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Little imaginal discs, a Trithorax group member, is a constituent of nuclear matrix of *Drosophila melanogaster* embryos

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Nuclear Matrix (NuMat) is the structural and functional framework of the nucleus. It has been shown that attachment of chromatin to NuMat brings significant regulation of the transcriptional activity of particular genes; however, key components of NuMat involved in this process remain elusive. We have identified Lid (Little imaginal discs) as one of the components of NuMat. It belongs to the TrxG group of proteins involved in activation of important developmental genes. However, unlike other activator proteins of TrxG, Lid is a Jumonji protein involved in H3K4me3 demethylation. Here, we report the association of Lid and its various domains with NuMat which implicates its structural role in chromatin organization and epigenetic basis of cellular memory. We have found that both N and C terminal regions of this protein are capable of associating with NuMat. We have further mapped the association of individual domains and found that, PHD, ARID and JmjC domains can associate with NuMat individually. Moreover, deletion of N-terminal PHD finger does not alter Lid's NuMat association implying that although it is sufficient, yet, it is not necessary for Lid's structural role in NuMat. Based on our findings, we hypothesize that C terminal region of Lid which contains PHD fingers might be responsible for its NuMat association via protein–DNA interactions. However, for the N terminal region harboring both a PHD and an ARID finger, Lid anchors to the NuMat via both protein-protein and protein-DNA interactions. The association of JmjC domain with NuMat is the first report of the association of a demethylase domain with NuMat suggesting that Lid, a demethylase, being part of NuMat might be involved in regulating the chromatin dynamics via its NuMat association.

Keywords. ARID; demethylase; Jumonji; Little imaginal discs (Lid); nuclear matrix; PHD; trithorax; TrxG

Abbreviations: ARID, AT-rich interaction domain; JmjC, Jumonji C; JmjN, Jumonji N; Lid, Little imaginal discs; MAR, matrix attachment regions; NuMat, nuclear matrix; PHD, plant homeodomain; TrxG, trithorax group; TSS, transcription start sites

1. Introduction

Regulation of gene expression is a highly complex process. It not only depends upon the presence or absence of *trans*-factors binding onto the chromatin but also upon the spatial and temporal higher order chromatin organization in the nucleus. There are convincing evidences to believe that Nuclear Matrix (NuMat), the functional and structural framework of the nucleus helps to bring about the latter process. NuMat is defined as the salt, nuclease and detergent resistant fibrillar meshwork of the nucleus concealed beneath the dense mass of chromatin and is known to be composed of proteins, DNA and RNA (Berezney and Coffey 1974). Quite a number of studies in last few decades focused on the morphological and biochemical properties of NuMat. However, due to the lack of high throughput methods, a complete molecular characterization of NuMat and the role of its components in higher order chromatin organization and regulation of gene expression has lagged behind.

We identified a large number of NuMat proteins in our previous work in *D. melanogaster* using high-throughput proteomic analysis (Kallappagoudar *et al.* 2010; Varma and Mishra 2011). These proteins belonged to a variety of functional classes reflecting the functional diversity of NuMat. One of the many functionally important proteins identified, was the TrxG proteins. These are evolutionary conserved proteins and regulate the expression of the genes controlling development. A classic example of genes controlling development in fly are the HOX genes and TrxG proteins are the activator proteins required to maintain these genes in an active state of expression (Schuettengruber *et al.* 2007).

An interesting TrxG protein that we identified in the NuMat proteomic studies was Little imaginal discs (Lid) (Kallappagoudar *et al.* 2010). *D. melanogaster lid* gene is

essential for development. While, genetically, Lid is identified as a TrxG protein, enzymatically it belongs to the Jumonji group of proteins (Gildea *et al.* 2000; Eissenberg *et al.* 2007; Lee *et al.* 2007). Jumonji domain containing proteins are known to possess histone demethylase activity (Hou and Yu 2010). Consistent with this observation, Lid is also shown to possess demethylase activity specifically towards trimethylated histone H3 lysine 4 (H3K4me3) (Eissenberg *et al.* 2007; Lee *et al.* 2007). Modifications of histones at specific residues serve as regulatory marks that are subservient to the control of transcription and chromatin organization. In general, trimethylation marks at H3K4, H3K36 and H3K79 are found in transcriptionally active euchromatic regions, whereas, H3K9me3/me2, H4K20me3 and H3K27me3 are associated with transcriptionally silenced chromatin. In particular, H3K4me3 marks are enriched at the transcription start sites (TSS) of active genes and are important for transcriptional activation. H3K4me2 marks are also enriched at TSS and show a broader distribution pattern whereas H3K4me1 marks are enriched mostly at transcriptional enhancers (Pedersen and Helin 2010). Recently, a study reported that Lid specifically localizes at TSS of actively transcribed developmental genes and regulates the H3K4me3 dynamics of these loci (Lloret-Llinares *et al.* 2012).

Another remarkable feature of Lid is the presence of a number of domains with diverse functions. It harbors two Jumonji domains namely JmjN domain and JmjC domains. JmjC domain is the catalytic domain of this protein and is involved in H3K4me3 demethylation. It was defined on the basis of amino acid similarities among Jarid2, Jarid1C and Jarid1A proteins. JmjC domain forms an enzymatically active domain by folding into eight β-sheets and co-ordinates with Fe (II) and α-ketoglutarate for its action (Klose *et al.* 2006). Many proteins containing JmjC domain also contain JmjN domain. In some proteins, these two domains are separated from each other by other domains as is the case with Lid and in some proteins these are next to each other. Though there are some studies that provide cues to the involvement of JmjN domain in stability and transcriptional activity of proteins having both of these domains, the exact function of JmjN domain is poorly understood (Huang *et al.* 2010; Quan *et al.* 2011). The other domains are ARID (AT-rich interaction domain), a C_5HC_2 zinc finger domain and three PHD (plant homeodomain) fingers. ARID is a highly conserved AT-rich interaction domain and was first recognized in mouse Bright and *D. melanogaster* dead ringer protein. It is a ∼ 90 residue domain and is present widely in proteins involved in cell growth regulation, development and tissue specific gene expression (Dallas *et al.* 2000). Zinc fingers are zinc binding motifs containing conserved cysteine and histidine residues. These motifs are present in wide variety of eukaryotic proteins and are implicated in sequence specific DNA binding, protein-protein interaction and RNA interaction (Gamsjaeger *et al.* 2007). PHD finger is a common structural motif found in a wide variety of all eukaryotic proteins. It is a Zn^{2+}-binding domain of ∼ 60 amino acid length which is known to play an important role in protein-protein interactions and is also implicated in reading of histone methylation marks (Kortschak *et al.* 2000; Bienz 2006; Gamsjaeger *et al.* 2007; Hou and Yu 2010; Sanchez and Zhou 2011). Though these studies elegantly delineate the role of Lid in regulation of gene expression; the mechanism of its action in context of three dimensional nuclear organization remains unknown. Therefore, in order to shed light on the role of Lid in structural organization of chromatin and epigenetic basis of cellular memory, we mapped the NuMat targeting regions of Lid and found that both N and C terminal regions of Lid associate with NuMat. We have further mapped the NuMat association of different domains of Lid and have found that PHD, ARID and JmjC domains are NuMat associating domains.

2. Materials and methods

2.1 *NuMat preparation from S2 cells*

The cells were collected and spun at 900*g* for 5 min at 4°C. The cells were washed in the nuclear isolation buffer (NIB: 3.75 mM Tris-HCl (7.4), 0.5 mM EDTA-KOH, 20 mM KCl, 0.125 mM Spermidine (added fresh), 0.05 mM Spermine (added fresh), and 0.1 mM PMSF) at 900*g*, 4°C at 5 min. The cells were suspended in 2 ml of NIB. After 5 min at room temperature, the cells were passed 10 times through a 22-gauge needle. The nuclear pellet was collected by centrifugation at 3000*g* for 10 min at 4°C. The pellet obtained was washed twice by re-suspending it completely in NIB and spinning at 3000*g* for 10 min at 4°C. The nuclear pellet thus obtained was suspended in 1 ml of 0.25 M sucrose in NIB. Nuclear OD was checked at A260 by lysing an aliquot of 5 µl nuclei in 0.5%SDS. The nuclear pellet was then suspended at a concentration of 10 ODU/ml in 0.25 M sucrose in NIB. After taking out an aliquot for nuclei separately, the rest of the nuclear suspension was kept at 37°C for 20 min for nuclei stabilization. After 20 min incubation, the nuclei were pelleted at 3000*g* at 4°C. This nuclear pellet was incubated in digestion buffer at a concentration of 10 ODU/ml (20 mM Tris pH 7.4, 20 mM KCl, 70 mM NaCl, 10 mM $MgCl_2$, 0.125 mM Spermidine, 0.05 mM Spermine, 0.1 mM PMSF, 0.1 µM Aprotinin, 0.5% Triton X-100, and 40 U/µl DNase I) at 4°C for 1 h. The digested nuclei were pelleted by centrifuging at 3000*g* at 4°C. The digested nuclei were extracted at the concentration of 1 ODU/ml. The first extraction was done for 5 min with 0.4 M NaCl and then another 5 min by increasing the salt concentration to 2 M in extraction buffer (10 mM Hepes pH 7.5, 4 mM KCl, 4 mM EDTA, 0.5 mM Spermidine, 0.1 mM PMSF, 0.1 µM

Aprotinin, 0.5% Triton X-100) at room temperature. The final pellet obtained was washed twice with wash buffer (5 mM Tris pH 7.5, 20 mM KCl, 1 mM EDTA, 0.25 mM Spermidine, 0.1 mM Spermine, 0.1 mM PMSF, 0.1 µM Aprotinin) by spinning at 3000*g* at 25°C. The final pellet was stored at −70°C or used for further analysis.

2.2 *NuMat association assay*

NuMat association assay was developed in S2 cells, the semi-adherent cell line derived from *D. melanogaster* embryos, to study the association of any protein or a region of the protein with NuMat. The full-length protein and different regions of the protein tagged N-terminally with either FLAG or EGFP were transfected and expressed in these cells. The cells were harvested at appropriate time and were processed for NuMat isolation. The NuMat thus prepared, was probed with the suitable antibody on Western blots to follow the NuMat association of the various constructs expressed.

2.3 *Transfection of S2 cells*

S2 cell transfections were done using transfection reagent (Qiagen 301425). 10^6 cells/ml in S2 cell culture medium were plated in each well of the 6-well plate 24 h prior to transfection. For each well, 1 µg/2 µl of plasmid DNA was mixed with 94.8 µl of enhancer buffer and 3.2 µl of enhancer in a microfuge tube. The solution was mixed by pipetting and incubated at room temperature for 5 min. 4 µl of effectene reagent was added to the tube now, mixed by pipetting and incubated at room temperature for 10 min. 500 µl of fresh S2 medium was added to each reaction mixture. In the meanwhile, 500 µl of the medium was aspirated out from each well. The reaction mixture thus prepared was added drop-wise into each well. The 6-well plates were incubated in 25°C incubator. All the steps were carried out in laminar hood.

2.4 *Immunostaining and confocal imaging of S2 cells*

For the purpose of immunostaining, S2 cells were plated onto cover slips 24 h prior any other processing. Cover slips for this purpose were dipped in absolute alcohol, flamed and then placed in the wells of the 6-well plate. S2 cells were pipetted onto the coverslips and after 5 min, 1 ml of medium were added in the well. For the study of the localization of different constructs, the plasmid DNA was transfected onto these cover slips similar to above explained method. For immunostaining, the entire medium from the wells was aspirated out. The cover slips were washed by adding 1 ml of phosphate buffer saline (PBS) in the wells containing coverslips 3–4 times. All the steps before fixation of the cells were performed gently as S2 cells are semi adherent cell lines and there is high probability of these to come out of the cover slips. Therefore, each time washing was done before fixation, the solution was added from the side of the well, avoiding direct splashing over the cover slips. After washing, cells were fixed by adding 1 ml of 4% formaldehyde in PBST (PBS + 0.1% Triton X-100) for 5 min. The cells were then washed 4–5 times with PBS and additionally till the smell of the formaldehyde had gone completely. Primary antibody was then added in the required dilution in PBT (PBS + 0.1% Triton X-100 +0.2% BSA and incubated for 14–16 h at 4°C. After primary incubation, the cover slips were washed 3 times for 5 min each in PBT and then secondary antibody was added in the required dilution for 3 h. After secondary incubation, the coverslips were again washed 3 times for 5 min each in PBT. Clean glass slides were taken, and the coverslips were inverted onto 10 µl of DAPI with mounting media placed on these slides. The edges were sealed using transparent nail paint. The cells were imaged using appropriate excitation wavelength of laser light on a Leica SP8 confocal microscope. Images were processed using Leica Application Suite Advanced Fluorescence (LAS AF) software.

2.5 *Plasmids*

The vector pOT2 (LD40310) carrying the cDNA sequence of Lid protein was taken from DGRC (Drosophila Genome Research Center). PFPc19 vector is a pCaSpeR4-based vector and was used for cloning all the fragments amplified from pOT2 vector. This vector contains an N-terminal Flag tag (MDYKDDDK) and hence all the protein fragments were N-terminally Flag tagged (Pathak *et al.* 2007). The full-length (FL), fragment 1 (F1), fragment 2 (F2), fragment 3 (F3) and ΔPHD amplicons of Lid were cloned between BamH1 and XbaI sites. pRmHa-EGFP-C2 vector was used for tagging proteins N-terminally with EGFP. This vector harbors an inducible promoter called metallothionein promoter (MT) (Bunch *et al.* 1988). It carries an N terminal EGFP-C2 tag and hence all the clones were N-terminally tagged with EGFP. The amplicons were cloned between *Bgl*II and *Sal*I restriction sites.

2.6 *Gel electrophoresis and Western blotting*

The NuMat protein preparations were analyzed by SDS-PAGE. For Western analysis, the proteins were transferred to PVDF membranes (Millipore IPVH00010) and then probed with Lamin Dm0 (DSHB ADL101), H3 (Santa Cruz

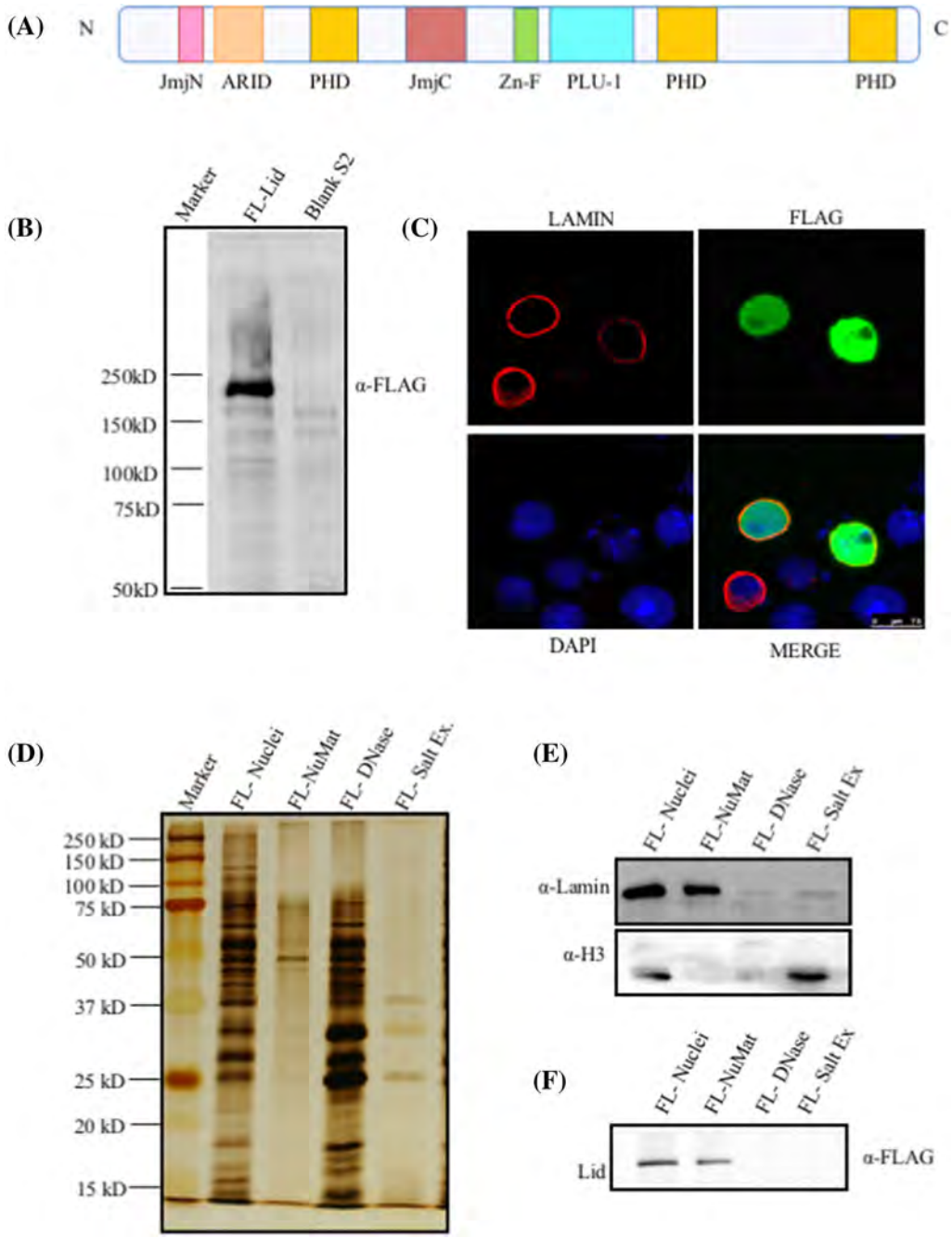

Figure 1. Lid is a NuMat component. (**A**) Domain map of *D. melanogaster* Lid showing all the annotated domains. (**B**) Full-length Lid protein (∼204kD, FL-Lid) tagged with an N-terminal FLAG tag was expressed in S2 cells and the total cell extracts were analyzed by western blotting with α-FLAG antibody. Blank S2 cells represent the control set of untransfected cells. (**C**) Immunostaining showing full-length Lid expression in S2 cells. While Lamin (red) is present at the nuclear periphery, Lid tagged with N-terminal FLAG (green) is present completely inside the nucleus. DAPI (blue) shows the DNA in the nucleus. (**D**) Silver stain profile of extracts at each step of NuMat preparation from S2 cells transfected with FL-Lid tagged with an N-terminal FLAG tag. (**E**) Western blot with α-Lamin and α-H3 for the quality control of NuMat preparation. Lamin, the known NuMat protein is retained in the NuMat preparation, whereas, H3 the non-NuMat protein is removed in the soluble fraction. (**F**) Western blot with α-FLAG shows the NuMat association of Lid.

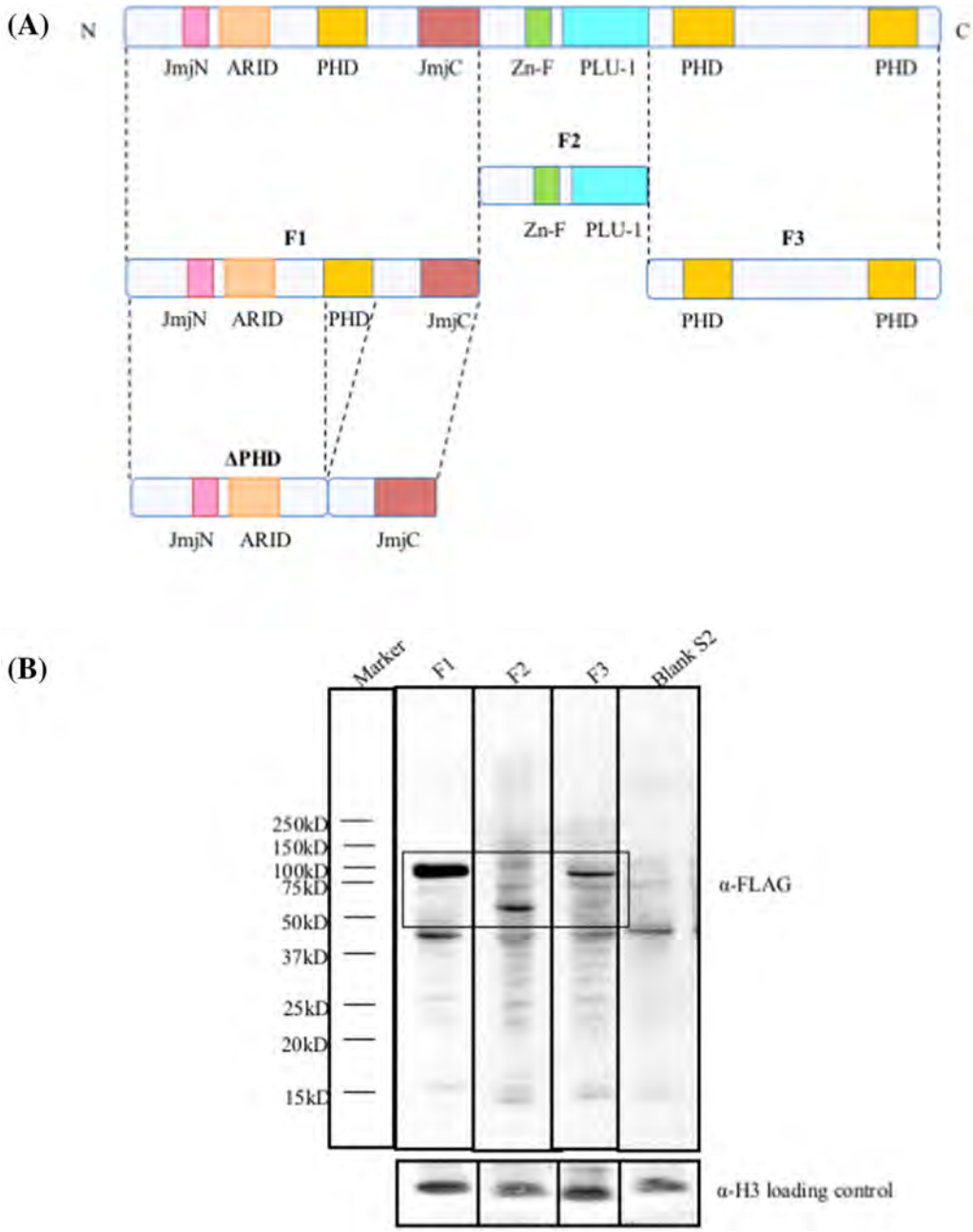

Figure 2. F1, F2 and F3 fragments of Lid are expressed is S2 cells. (**A**) A schematic diagram of different fragments of Lid tagged with N-terminal FLAG tag. The whole Lid protein is divided into three different regions based on the combinations of domains. The first fragment (F1) which is the N-terminal fragment of Lid contains JmjN, ARID, PHD and JmjC domains. The middle fragment called the second fragment (F2) contains zinc finger domain and PLU-1 motif. The third fragment (F3) which is the C-terminal fragment contains 2 PHD fingers. The ΔPHD fragment is the one from which PHD domain has been deleted. (**B**) Western blot with α-FLAG showing expression of the three fragments of Lid (F1, F2 and F3) transfected into S2 cells. Blank S2 cells represent the control set of cells without any transfection. The expected sizes of F1, F2 and F3 are 81kD, 57kD and 66kD, respectively.

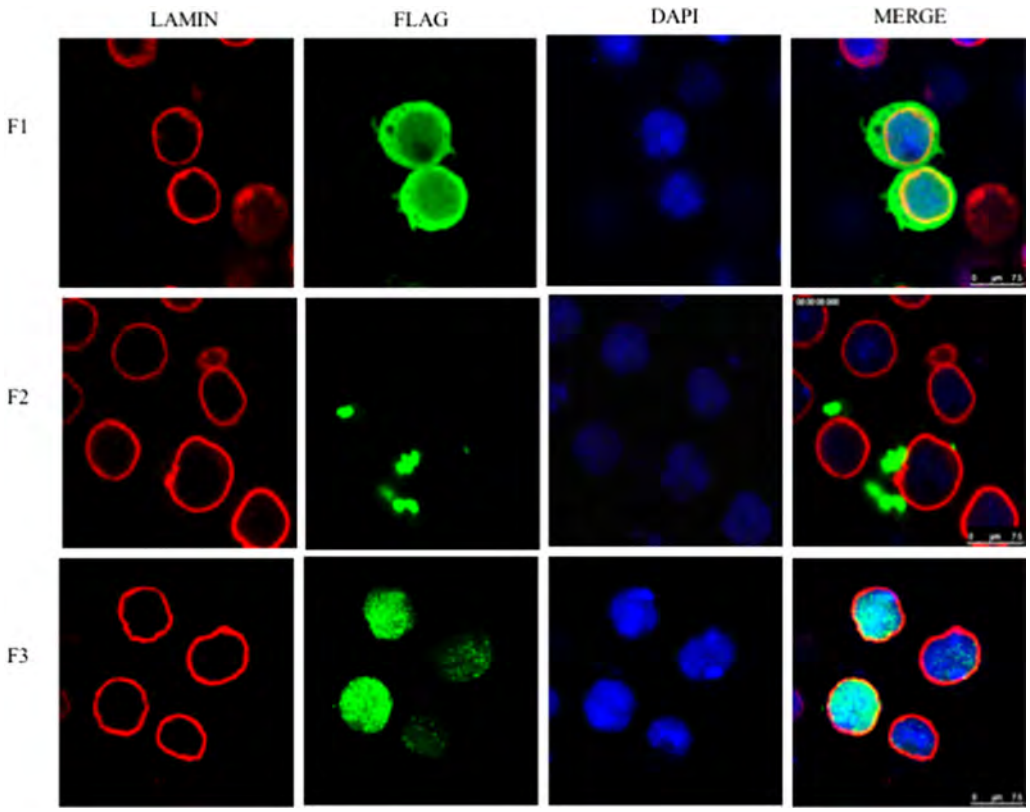

Figure 3. Subcellular localization of various regions of Lid. To follow the subcellular localization of various regions of Lid, the different constructs were expressed and immunostained in S2 cells. Nuclear periphery is stained with Lamin (red) and the cells expressing the transfected protein are stained with FLAG (green). DAPI (blue) shows the DNA in the nuclei. While F1 shows both a nuclear and cytoplasmic localization, F2 is seen forming cytoplasmic aggregates. F3 region of Lid is completely nuclear in localization.

Biotechnologies sc-10809), FLAG (Sigma F3165) and GFP (Abcam ab290).

3. Results

3.1 *Lid is a NuMat component*

We had identified Lid as one of the proteins in our NuMat proteome analysis of 0–16 h old *D. melanogaster* embryos (Kallappagoudar *et al.* 2010). Figure 1A shows the map of Lid protein with all its annotated domains. To validate, that, this is a bonafide NuMat component, we expressed the full-length Lid protein with an N-terminal FLAG tag in S2 cells (figure 1B) and looked for its subcellular localization by immunofluorescence. We found that Lid localizes only in the nucleus (figure 1C). To look further for its NuMat association, we expressed it in S2 cells and isolated NuMat from these transfected cells as described in materials and methods section. NuMat is the DNase and salt resistant fraction of the nucleus consisting of high molecular weight proteins. In order to have consistent NuMat preparations, we applied quality controls on each preparation. To this end, we first performed silver staining of all the different fractions obtained during the preparation and confirmed the presence of high molecular weight protein bands in the NuMat fraction (figure 1D). Secondly, we performed western blot analysis to look for the presence of known NuMat proteins and absence of non-NuMat proteins. Lamin is a known NuMat protein which is retained in the NuMat fraction and histones are extracted away in the salt fractions (figure 1E). Western with α-FLAG antibody was used on such preparation to see the presence of Lid, which turned out to be a bonafide NuMat component (figure 1F).

3.2 *Mapping the NuMat targeting region of Lid*

To map the NuMat targeting region of Lid, we made different constructs as shown in figure 2A. These constructs were tagged N-terminally with FLAG and expressed in S2

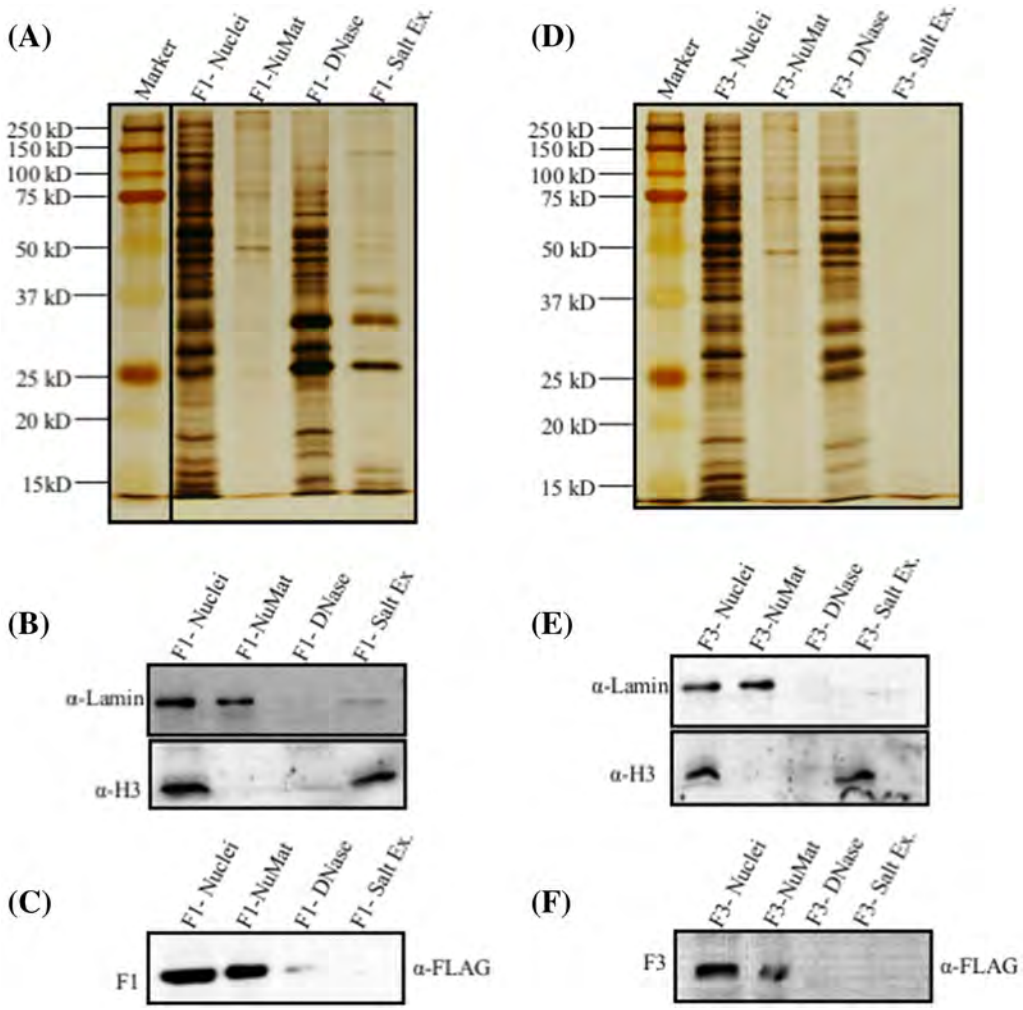

Figure 4. F1 and F3 regions of Lid are NuMat associated. (**A**) Silver stain profile of extracts at each step of NuMat preparation from S2 cells transfected with F1 region of Lid tagged with an N-terminal FLAG tag. (**B**) Western blot with α-Lamin and α-H3 for the quality control of NuMat preparation. Lamin, the known NuMat protein is retained in the NuMat preparation, whereas, H3 the non-NuMat protein is removed in the soluble fraction. (**C**) Western blot with α-FLAG shows the NuMat association of F1. (**D**) Silver stain profile of extracts at each step of NuMat preparation from S2 cells transfected with F3 region of Lid tagged with N-terminal FLAG tag. (**E**) Western blot with α-Lamin and α-H3 for the quality control of NuMat preparation. (**F**) Western blot with α-FLAG shows the NuMat association of F3 region of Lid.

cells (figure 2B). We looked for the subcellular localization of these constructs and found that F1 region shows both nuclear and cytoplasmic localization, F2 region forms cytoplasmic aggregates and F3 shows a complete nuclear localization (figure 3). We chose the fragments that showed nuclear localization to look for their NuMat association. Therefore, we expressed F1 and F3 fragments in S2 cells and isolated NuMat from these transfected cells and performed the quality controls of all the preparations (figure 4A, B, D, E). Western blot with α-FLAG antibody on these NuMat preparations showed that both F1 and F3 have NuMat association potential (figure 4C, F).

3.3 *PHD finger domain of F1 fragment of Lid is not essential for NuMat targeting*

Both the fragments of Lid that associate with NuMat contain well-annotated domains of which PHD finger domain is common, F1 has one while F3 has two PHD fingers. To test if this common feature of the two fragments was behind their NuMat association and, therefore, that of Lid, we decided to delete PHD finger. We chose F1 for this as it has only one PHD finger and less alterations were needed to address this question. The PHD-finger-deleted version of F1 (ΔPHD) was checked for its

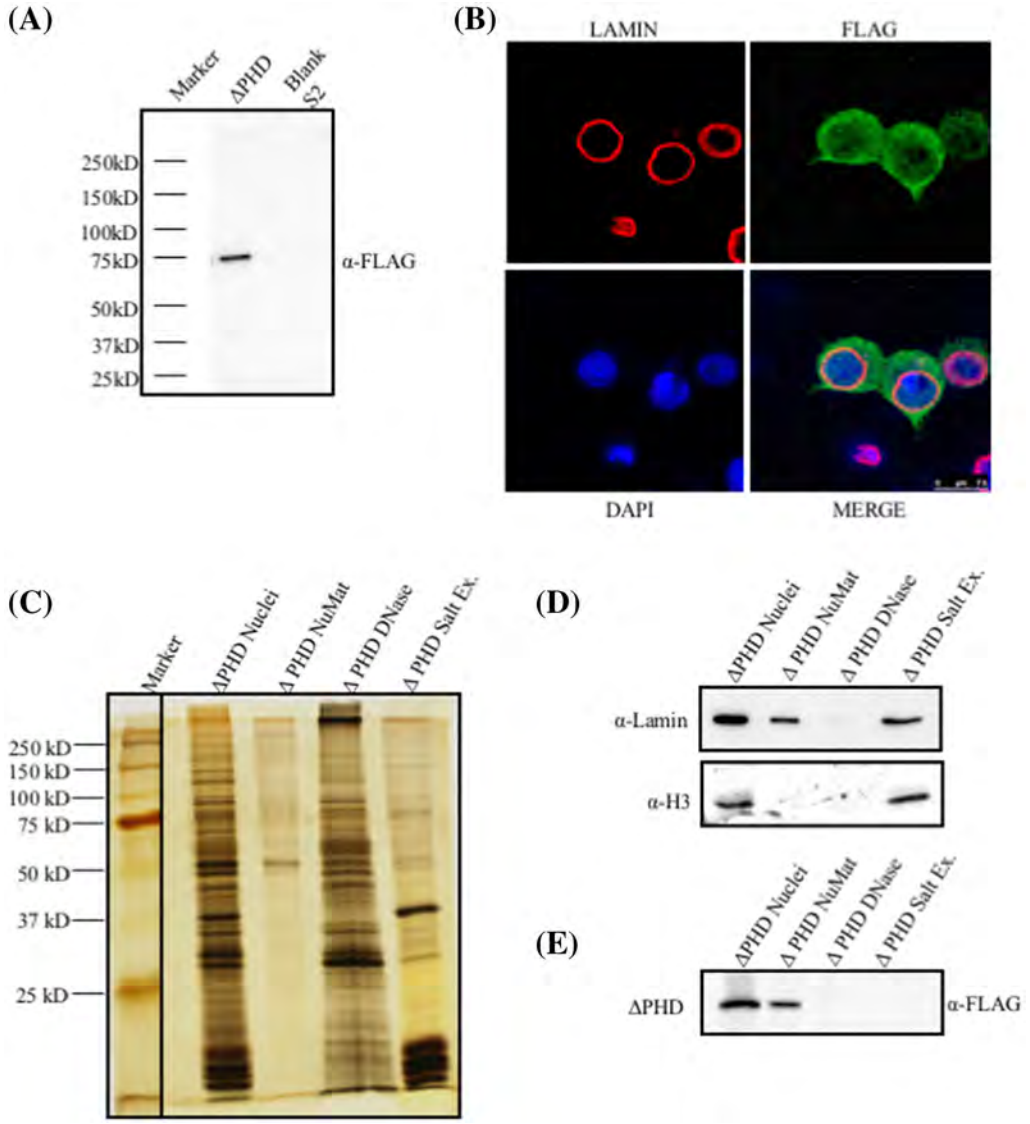

Figure 5. PHD finger is not essential for NuMat association of N-terminal region of Lid. (**A**) Western blot showing the expression of ΔPHD fragment of Lid. The blot is probed with α-FLAG antibody. ΔPHD is ∼75 kD. (**B**) ΔPHD region of Lid is expressed and immunostained in S2 cells. While Lamin (red) is present at the nuclear periphery, ΔPHD Lid tagged with N-terminal FLAG (green) shows both nuclear and cytoplasmic localization. DAPI (blue) shows the DNA in the nucleus. (**C**) Silver stain profile of extracts at each step of NuMat preparation from S2 cells transfected with ΔPHD Lid tagged with an N-terminal FLAG tag. (**D**) Western blot with α-Lamin and α-H3 for the quality control of NuMat preparation. Lamin, the known NuMat protein is retained in the NuMat preparation, whereas H3 the non-NuMat protein is removed in the soluble fraction. (**E**) Western with α-FLAG shows the NuMat association of ΔPHD region of Lid.

expression in S2 cells (figure 5A) and followed its subcellular localization. We found that like F1, ΔPHD version of this fragment shows both nuclear and cytoplasmic localization (figure 5B). We then prepared NuMat from S2 cells transfected with ΔPHD fragment and performed the quality control assays (figure 5C, D) followed by western blot analysis and found that ΔPHD fragment is also NuMat associated (figure E). These results suggest that PHD finger domain is not essential for NuMat association of N-terminal region (F1) of Lid.

3.4 *Identification of the NuMat targeting domain of Lid*

Since PHD finger was dispensable for NuMat association in N-terminal region of Lid, we decided to test if domains other than PHD finger could be responsible for NuMat association. For this purpose, we tagged different domains of F1 fragment, JmjN, ARID, PHD and JmjC, at the N-terminal of EGFP and expressed in S2 cells and confirmed their expression in S2 cells (figure 6A). The subcellular localization showed nuclear as well as

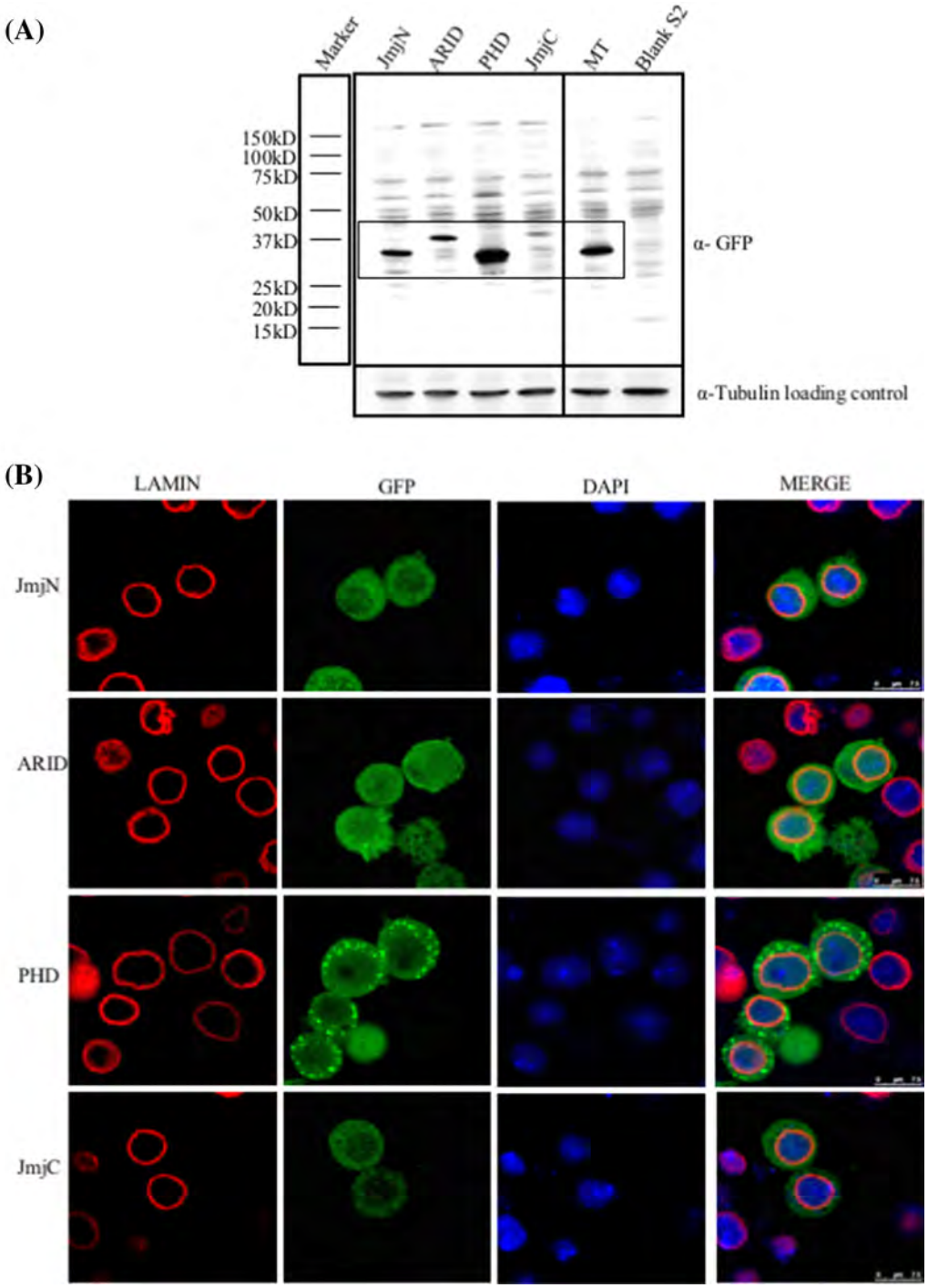

Figure 6. Expression and subcellular localization of various domains of Lid. (**A**) The EGFP tagged JmjN, ARID, PHD and JmjC constructs are transfected and expressed in S2 cells. Western blot analysis with α-GFP shows the expression of the constructs at appropriate molecular weight (JmjN ∼35 kD, ARID ∼40 kD, PHD ∼36 kD, JmjC ∼44kD, EGFP vector alone ∼30kD). (**B**) To follow the subcellular localization of various domains of Lid, the different constructs are expressed and immunostained in S2 cells. Nuclear periphery is stained with Lamin (red) and the cells expressing the individual domains of Lid are stained with FLAG (green). DAPI (blue) shows the DNA in the nuclei. All the domains (JmjN, ARID, PHD and JmjC) show both nuclear and cytoplasmic localization. PHD finger is seen forming some speckle like structures in the cytoplasm in addition to the nuclear localization.

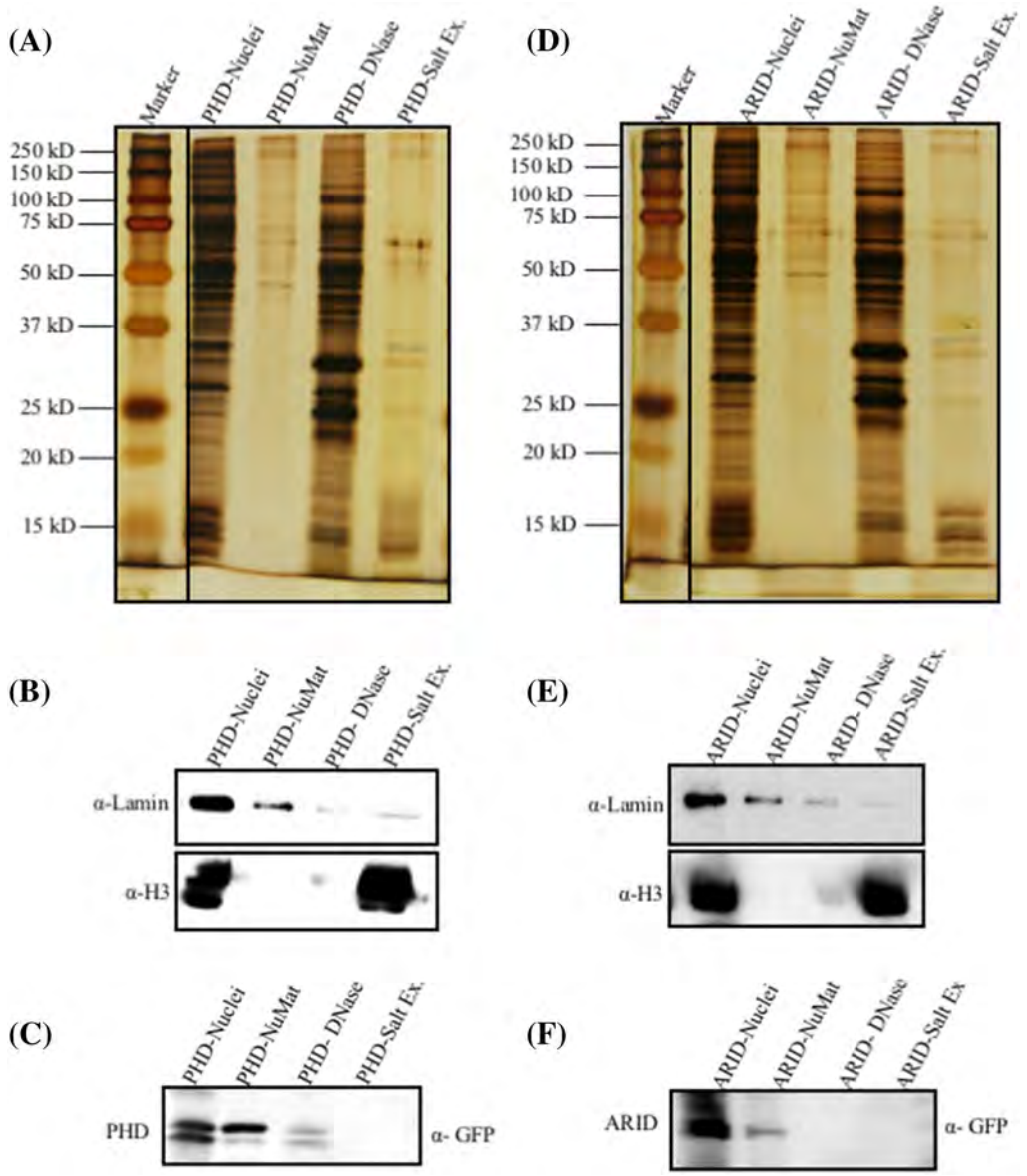

Figure 7. PHD and ARID are NuMat associating domains. (**A**) Silver stain profile of extracts at each step of NuMat preparation from S2 cells transfected with PHD finger of Lid tagged with N-terminal EGFP. (**B**) Western blot with α-Lamin and α-H3 for the quality control of NuMat preparation. Lamin, the known NuMat protein is retained in the NuMat preparation, whereas, H3 the non-NuMat protein is removed in the soluble fraction. (**C**) Western blot with α-GFP showing the NuMat association of PHD finger of Lid. (**D**) Silver stain profile of extracts at each step of NuMat preparation from S2 cells transfected with ARID domain of Lid tagged with N-terminal EGFP. (**E**) Western blot with α-Lamin and α-H3 for the quality control of NuMat preparation. (**F**) Western blot with α-GFP showing the NuMat association of ARID domain of Lid.

cytoplasmic localization of EGFP from all constructs (figure 6B). NuMat was isolated from the cells transfected with these constructs and probed in western blot with α-GFP antibody. We found that PHD finger, ARID and JmjC domains show NuMat association, while JmjN domain does not (figures 7 and 8). These findings suggest that multiple domains in Lid can target it to the NuMat.

4. Discussion

A large number of studies in last few years have uncovered the role of nuclear architecture and higher order chromatin organization in gene expression. NuMat, an important feature of the nuclear architecture is believed to play a key role in gene expression. Very few components of NuMat, however, are characterized precisely in the context of their structural role in

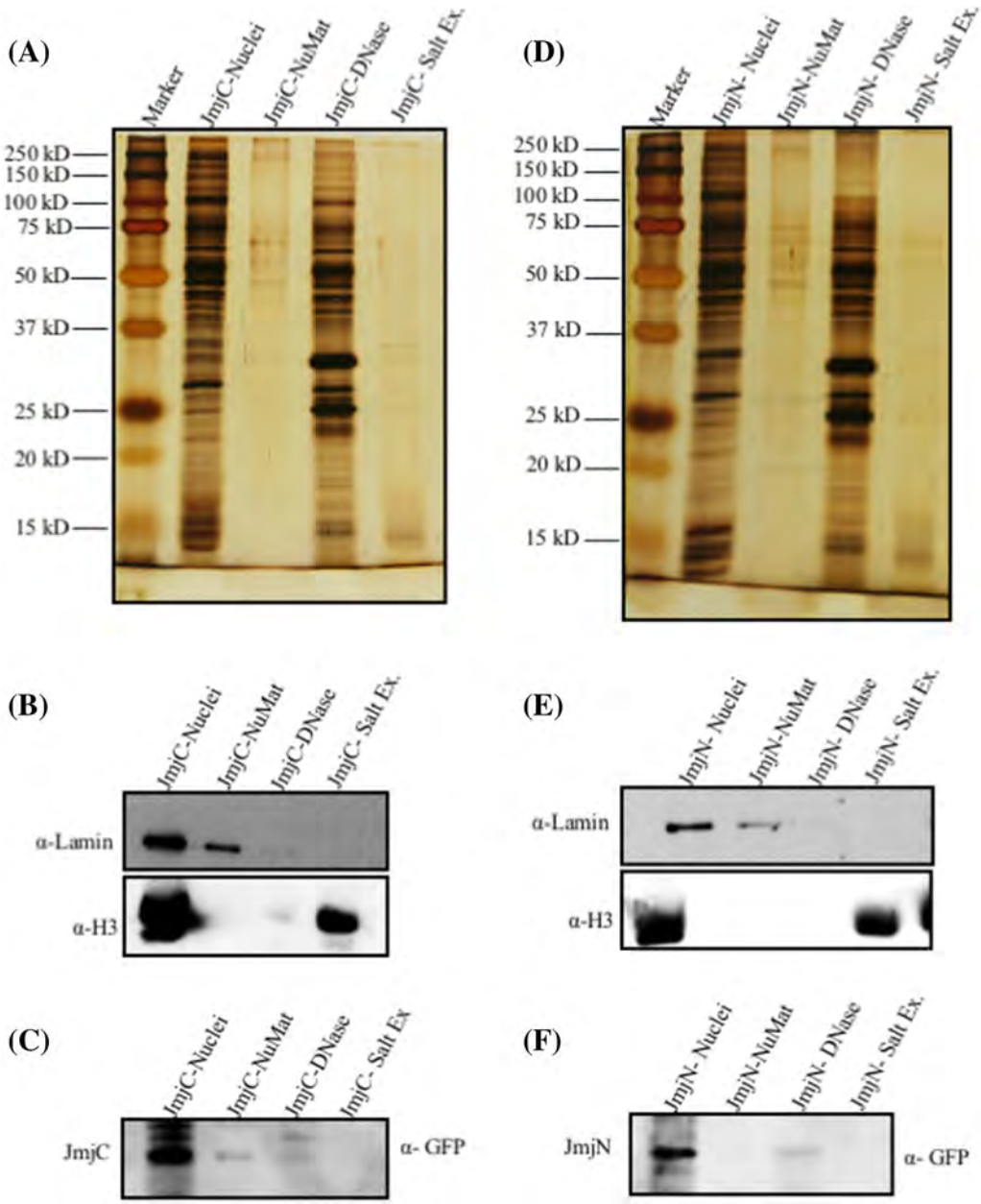

Figure 8. JmjC but not JmjN is NuMat associating domain. (**A**) Silver stain profile of extracts at each step of NuMat preparation from S2 cells transfected with JmjC domain of Lid tagged with N-terminal EGFP. (**B**) Western blot with α-Lamin and α-H3 for the quality control of NuMat preparation. Lamin, the known NuMat protein is retained in the NuMat preparation, whereas, H3 the non-NuMat protein is removed in the soluble fraction. (**C**) Western with α-GFP showing the NuMat association of JmjC domain of Lid. (**D**) Silver stain profile of extracts at each step of NuMat preparation from S2 cells transfected with JmjN domain of Lid tagged with N-terminal EGFP. (**E**) Western blot with α-Lamin and α-H3 for the quality control of NuMat preparation. (**F**) Western blot with α-GFP showing that JmjN domain of Lid is not NuMat associated.

organizing chromatin in three-dimensional nuclear milieu (Pathak *et al.* 2007). We chose a TrxG protein Lid, that was shown earlier to be a NuMat component, for this purpose (Kallappagoudar *et al.* 2010). Lid has an array of well-annotated domains, which provided leads to alter different domains and look for their role in its NuMat association. Our results indicate that there are multiple means by which Lid can be targeted to NuMat. Distinctly annotated domains, viz., JmC, ARID and PHD finger domains but not the JmjN domain, are sufficient for its NuMat association. Considering different functions of these domains, we speculate that Lid, by these domains, may act as an adapter molecule that converges

different chromatin features to the NuMat context and links structure and function of nuclear architecture.

PHD finger, that we show as one of the NuMat targeting domains, is known to be transcriptional co-activator as well as sequence specific histone reader involved in regulation of gene expression (Sanchez and Zhou 2011). Since one of the key features of NuMat are the DNA sequences called matrix associated regions (MARs), one possible scenario is that Lid binds to specific chromatin marks through one or more of its PHD fingers and this 'Lid bound chromatin' is brought in association with NuMat and, therefore with MARs to interact with the entire repertoire of other proteins including 'transcription factories' required for gene expression. Several lines of studies can be interpreted in this new context. For example, several interactors of Lid, viz., Rpd3, Mrg15, dMyc and Ash2 (Secombe *et al.* 2007; Lee *et al.* 2009) well-known regulators of gene expression are also constituents of NuMat proteome (Kallappagoudar *et al.* 2010). A recent study on the genome wide localization of Lid shows that it localizes at the transcription start sites (TSS) of developmental genes and acts in concert with Ash2, which is another TrxG protein and an important component in transcriptional regulation (Lloret-Llinares *et al.* 2012). We have earlier shown that TSS forms a major part of MARs in fly genome which often maps to paused RNA Polymerase II (Pathak *et al.* 2014). Taken together, these observations suggest that Lid and its interactors act in the context of NuMat which brings in the proximity of MARs associated features including H3K4me3 marks on TSS. The C-terminal third PHD finger of Lid involved in recognizing H3K4me2/H3K4me3 marks is crucial for its interaction with dMyc (Li *et al.* 2010). Therefore, presence of multiple domains of distinct function in Lid enables it to function as an adaptor molecule that brings in a combination of chromatin features and set of regulatory proteins in the context of NuMat. In fact, a recent study where Lid was shown to regulate chromosome architecture independent of its histone demethylase activity supports our hypothesis of Lid's role in acting as an adapter molecule between chromatin and NuMat (Zhaunova *et al.* 2016). One of the domains of Lid that can target it to NuMat is ARID, a domain implicated in binding to AT-rich DNA sequences (Kortschak *et al.* 2000). Interestingly, often NuMat associated parts of the genome, MARs, are found to be AT-rich (Gasser and Laemmli 1986). It is therefore possible that one of the ways Lid targets to NuMat is via its binding to the DNA sequence associated with NuMat. Two ARID domain containing proteins that are known to bind to MAR associated sequences are mouse protein Bright that binds to AT tracts of 20–40 base pairs and *D. melanogaster* dead ringer (DRI) protein which binds to ATTA motif (Dallas *et al.* 2000).

Another important and novel finding of our study is the association of JmjC domain with NuMat. JmjC domain is a unique domain present in a class of histone demethylases which requires Fe (II) and 2-oxoglutarate for demethylation of histone marks (Takeuchi *et al.* 2006). As opposed to the earlier concept, it is well known now that histone methylation marks are reversible and thus, histone demethylases are instrumental in processes such as development and diseases (Benevolenskaya 2007). It is also well established that histone modifications are indispensable for organization of chromatin in interphase nuclei and compartmentalization (Bartova *et al.* 2008). However, there are very few reports, which suggest the association of histone modification enzymes with NuMat. For example, it has been shown that both histone acetyltransferases and histone deacetylases are associated with NuMat and carry out the acetylation-deacetylation of core histones of the chromatin which is bound to the NuMat (Davie 1997). In this context, the association of NuMat with a demethylase catalytic domain is interesting and opens new lines of investigation.

In conclusion, we have identified a chromatin remodeler protein, Little imaginal discs (Lid) as one of the NuMat components and have mapped its NuMat targeting regions. We find that Lid can associate with NuMat by multiple means, which include ARID, PHD and JmjC domains. We also show that although PHD finger is not necessary, it is sufficient for NuMat association. We speculate that Lid may act as an 'adapter' protein between NuMat and chromatin and, owing to the presence of a variety of domains, it can interact with the different chromatin marks to bring such regions in the context of NuMat. Further studies will be needed to address the exact contribution of Lid to the structure of NuMat, if any. It will also be interesting to explore if the demethylase function of JmjC domain is of functional relevance in the NuMat context.

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