S2 cells endogenous dSmyd4 showed a nuclear preference (Fig 4B). However, in late stage Drosophila embryos dSmyd4 was restricted to muscle fibres and staining was strongly localised to the cytoplasm (Fig 4C). We used in situ hybridisation to confirm whether dSmyd4 expression was restricted to the muscle lineage. dSmyd4 mRNA was expressed throughout the embryonic mesoderm from stage 10 (Fig 4D–K). dSmyd4 was observed in visceral, cardiac and somatic muscle precursors and in late embryogenesis dSmyd4 was strongly expressed in the somatic musculature. This expression pattern indicates that dSmyd4 may play a role in muscle development or function.

dSmyd4 loss-of-function causes lethality

We used the Gal4-UAS system [26] to induce RNAi against dSmyd4 in different tissues. Two independent insertions of the UAS-RNAi construct were tested, CG14122R-1 and CG14122R-3. In the presence of Gal4 these constructs produce long RNA hairpins that are processed to produce short interfering RNAs. The viability of flies carrying one copy of the UAS-RNAi construct and one copy of a Gal4 driver is summarised in Table 1. Crosses with wild type (yellow white) flies that do not express Gal4 were used as a negative control. Ubiquitous RNAi using Act5C-Gal4 or Da-Gal4 resulted in severe levels of lethality. To reduce the possibility that an off-target effect was responsible for this phenotype we specifically induced RNAi in all muscle tissue using 24B-Gal4 and Myo2-Gal4. Crosses between these drivers and the stronger UAS-RNAi insertion, CG14122R-3, also resulted in lethality. However, inducing RNAi with the neuronal driver Elav-Gal4 had no effect on viability with either UAS-RNAi line.
Mesodermal knockdown of dSmyd4 causes eclosion failure

We examined the protein level of dSmyd4 when RNAi was induced with the muscle-specific driver 24B-Gal4. Inducing RNAi in the mesoderm was sufficient to knockdown almost all dSmyd4 protein expression in adult flies (Fig 5A). The level of a high molecular weight band recognised by the antibody was also reduced, suggesting that this is a modified form of dSmyd4. When RNAi was induced ubiquitously or in the mesoderm we observed large numbers of dead pupae. These flies had died just prior to, or during, eclosion, the stage when adult flies escape from the pupal case. When RNAi was induced in the mesoderm using 24B-Gal4, fewer than 20% of flies were able to eclose (Fig 5B). Many flies initiated the eclosion process, but became trapped and died partially emerged from the case (Fig 5C). Eclosion requires peristaltic movement of the abdominal muscles to enable flies to escape from the pupal case [27]. Most knockdown flies were able to perform rupture of the pupal case and those flies that escaped far enough also performed normal wing expansion. This phenotype resembled that of temperature sensitive dMef2 allelic combinations, raised to the non-permissive temperature during larval development [28]. dMef2 is expressed throughout muscle tissue and is required for embryonic muscle development [29,30]. The majority of flies lacking dMef2 during adult myogenesis survive until the late pupal stage but fail to eclose fully [28]. The similarities between the eclosion failure phenotype and expression patterns of dMef2 and dSmyd4 suggest that dSmyd4 is also required for correct muscle function during eclosion.

Discussion

The Drosophila Smyd family

The Drosophila genome contains eleven Smyd genes, more than have previously been annotated in the human or mouse genomes. The large number of family members may allow these proteins to assume a repertoire of functions, or ensure redundancy between family members during development. Further analysis of vertebrate genomes may also reveal larger numbers of Smyd proteins than had previously been anticipated. Studies in vertebrates show that individual Smyd proteins control gene expression in order to fulfil varied functions during development. The tissue specific expression patterns of Drosophila Smyd family members suggest that these proteins may play equivalent roles in the development of specific tissues in this species.

Conserved mechanisms of repression and localisation of Smyd proteins

dSmyd4 represses transcription and recruits HDACs in a manner analogous to vertebrate Smyd1 and Smyd2 [9,16]. This study gives additional insight into the HDAC co-repressors that are involved in repression by dSmyd4. We have shown that dSmyd4 interacts with both dHDAC3 and Ebi, components of the SMRTER co-repressor complex. This contrasts with mammalian Smyd2 protein, which interacts with the Sin3A-HDAC complex [9]. We were unable to detect an interaction between dSmyd4 and HDAC1-containing NuRD, CoREST and Sin3A co-repressors by immunoprecipitation. Nevertheless, a common feature of dSmyd4 and vertebrate Smyd2 and Smyd1 is the association of a potential methyltransferase with histone deacetylase activity in a single complex. This implies that a primary role of these proteins is to coordinate changes in modification status at their target sites.

We have described a direct interaction between dSmyd4 and the N-terminal regions of dHDAC1 and dHDAC3. There is a high level of identity between Drosophila and vertebrate class I HDACs, especially at the N-termini where this interaction occurs, therefore this interaction may be relevant to recruitment of HDACs by Smyd family members in other species. The recruitment of HDAC co-repressor complexes by MYND domains is also of clinical importance. AML/MTG8 fusions lead to the aberrant recruitment of HDAC co-repressor complexes in the development of leukaemia [13]. The MTG8 MYND domain interacts with components of these complexes, but the interaction between the MYND domain of MTG8 and HDACs is poorly described. The novel interaction described here may also apply to other interactions such as these.

The cytoplasmic over-expression pattern of dSmyd4 resembles that of vertebrate Smyd2 [9], providing another parallel between vertebrate and invertebrate Smyd proteins. However, a more relevant indicator of biological function is the distribution of
endogenous protein. We show here that endogenous dSmyd4 is predominantly nuclear in S2 cells. The strong cytoplasmic localisation of dSmyd4 in embryos suggests that in addition to its activity as a transcriptional repressor, dSmyd4 may perform additional cytoplasmic functions, for example the methylation of non-histone substrates. This raises additional parallels with Smyd2, since a cytoplasmic role has been suggested for this protein [6]. The cell-type dependent localisation of endogenous dSmyd4 raises interesting questions about how the localisation of dSmyd4 is regulated. The subcellular localisation of human Smyd3 is regulated in a cell cycle dependent manner and analogous developmental regulation may be required for the function of other Smyd proteins such as dSmyd4 [7].

**Table 1. Viability of Drosophila with induced RNAi (%)**

| Gal4 driver line | UAS-RNAi line | R1/CyO GFP w+ | R3/CyO GFP w+ |
|------------------|----------------|----------------|----------------|
|                  | yw             | 92             | 105            |
| Elav-Gal4        | 106            | 101            |                |
| Da-Gal4          | 1              | 0              |                |
| Act5C-Gal4/TM6b Tb | 10             | 7              |                |
| 24B-Gal4         | 99             | 5              |                |
| dMef2-Gal4       | 101            | 76             |                |

Percentage viability is calculated as the number of unbalanced adult escapers recovered as a percentage of the number of CyO adult escapers from UAS-RNAi/CyO GFP w+ x Gal4 crosses. For the UAS-RNAi/CyO GFP w+ x Act5C-Gal4/TM6b cross the percentage is calculated as the number of unbalanced adult escapers recovered as a percentage of half the number of CyO adult escapers. Total progeny from each cross was >100.

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**Figure 4. dSmyd4 is expressed in the embryonic mesoderm.** A, dSmyd4-FLAG was over-expressed in S2 cells and visualised by immunofluorescence using anti-FLAG. B, Endogenous dSmyd4 in S2 cells was visualised by immunofluorescence using anti-dSmyd4. C, Endogenous dSmyd4 in late stage Drosophila embryos was visualised by immunofluorescence using anti-dSmyd4. D–K, In situ hybridisation of dSmyd4 mRNA in developing Drosophila embryos, anterior to the left. D, F, H and J are lateral views; E, G, I and K are dorsal views. D and E, stage 10 embryo, weak expression of dSmyd4 throughout the mesoderm. F and G, stage 11 embryo, dSmyd4 expression in specified mesoderm. vmp, visceral muscle precursor; cp, cardiac precursor. H and I, stage 12 embryo, dSmyd4 was expressed at high levels in the somatic muscle (sm) and expression was maintained in visceral muscle (vm). J and K, stage 14 embryo.

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Requirement for dSmyd4 in development

Knockdown of dSmyd4 in muscle tissue resulted in reduced rates of survival. dSmyd4 was expressed during embryogenesis, yet the majority of knockdown flies died at the late pupal stage suggesting that there is a greater requirement for dSmyd4 in processes involved in adult myogenesis. This may be due to redundancy between Smyd proteins during embryogenesis since CG8503 and CG18136 are also expressed in muscle tissue at this time [24]. The majority of knockdown flies were not able to escape from the pupal case but performed other eclosion behaviours normally. The neural networks and signalling required for eclosion therefore appear to be intact, indicating that dSmyd4 is likely to play a role in controlling muscle development or function. Identifying the precise nature of the eclosion defect caused by dSmyd4 knockdown will be an important step in understanding the function of this and other Smyd proteins in the development of multicellular organisms. Much is known about the transcription factors involved in Drosophila muscle development [29,30,31,32] but little is understood about how chromatin structure is regulated.
during this process, dSmyd4 is a good candidate to direct chromatin remodelling during muscle development. Smyd1 is required for cardiac development in vertebrates [8,16] and a number of other Drosophila Smyd proteins appear to be specifically expressed in muscle. These results suggest that members of the Smyd family play conserved roles in muscle development in both vertebrate and invertebrate species. Drosophila provides a tractable system for the analysis of gene function, for example testing genetic interactions with other genes involved in muscle development. Analysis of mutants in dSmyd4 and other Smyd genes using this approach may also shed light on conserved aspects of Smyd function in vertebrates.

This study presents the first analysis of both Smyd proteins in Drosophila and of a Smyd4 homologue. It appears that aspects of mechanism and function are conserved between Drosophila and vertebrate Smyd proteins. The repression of transcription by SMRTER complex recruitment and the requirement of dSmyd4 for survival highlight the importance of this protein family as transcriptional modulators of developmental processes.

Materials and Methods

Identification of Drosophila Smyd homologues

BLAST searches against the Drosophila annotated proteins database were performed using each of the human Smyd proteins. Candidate Smyd proteins were analysed for the presence of SET and MYND domains by reference to Uniprot and direct comparison with consensus sequences.

Cloning

cDNA clones from the Berkeley Drosophila Genome Project [33] were obtained from Geneservice (Cambridge, UK): CG11160, RE25548; CG12119, RE62495; CG14122, RE32936; CG14590, AT24727; CG1868, LD26420; CG7759, HL04910; CG8378, LD29892; CG8503, GH11294; dHDAC1, GM14158; dHDAC3, LD23745. Coding regions were amplified by PCR and cloned into the S2 expression vector pRmHa3.

S2 cell culture

Drosophila S2 cells (Drosophila Genomics Resource Centre) were grown at 25°C in Schneider's Medium supplemented with 10% foetal calf serum and antibiotics. S2 cells were split the day before transfection and were plated at a density of 0.5 × 10^5 per well of a 24 well plate on the day of transfection. Cells were transfected using FuGENE HD (Roche). Expression from pRmHa3 was induced with 0.7 mM CuSO4.

LexA dependent reporter assay

Drosophila S2 cells were transfected with 50 ng pAc5.1 (Invitrogen) encoding dSmyd4 fused to LexA at the C-terminus, or LexA alone. Cells were co-transfected with 100 ng pRLAct5C, encoding Renilla luciferase downstream of an Actin5C promoter and 500 ng pGL2LexA, encoding firefly luciferase downstream of four interspersed LexA sites/Drosophila heat shock elements and a minimal hsp70 promoter. The constructs were based on pRL and pGL2 respectively (Promega). Cells were harvested two days after transfection. The Dual-Luciferase Reporter system (Promega) was used to measure firefly and Renilla luciferase luminescence according to the manufacturer's directions. Each firefly luciferase reading was normalised to its partner Renilla luciferase reading to control for cell number/viability and transfection efficiency. Results were the mean of three transfections and the mean value for LexA alone was set to an arbitrary value of one.

Co-immunoprecipitation

S2 cells expressing dSmyd4-FLAG and dHDAC1-HA or dHDAC3-HA were lysed in IPB250 (20 mM Tris pH 8.0, 250 mM NaCl, 0.4% NP-40, 1 mM DTT, 1 mM EDTA and Roche protease inhibitors). S2 cells expressing dSmyd4-FLAG and dMi2-HA, Ebi-HA, Sin3A-HA or CoREST-HA were lysed in IPB150 (20 mM Tris pH 8.0, 150 mM NaCl, 0.1% NP-40, 1 mM DTT, 1 mM EDTA and Roche protease inhibitors). Cell extracts were incubated with anti-FLAG M2 sepharose (Sigma) for 2 h at 4°C. Sepharose was washed extensively in the lysis buffer and
proteins were eluted in 500 ng/μL FLAG peptide (Sigma). Bound proteins were visualised by western blotting with anti-FLAG M2 (1:5000; Sigma) and anti-HA (1:2000; Roche).

HDAC pulldowns
The region encoding the MYND domain fragment of dSmyd4 (amino acids 208–377) was cloned into pGEX4T1 (Pharmacia) to generate a N-terminal GST fusion. GST-MYND was purified in phosphate buffered saline (PBS) with 0.1% Triton X-100 on glutathione sepharose. 35S-labelled dHDAC1 and dHDAC3 were generated by in vitro translation using TNT Quick Coupled Transcription/Translation System (Promega). 10 μg GST-MYND bound to glutathione sepharose was incubated with 35S-labelled dHDAC1 or dHDAC3 in PDB (20 mM Tris pH 8.0, 200 mM NaCl, 50 mM KCl, 0.5% Triton X-100, 1 mM EDTA) for 2 h at 4°C. Sepharose was washed extensively with PDB. Bound proteins were separated by SDS PAGE and visualised using a Typhoon scanner (GE Healthcare). dHDAC1-his6 and dHDAC3-his6 were expressed from pET28 (Novagen) and were purified on Ni-NTA agarose (Qiagen) according to the manufacturer’s directions. dHDAC1-his6 or dHDAC3-his6 in PDB (20 mM Tris pH 8.0, 150 mM NaCl, glutathione sepharose was incubated with 20 mM Tris pH 8.0, 150 mM NaCl, 0.1% Triton X-100, 1 mM EDTA) and 10 min at room temperature. 10 μg GST-MYND bound to glutathione sepharose was incubated with 20 μg dHDAC1-his6, or dHDAC3-his6 in HisPDB (20 mM Tris pH 8.0, 150 mM NaCl, 50 mM KCl, 0.5% Triton X-100, 1 mM DTT, 1 mM EDTA) for 2 h at 4°C. Sepharose was washed extensively with PDB. Bound proteins were separated by SDS PAGE and visualised by western blotting with anti-polyhis (1:4000; Sigma).

Generation of anti-dSmyd4
A polyclonal antibody against dSmyd4 was raised in rabbits using the GST-MYND protein fragment. Immunisation was performed by Eurogentec. GST-MYND was blotted onto nitrocellulose membrane and incubated with the final bleed performed by Eurogentec. GST-MYND was purified in (amino acids 208–377) was cloned into pGex4T1 (Pharmacia) to generate an N-terminal GST fusion. GST-MYND was purified in phosphate buffered saline (PBS) with 0.1% Triton X-100 on glutathione sepharose. 35S-labelled dHDAC1 and dHDAC3 were generated by in vitro translation using TNT Quick Coupled Transcription/Translation System (Promega). 10 μg GST-MYND bound to glutathione sepharose was incubated with 35S-labelled dHDAC1 or dHDAC3 in PDB (20 mM Tris pH 8.0, 200 mM NaCl, 50 mM KCl, 0.5% Triton X-100, 1 mM DTT, 1 mM EDTA) for 2 h at 4°C. Sepharose was washed extensively with PDB. Bound proteins were separated by SDS PAGE and visualised using a Typhoon scanner (GE Healthcare). dHDAC1-his6 and dHDAC3-his6 were expressed from pET28 (Novagen) and were purified on Ni-NTA agarose (Qiagen) according to the manufacturer’s directions. Embryos were fixed with 4% formaldehyde. Embryos were fixed and deviellinised according to Kosman et al. [34]. The following antibody dilutions were used for stainings: anti-FLAG M2 (Sigma), 1:2000; anti-HA 3F10 (Roche), 1:500; anti-dSmyd4, 1:250 for S2 cells, 1:50 for embryos. Alexafluor conjugated secondary antibodies (Molecular Probes) were used at 1:250. Nuclei were visualised with DAPI (500 ng/mL). Embryos and S2 cells were imaged using confocal microscopy.

In situ hybridisation
Full-length dSmyd4 cDNA was used to generate a digoxigenin-labelled anti-sense probe using the DIG labelling kit (Roche). dSmyd4 transcripts were hybridised with digoxygenin-alkaline phosphatase and NBT/BCIP (Roche). Embryos were staged according to Campos Ortega and Hartenstein [35].

Fly stocks and RNAi
Fly stocks were maintained at 25°C on standard medium. RNAi was induced using the UAS-Gal4 system [26]. CG14122R-1 and CG14122R-3 UAS-RNAi lines were obtained from the RNAi fly project, NIG/MITILS, Japan. UAS-RNAi stocks balanced with CyO GFP w+ were crossed to a variety of Gal4 expressing drivers: Act5C-Gal4 (Y. Hiromi, Bloomingston Stock centre); Da-Gal4 and 2AB-Gal4 (gifts from M. Bienz); Elav-Gal4, Mef2-Gal4 (a gift from S. Nuelsman). Viability of flies containing one copy of the UAS-RNAi insertion and one copy of a Gal4 driver was compared to that of CyO flies from the same cross. To analyse the elesion defect non-GFP larvae from the crosses CG14122R-3/CyO GFP w+ x 2B-Gal4 and CG14122R-3/CyO GFP w+ x w were selected and allowed to develop.

Supporting Information
Table S1 Domain annotation of Drosophila Smyd homologues
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Table S2 Subcellualr localisation of Drosophila Smyd proteins
Table S3 Anti-dSmyd4 specifically recognises dSmyd4 in western blots and immunofluorescence.

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