SON protects nascent transcripts from unproductive degradation by counteracting DIP1

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Abstract

Gene expression involves the transcription and splicing of nascent transcripts through the removal of introns. In Drosophila, a double-stranded RNA binding protein Disco-interacting protein 1 (DIP1) targets INE-1 stable intronic sequence RNAs (sisRNAs) for degradation after splicing. How nascent transcripts that also contain INE-1 sequences escape degradation remains unknown. Here we observe that these nascent transcripts can also be bound by DIP1 but the Drosophila homolog of SON (Dsn) protects them from unproductive degradation in ovaries. Dsn localizes to the satellite body where active decay of INE-1 sisRNAs by DIP1 occurs. Dsn is a repressor of DIP1 posttranslational modifications (primarily sumoylation) that are assumed to be required for efficient DIP1 activity. Moreover, the pre-mRNA destabilization caused by Dsn depletion is rescued in DIP1 or Sumo heterozygous mutants, suggesting that Dsn is a negative regulator of DIP1. Our results reveal that under normal circumstances nascent transcripts are susceptible to DIP1-mediated degradation, however intronic sequences are protected by Dsn until intron excision has taken place.

Author summary

During transcription, nascent RNAs are exposed to various RNA degradation machineries in the nucleus. Nascent RNAs undergo a process called splicing that removes noncoding sequences (known as introns) in order to produce protein-coding messenger RNAs. In the vinegar fly Drosophila, introns that contain a transposable sequence known as INE-1 are recognized and degraded by a protein called DIP1. This process usually happens after splicing so that DIP1 does not degrade nascent RNAs. How such a target specificity and temporal control are achieved is not known. Here we found that nascent RNAs are already being recognized by DIP1. However, its activity is inhibited by the SON protein that also binds to nascent RNAs. After splicing, the inhibition of DIP1 by SON is relieved, allowing a spatial and temporal control of DIP1 activity. This regulation is important as it prevents unspecific decay of nascent RNAs that can drastically affect gene expression.
Introduction

The first few steps of gene expression include the production of nascent transcripts and the removal of introns via the splicing reaction. The nucleus contains numerous RNA decay machineries, and thus nascent transcripts need to be protected by various mechanisms to ensure productive gene expression [1]. Certain intronic sequences in the excised introns can target them for degradation. In principle, nascent transcripts that also contain the same intronic sequences can also be subjected to decay. In budding yeast, certain double-stranded RNA (dsRNA) stem-loop structures trigger RNase III-mediated degradation of both the excised introns and unspliced pre-mRNAs [2]. Whereas in fission yeast, decay-promoting introns target unspliced pre-mRNAs for degradation by recruiting the exosome specificity factor Mmi1 [3]. For productive gene expression, decay-promoting introns should only trigger degradation of excised introns and not nascent transcripts. How nascent transcripts avoid such degradation is unknown.

Stable intronic sequence RNAs (sisRNAs) are intron-containing transcripts that are relatively more stable than their excised counterparts or those that undergo nonsense-mediated decay [4–9]. They have been shown to regulate various biological processes such as germline stem cell (GSC) maintenance and embryonic development [10,11]. The *Drosophila* chromosome four contains an extremely high abundance of INE-1 sequences in the introns [12]. INE-1 belongs to class of transposable element abundant in *Drosophila* [12–14]. As a result, the fourth chromosome is a region where a high density of INE-1 sisRNAs is being produced. Here, a double-stranded RNA binding protein Disco-interacting protein 1 (DIP1) binds and degrades INE-1 sisRNAs [15]. This leads to the formation of microscopically visible DIP1-positive nuclear bodies known as satellite bodies around the fourth chromosomes [15]. DIP1 only degrades INE-1 sisRNAs after splicing as pre-mRNAs containing INE-1 sequences were unaffected in DIP1 mutants [15]. It is not understood how such a target specificity is achieved (Fig 1A).

In this study, we report the conserved protein SON (or Dsn in *Drosophila*) acts to protect nascent transcripts containing INE-1 from being degraded by DIP1. Our results show that nascent transcripts are bound to DIP1. However, the presence of Dsn inhibits DIP1 at the level of RNA decay activity until intron excision is completed. Thus, Dsn acts as a ‘timer’ to ensure that intronic sequences are only subjected to degradation after splicing in order for productive gene expression to take place.

Results

Dsn is a novel satellite body component

We previously reported that Dsn regulates GSCs by repressing the expression of *regena* (*rga*), which encodes for NOT2 (a component of the CCR4-NOT complex) required for the maintenance of GSCs [16–19]. To examine the localization of endogenous Dsn, we attempted to generate antibodies against Dsn using bacteria-expressed GST-tagged Dsn and peptide sequence of Dsn, but were unsuccessful. We therefore generated transgenic flies over-expressing FLAG-Dsn. When driven by a germline driver (*MTD-Gal4*), FLAG-Dsn rescued the *dsn* mutant phenotype (S1 Fig, discussed later), verifying that our FLAG-Dsn transgene produced a fully functional protein. We observed that FLAG-Dsn localized around the presumed fourth chromosomes in the ovarian nurse cells, reminiscent of the satellite body. Co-staining with the satellite body marker DIP1 confirmed that FLAG-Dsn is a satellite body component as both proteins co-localized around the presumed fourth chromosomes in the nurse cell nucleus (Fig 1B, arrowheads). Specificity of the staining was verified by the lack of signals in the somatic...
follicle cells (Fig 1B, arrows), and the non-transgenic control (MTD-Gal4/CyO) (Fig 1B). Although it should be noted that due to over-expression, the localization of FLAG-Dsn may not precisely reflect that of endogenous Dsn, we believe that the protein reflects its endogenous localization due to its ability to rescue the dsn mutant phenotype.

To investigate further, we examined the localizations of DIP1 and FLAG-Dsn more closely by super-resolution deconvolution (STED) microscopy. Under the super-resolution microscope, the localization patterns of DIP1 and FLAG-Dsn were better resolved. Interestingly, DIP1 and FLAG-Dsn did not overlap completely. Fig 1C shows a representative single optical section of the satellite bodies. Four different regions of the satellite bodies are presented. Measurements of signal intensities showed that DIP1 and FLAG-Dsn only partially overlapped, where they appeared associated closely with each other in a network (Fig 1C).

Dsn promotes the stability of INE-1 containing pre-mRNAs

DIP1 acts to repress INE-1 sisRNAs after splicing as DIP1 mutant ovaries exhibited an increase in INE-1 sisRNAs but no change in the INE-1 containing pre-mRNAs [15]. Since Dsn is also a satellite body component, we examined if Dsn performs the same function as DIP1. We used a mutant allele CG8273GS7314 that contains a transposon insertion at the 5’ UTR of dsn (or CG8273) leading to a dramatic loss (over 90% reduction) of dsn mRNA expression [16]. In contrast to DIP1 mutants, dsn mutant ovaries had a decrease in INE-1 sisRNAs and mRNAs from genes harboring INE-1 sequences in their introns (CG32000 and CG2316) (Fig 2A and 2B). To examine whether if pre-mRNA levels were affected, we performed RT-qPCR using primers that flank the exon-intron junctions, and found a consistent decrease in CG32000, CG2316 and ANK pre-mRNAs (Fig 2C). In contrast, the pre-mRNAs of genes that do not contain INE-1 sequences (Maverick and Myoglianin) were unaffected in dsn mutant ovaries (Fig 2C). Although there was a decrease in ANK pre-mRNA, we did not observe a similar decrease in ANK mRNA in dsn mutant ovaries, which may suggest a feedback mechanism regulating ANK mRNA. Together, these results indicate that Dsn specifically regulates pre-mRNAs containing INE-1.

In dsn mutant ovaries, both the levels of pre-mRNAs and mRNAs were down-regulated, therefore excluding the possibility that Dsn regulates splicing. Next, we wondered if Dsn regulates the stability of pre-mRNA. We performed transcription inhibition assays and measured the levels of a relatively abundant pre-mRNA from CG32000 over time by RT-qPCR. Ovaries were first treated with alpha-amanitin to inhibit transcription for a period of 0.5 hr. We observed that in dsn mutant ovaries, the CG32000 pre-mRNA had a higher magnitude of decrease than in controls (Fig 2D). As a control, we examined the Marverick pre-mRNA and did not observe any difference between wild-type and dsn mutants (S2 Fig). We repeated the experiment with another transcription inhibitor Actinomycin D under a longer incubation period, and similar results were obtained (Fig 2E), suggesting that pre-mRNA was less stable in dsn mutant ovaries. Together, our results suggest that Dsn is required to ensure robust expression of INE-1 containing pre-mRNA, at least in part, by ensuring their stability (Fig 2F).
DIP1 binds to nascent transcripts in a Dsn-independent manner

It was surprising that although Dsn and DIP1 localize to satellite bodies, *dsn* and DIP1 mutants displayed contrasting phenotypes suggesting different functions. Since Dsn promotes the expression of INE-1 containing pre-mRNAs, and DIP1 had been found to degrade INE-1 sisRNAs, we explored the possibility that Dsn may inhibit DIP1 from degrading pre-mRNAs.

We checked if Dsn regulates the expression of DIP1. RT-qPCR experiment revealed that DIP1 mRNA was unchanged in *dsn* mutant ovaries as compared to controls (Fig 3A). Therefore, we asked if Dsn is required for the localization of DIP1 to satellite bodies. By staining control and *dsn* mutant ovaries with an antibody against DIP1, we did not observe any difference in the localization of DIP1 to the satellite bodies (Fig 3B). This result indicates that Dsn is not required for the localization of DIP1 to satellite bodies.

One possible mechanism is that Dsn could inhibit the binding of DIP1 to nascent transcripts to prevent them from being degraded. Alternatively, Dsn may regulate the activity of DIP1 on nascent transcripts. We performed RNA-immunoprecipitation (RNA-IP) using DIP1 antibody and found that DIP1 generally binds more strongly to INE-1 containing CG32000,
Fig 3. DIP1 binds to nascent transcripts in a Dsn-independent manner. (A) RT-qPCR showing no change in the expression of DIP1 mRNA in dsn mutant versus control ovaries. N = 3 biological replicates. (B) Confocal images showing the localization of DIP1 (green) in the control and dsn mutant nurse cell nuclei. Scale bar: 7 μm. Arrowheads point to the heterochromatin of the fourth chromosomes. (C) RT-qPCR showing enrichment of CG32000, CG2316 and ANK pre-mRNAs in three independent DIP1 immunoprecipitation experiments. Actin5C was used as a negative control for non-specific pull down. (D) RT-PCR showing the knockdown efficiency of dsn in dsn RNAi versus control S2 cells. Actin5C was used as a loading control. (E) RT-qPCR showing no change in the binding of DIP1 to CG32000 pre-mRNA in dsn RNAi versus control S2 cells. N = 3 biological replicates. (F) RT-qPCR showing no change in the expression of CG32000 pre-mRNA in dsn RNAi versus control S2 cells. N = 3 biological replicates. (G) RT-qPCR showing no change in the binding of DIP1 to CG32000, CG2316 and ANK pre-mRNA in dsn mutant versus control ovaries. N = 3 biological replicates.

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CG2316 and ANK pre-mRNAs than to a control actin5C mRNA (Fig 3C). As a positive control, we detected an enrichment of INE-1 sisRNAs in the precipitates in S2 cells (S3 Fig). This result was surprising as it showed that nascent transcripts are already bound to DIP1 under normal conditions. We next knocked down the expression of dsn by RNAi in S2 cells (Fig 3D) and asked if it led to an increase in the binding of DIP1 to nascent transcripts. To our surprise, we did not observe any changes in the binding of DIP1 to CG32000 pre-mRNA (Fig 3E). To determine if Dsn regulates nascent transcripts in S2 cells, we examined the expression of CG32000 pre-mRNA and found no change in its expression in dsn RNAi S2 cells (Fig 3F). Therefore, Dsn appears to be dispensable for the regulation of nascent transcripts in S2 cells.

We therefore repeated the DIP1 immunoprecipitation experiments using ovarian lysates where Dsn activity was needed for pre-mRNA stability. Similar to the results from S2 cells, we did not observe an increase in the binding of DIP1 to pre-mRNAs (CG32000, CG2316 and Ank) in dsn mutant ovaries when compared to wild-type controls (Fig 3G). Together, our results suggested that under normal circumstances, nascent transcripts are already bound by DIP1 independently of Dsn.

**Dsn counteracts the activity of DIP1**

We next considered the alternative hypothesis that Dsn may regulate the activity of DIP1 on nascent transcripts. On the FlyBase, DIP1 was found to interact with Lesswright (Lwr) via a yeast-two-hybrid screen. *Drosophila lwr* encodes for the Ubc9 protein, which is a Sumo conjugating enzyme responsible for sumoylation of its targets [20,21]. In *Drosophila*, sumoylation has been found to regulate the activities of various proteins in a dynamic manner [22]. To investigate if DIP1 is sumoylated in vivo, we immunoprecipitated DIP1 in ovarian and S2 cell lysates, and performed western blotting using an antibody detecting Sumo protein. In both lysates, we detected a specific band of ~55 kDa, which is a size that is consistent with sumoylated DIP1 (DIP1: 44 kDa + Sumo: 10 kDa = 55 kDa) (Fig 4A, arrowhead), indicating that DIP1 is indeed sumoylated in vivo. Interestingly, immunostaining of ovaries revealed that Sumo and DIP1 co-localized at the satellite bodies (Fig 4B), suggesting that the activity of DIP1 at the satellite bodies may be regulated by sumoylation.

By performing western blotting, we observed that the majority of the DIP1 protein was not sumoylated in ovaries (Fig 4C, ~44 kDa predicted size), however some DIP1 protein also appeared as slower-migrating (presumably sumoylated) forms (Fig 4C, arrowhead). The appearance of these slower-migrating DIP1 was reduced in lwr and smt3 heterozygous mutant ovaries, indicating that they represent sumoylated forms of DIP1 (Fig 4C). The non-sumoylated form of DIP1 protein was unchanged in dsn mutant ovaries (Fig 4D, arrow, ~44 kDa predicted size). In two out of three biological replicates, the abundance of the sumoylated forms of DIP1 was up-regulated in dsn mutant ovaries (Fig 4D). We believe that the extent of up-regulation of sumoylation is greater in Expt 2 and 3 than in Expt 1. In Expt 1, the upper-most band in the dsn mutant lane was stronger than that in the control lane, suggesting that DIP1 is more heavily modified in the mutants than controls. Thus, the result is still consistent with that of Expt 2 and 3.

In contrast, we observed little change in sumoylated DIP1 in S2 cells after dsn RNAi (Fig 4E). This could be due to the fact that majority of the DIP1 was already sumoylated in S2 cells (Fig 4E), explaining why we did not observe any change in CG32000 pre-mRNA after Dsn knockdown (Fig 3F). Taken together, our data indicated that Dsn represses the sumoylation of DIP1 at the protein level.

To confirm that the decrease in nascent transcripts in dsn mutants was indeed caused by the increase in activity of DIP1, we asked if reducing a copy of DIP1 was able to rescue the
Fig 4. Dsn counteracts the activity of DIP1 via repressing DIP1 sumoylation. (A) Western blots showing the presence of sumoylated DIP1 after immunoprecipitation of DIP1 in ovaries and S2 cells. (B) Confocal images showing the enrichment of Sumo proteins (green) at the satellite bodies, co-localizing with DIP1 (red) in wild type ovaries. DAPI (white). (C) Western blot showing the reduction of slower-migrating forms of DIP1 in lwr/CyO and smt3/CyO ovaries compared to control. Actin5C was used as a loading control. (D) Western blot showing an increase in the
phenotype in dsn RNAi ovaries. Consistent with dsn mutant ovaries, we also observed a decrease in the expression of CG32000 pre-mRNA in vasa-Gal4>dsn RNAi versus vasa-Gal4 and no change in CG32000 pre-mRNA expression in vasa-Gal4 versus DIP1 +/-; vasa-Gal4>dsn RNAi. *p < 0.05, two-tailed t test. N = 3 biological replicates. (G) RT-qPCR showing upregulation of CG32000 pre-mRNA in smt3/+;dsn/dsn versus dsn/dsn. Two-tailed t test. N = 2 biological replicates.

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abundance of slower-migrating DIP1 protein in dsn mutant versus control ovaries in 3 biological replicates. Actin5C was used as a loading control. (E) Western blots showing the increase in sumoylated DIP1 (arrowhead) in dsn RNAi S2 cells. Actin was used as a loading control in the input lanes. (F) RT-qPCR showing downregulation of CG32000 pre-mRNA in vasa-Gal4>dsn RNAi versus vasa-Gal4 and no change in CG32000 pre-mRNA expression in vasa-Gal4 versus DIP1 +/-; vasa-Gal4>dsn RNAi. *p < 0.05, two-tailed t test. N = 3 biological replicates. (G) RT-qPCR showing upregulation of CG32000 pre-mRNA in smt3/+;dsn/dsn versus dsn/dsn. Two-tailed t test. N = 2 biological replicates.

**Discussion**

SON encodes for an evolutionary conserved protein that binds to nascent transcripts and localizes to nuclear speckles [23–27]. Various functions have been assigned to SON, which includes regulation of splicing and transcription [23,24,28,29]. Besides that, SON has been implicated in various processes such as stem cell self-renewal and differentiation and cell cycle progression [16,23,25,29]. In human, mutations in SON have been linked to brain developmental defects and leukemia [28,30,31].

In this study, we uncovered a novel role for *Drosophila* homolog of SON (Dsn) in protecting nascent transcripts from unproductive degradation by DIP1 (Fig 5). During transcription, nascent transcripts that contain INE-1 sequences in the introns are bound by both Dsn and DIP1. Dsn inhibits the activity of DIP1, and shields the nascent transcripts from entering the decay pathway. Upon splicing, the intron is excised and Dsn would be released from the transcripts, which leads to the alleviation of the inhibition by Dsn. As a consequence, the INE-1 sisRNA is degraded by DIP1. Conceptually, Dsn acts as a ‘timer’ to ensure that nascent transcripts are fully spliced before the decay activity of DIP1 kicks in. Based on the fact that SON is highly conserved in mammals and mammalian SON had been shown to bind to nascent transcripts, we envision that this novel role of SON may be conserved.

Our model is consistent with the prevailing idea that nascent transcripts are highly susceptible to decay, and cells have evolved active mechanisms to safeguard nascent transcripts [1]. One such example is the process of telescripting whereby U1 snRNA protects the nascent transcript from cryptic intronic cleavage and polyadenylation [32].

How does Dsn regulate the activity of DIP1? We suggest that Dsn regulates the activity of DIP1 via its sumoylation. In dsn mutants, there is a strong correlation between the increase in sumoylated DIP1 and increase in DIP1 activity. This observation suggests that DIP1 activity may be influenced by sumoylation. Our data suggest that sumoylation is the primary modification that is responsible for the subsequent modification of DIP1. We show that reduction of sumoylation pathway genes (lwr and smt3) led to an overall reduction of DIP1 modification (Fig 4C). Thus, without sumoylation, other forms of posttranslational modifications are also dramatically reduced.

We hypothesize that the satellite body serves as a protective zone where nascent transcripts are bound and protected by Dsn. An interesting hypothesis is that Dsn limits the concentration of modified forms (including sumoylation) of DIP1 so that it is not sufficient to degrade transient nascent transcripts before splicing occurs. After splicing, the introns are released
from the pre-mRNAs and the repressive effect of Dsn is alleviated. Sumoylation of DIP1 then
activates its degradation activity. Sumoylation of DIP1 may influence its conformation and
binding partners, thereby modulating its activity [33]. Future work will aim to address the
molecular mechanism on how DIP1 activity is regulated by sumoylation.

Although we did not observe any differences in binding of DIP1 to nascent transcripts
between wildtype and dsn mutant ovaries, we cannot totally exclude the possibility that Dsn
may regulate the binding activity of DIP1 to nascent transcripts. As DIP1 binds to and
degradates nascent transcripts, we envision the regulation of DIP1 binding and decay as a
dynamic and coordinated event. Thus, it is possible that the binding of DIP1 to nascent tran-
scripts may be coupled to its decay activity.

One interesting observation was that the repressive activity of Dsn on DIP1 was seen in ova-
ries but not in S2 cells. Knockdown of dsn in S2 cells did not lead to a dramatic increase in
sumoylation of DIP1. This observation can be explained by the fact that most of the DIP1 pro-
tein is already being sumoylated in S2 cells, unlike the case in the ovaries. This difference
between S2 cells and ovaries may be due to an intrinsic difference in the activity of sumoyla-
tion pathway between these two cell types, thus making the ovaries more sensitive to changes
in Dsn activity.

In closing, our work encourages the use of satellite body as a model for studying RNA
metabolism. We envision that by identifying and characterizing more proteins/RNAs that
localize to the satellite body, we can in principle learn more about the intricate regulation of
RNA splicing and decay pathways.

Fig 5. Working model of Dsn and DIP1 in regulating the abundance of INE-1 containing pre-mRNA and INE-1 sisRNA in Drosophila. “S” stands for sumoylation.

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Materials and methods

Fly strains

yw flies were used as controls unless otherwise stated. The following strains were used in this study: MTD-Gal4 [34], FLAG- Dsn (this study), CG8273 [35] (dsn mutant) (Kyoto #201169), vasa-Gal4 (kind gift from Yukiko Yamashita), CG8273/dsn RNAi (TRiP HMS00114 Bloomington #34805), DIP1[EY02625], smt3 [34] /CyO (Bloomington #11378) and lwr [36] /CyO (Bloomington #11410). Flies were maintained at 25˚C. Before dissection, newly eclosed females were fed with wet yeast paste for 3 days at 25˚C. Generation of UASp-FLAG-Dsn transgenic flies was performed as previously described [15]. PCR of dsn full-length coding sequence (CDS) was performed using primers, CACC-dsn Fw (5’CACCATGACGGAGAACACAGA-GAAAGGG3’) and dsn Rv (5’CTAGCTGGGCGG AAGAATGCCTAA3’). Transgenic flies were generated by BestGene using P-element-mediated insertion.

Immunostaining

Immunostaining was performed as described previously [15]. Ovaries were fixed in 16% paraformaldehyde and Grace’s medium at a ratio of 2:1 for 10–20 min, rinsed and washed in PBX three times for 10 min each and pre-absorbed in PBX containing 5% normal goat serum for 30 min. Ovaries were incubated in primary antibodies overnight at room temperature, washed in PBX three times for 20 min each and incubated in secondary antibodies for 4 hr at room temperature. Finally, the ovaries were washed again in PBX three times for 20 min each before mounting on slides. The primary antibodies used were rabbit anti-DIP1 (1:300) [15], mouse anti-FLAG (1:500, M2 Sigma) and mouse anti-Sumo (1:500, DSHB 8A2). Images were taken with a Leica SP8 Inverted STED microscope and processed using Leica microscope software, LAS X.

Actinomycin D treatment

Actinomycin D treatment was performed as described previously [9]. Dissected ovaries were incubated in Grace’s medium containing 20 μg/ml actinomycin D with constant rocking at room temperature.

α-amanitin treatment

Dissected ovaries were incubated in Grace’s medium containing 20 μg/ml of α-amanitin with constant rocking at room temperature.

RNA extraction

Tissues were homogenized in 1.5 ml Eppendorf tubes using a plastic pestle and RNA was extracted using the TRIzol extraction protocol (Ambion) or the Direct-zol RNA miniprep kit (Zymo Research). RNA was quantified using a Nanodrop spectrophotometer to ensure equivalent loading for subsequent experiments.

RT-PCR

For standard RT-PCR, total RNA was reverse transcribed with random hexamers for 1hr using M-MLV RT (Promega). PCR was carried out using the resulting cDNA. For quantitative PCR (qPCR), SYBR Fast qPCR kit master mix (2X) ABI Prism (Kapa Biosystems, USA) was used and carried out on the Applied Biosystems 7900HT Fast Real-Time PCR system. Primers sequences for INE-1, CG32000 exon, CG2316 exon and DIP1 were reported previously [15].
For calculation of fold-change between controls and mutants/RNAi samples, changes in gene expression were normalized against actin5C as a loading control. For calculation of fold-change between DIP1 immunoprecipitations, the abundance of RNA in the immunoprecipitates was normalized against the abundance of the same transcript in the inputs. Primer sequences are CG32000 intron Fw (5’ TGGCAACAGTGTCCAATTA3’), CG32000 exon Rv (5’ TGACGCCACAAATGAAAC3’), CG2316 intron Fw (5’ TCTTTATTTTGAATGCGTTCCT3’), CG2316 exon Rv (5’ ACCCATATCTATCTCTTCC3’), ANK exon Fw (5’ TGATGTGACCCCATTACA CG3’), ANK exon Rv (5’ TGACCTGACAAATTCTC3’), Myoglianin intron Fw (5’ GCGGCCATTTGGTTATGGG3’), Myoglianin exon Rv (5’ AAAACATCGACCTTGC3’), Maverick intron Fw (5’ TGCCAAACCGTATAAGG3’), Maverick exon Rv (5’ AGGAAGCTCCCATGAATG3’).

Western blot
Western blotting was performed as previously described [11,15]. Ovaries were dissected in Grace’s medium and homogenized in 2X sample buffer containing β-mercaptoethanol. Primary antibodies used were rabbit anti-DIP1 (1:5000) [15], mouse anti-Sumo (1:1000, DSHB 8A2) and mouse anti-Actin (1:100, DSHB JLA20). Western blot detection was done digitally using the ChemiDoc Touch Imaging System (BioRad) and under non-saturating conditions.

Immunoprecipitation
S2 cells, which were obtained from Steve Cohen’s laboratory, were grown in serum-free medium. ~200 ovaries were dissected for each immunoprecipitation experiment. Immunoprecipitation was performed as previously described [15]. Cells or ovaries were lysed in protein extraction buffer (50mM Tris-HCl pH 7.5, 150mM NaCl, 5mM MgCl2, 0.1% NP-40) supplemented with Protease Inhibitor Cocktail (Roche). Lysates were blocked using protein A/G agarose beads (Merck Millipore) before incubating in rabbit anti-DIP1 (10 μl) overnight at 4°C. As a control, no antibody was added. Protein A/G agarose beads were then added and incubated for another 2 hr. After incubation, beads were washed in protein extraction buffer three times. Protein and RNA were extracted using 2X sample buffer containing β-mercaptoethanol and Direct-zol RNA miniprep kit (Zymo Research), respectively.

siRNA-mediated knockdown
For dsRNA knockdown, experiments were performed as described previously [35]. S2 cells were treated with 15 μg of dsRNA once for four days before cells were harvested. Primers used for generating the template for in vitro transcription were reported previously [16].

Supporting information
S1 Fig. FLAG-Dsn transgene produces a fully functional protein. (A) RT-qPCR showing over-expression of dsn mRNA in MTD>FLAG-Dsn versus MTD/CyO ovaries. N = 3 technical replicates. (B) CG32000 pre-mRNA expression was rescued in the dsn mutant rescue ovaries as compared to dsn mutant. A total of 2 independent experiments were done. (TIF)

S2 Fig. Stability of Marverick pre-mRNA is not affected in dsn mutant ovaries. Chart showing the levels of Marverick pre-mRNA in the ovaries of control and dsn mutant flies before and
after 0.5h of α-amanitin treatment. Error bars depict SD from three biological replicates.

(TIF)

S3 Fig. Positive control for DIP1 immunoprecipitation. Western blot showing enrichment of DIP1 in DIP1 immunoprecipitate in S2 cells. RT-PCR depicting enrichment of INE-1 sisRNA in DIP1 immunoprecipitate.

(TIF)

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