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Wash interacts with lamin and affects global nuclear organization

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Summary

The cytoplasmic functions of Wiskott-Aldrich Syndrome family (WAS) proteins are well established and include roles in cytoskeleton reorganization and membrane-cytoskeletal interactions important for membrane/vesicle trafficking, morphogenesis, immune response and signal transduction. Mis-regulation of these proteins is associated with immune deficiency and metastasis [1-4]. Cytoplasmic WAS proteins act as effectors of Rho family GTPases and polymerize branched actin through the Arp2/3 complex [1, 5]. Previously, we identified Drosophila *washout* (*wash*) as a new member of the WAS family with essential cytoplasmic roles in early development [6, 7]. Studies in mammalian cells and *Dictyostelium* suggest that WASH functions primarily in a multiprotein complex that regulates endosome shape and trafficking in an Arp2/3-dependent manner [8-11]. However, roles for classically cytoplasmic proteins in the nucleus are beginning to emerge, in particular as participants in the regulation of gene expression [12, 13]. Here we show that Drosophila Wash is present in the nucleus where it plays a key role in global nuclear organization. *wash* mutant and knockdown nuclei disrupt sub-nuclear structures/organelles and exhibit the abnormal wrinkled morphology reminiscent of those observed in diverse laminopathies [14-16]. We find that nuclear Wash interacts with B-type Lamin (Lamin Dm0), and like Lamin, Wash associates with constitutive heterochromatin. Wash knockdown increases chromatin accessibility of repressive compartments and results in a global redistribution of repressive histone modifications. Thus, our results reveal a novel role for Wash in modulating

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Author Contributions

JMV, HR-A, and SMP designed the study. JMV, HR-A, SMP, TRW, DS and VN performed experiments and analyzed data. VN, JVM, and DS performed the morphometric analyses. HR-A and JJD performed the bioinformatics analyses. HR-A, JMV, MG, and SMP wrote the manuscript. All authors participated in discussion of the results and commented on the manuscript.

nucleus morphology and in the organization of both chromatin and non-chromatin nuclear sub-structures.

Keywords

Wash; Wiskott Aldrich Syndrome; nuclear organization; Lamin; genome organization; chromatin accessibility; Drosophila

Results and Discussion

While examining cytoplasmic functions of Wash, we found that Wash is present in fly cell nuclear extracts (Figures 1A). In addition to localizing in the cytoplasm, Wash is present in the nucleus in Drosophila S2R+ cells and many embryo/larval tissues (e.g., salivary glands) (Figures 1B-1H'; data not shown) [7, 17]. Nuclear Wash is distributed in a punctuate pattern (Figures 1B-1C, 1F). This nuclear Wash staining is not observed in Wash knockdowns cells, *wash* mutant tissues, or when digitonin is used to permeabilize only the plasma membrane (Figures 1D, 1G, 1H-1I', and S1A-S1A'). Although Wash lacks a DNA binding domain, polytene chromosome staining shows that Wash associates (directly or indirectly) with ~500 chromatin regions (Figures 1J-1K').

To determine if nuclear Wash is biologically significant, we examined nuclear morphology in Wash RNAi-treated S2R+ cells. The effectiveness of Wash knockdown was established by western blot (Figure S1B) and confirmed by the disorganization of the cytoplasm of knockdown cells (Figures 1L-1O' and S1C-S1D'). Importantly, Wash depletion caused irregularly shaped nuclei in S2R+ cells without affecting expression of the nuclear lamina protein Lamin (nuclear shape concavity: 9.8±1.2% GFP RNAi versus 17.0±1.6% Wash RNAi, P=0.0003; Figures 1L-1P and S1C-S1E). This altered nuclear morphology phenotype is not due to cell culture manipulation or an indirect effect of cytoplasmic Wash knockdown, as salivary gland nuclei from $wash^{\Delta 185}$ mutants stained for Lamin also show dramatic morphological alterations in nuclear shape while retaining overall cell and gland shape (nuclear shape concavity: 2.7±0.68% wildtype versus 18.9±3.2% *wash*, P<0.0001; Figures 1Q-1X). Thus, Wash plays a key role in modulating nuclear shape in Drosophila.

We next explored if lack of Wash affects nuclear organization. *wash* mutants exhibit reduced nuclear volume (8243±473 μm^3 wildtype, 2797±146 μm^3 *wash*, P<0.0001) and lower DNA content (Figures S1F-S1H). DAPI staining of wildtype and *wash* polytene chromosome spreads showed that chromosomes in *wash* mutants are less organized (Figures 2A-2B), appear more twisted (Figures 2C-2D), and tend to easily break and/or fragment upon physical stress (Figures 2E-2F). We performed FISH on salivary glands using chromosome paints for the three larger chromosomes. While wildtype chromosomes occupy well-defined spaces (chromosome territories), chromosomes of *wash* mutants are positioned closer to the nuclear periphery, with chromosome 3 in particular tending to be more dispersed (Figures 2G-2H and S1I-S1K; Movie S1). We also examined the organization of diverse non-chromatin nuclear sub-structures, as well as different chromatin modifications. Strikingly, HP1, which accumulates at and marks the chromocenter, is present in the nucleus but accumulates only weakly at the chromocenter in *wash* mutants (Figures 2I-2J and S1L-

S1M). Similarly, Coilin and Fibrillarin (markers of cajal bodies and the nucleolus, respectively) and the nuclear envelope component Mtor exhibit non-specific nuclear localization in the absence of Wash (Figures 2K-2N and S1L-S1M). Importantly, these mis-localizations are specific, as lack of Wash does not affect localization of the dosage compensation complex (Mof) (Figures 2M-2N). The alterations of HP1, Coilin, Fibrillarin, and Mtor are likely due to their nuclear disorganization rather than protein loss, as protein levels remain roughly the same in *wash* mutants or knockdown cells (with the exception of Coilin; Figure S1L-S1M). We also examined histone modifications associated with repressive or active chromatin. Consistent with our previous results, H3K9me3, a histone modification recognized by HP1, is reduced in *wash* mutants (Figures 2O-2P and S1N-O). The repressive mark H4K20me2 and the active mark H3K4me3 are also lower in *wash* mutant nuclei (Figures 2Q-2T and S1P-S1S).

To further examine the requirement for Wash in the nucleus, we expressed a GFP-Wash fusion protein with and without its conserved nuclear localization sequence mutated under the control of its own endogenous Wash promoter in a $wash^{\Delta 185}$ mutant background (Figure 2U; see Supplemental Experimental Procedures). The wildtype version of these transgenics ($wash^{\Delta 185}$ P{$GFP\text{-}Wash^{WT}$}) is expressed in both the nucleus and cytoplasm, rescues the $wash^{\Delta 185}$ mutant to viability, and does not exhibit nuclear phenotypes (Figure 2V-V”, 2X, 2Z, 2AA, 2CC-DD and S1T). In transgenics with the version lacking the nuclear localization signal ($wash^{\Delta 185}$ P{$GFP\text{-}Wash^{+NES\Delta NLS}$}), the GFP-$Wash^{+NES\Delta NLS}$ fusion protein is expressed in the cytoplasm while largely excluded from the nucleus (Figure 2W’-W”, 2Y, 2Z, 2BB, 2CC-DD and S1T). These transgenics exhibit similar nuclear phenotypes as $wash^{\Delta 185}$ mutants, including disruption of nuclear morphology (Figure 2V-2Z and S1T) and disorganization of sub-nuclear structures/organelles (Figure 2AA-DD). Thus, Wash’s nuclear activity is required during development, where it plays a key role in global nuclear organization.

We identified a strong interaction between Wash and the Drosophila B-type lamin, Lamin Dm0, in a yeast two-hybrid screen (data not shown). Lamins are intermediate filament proteins that form a mesh lining the inner nuclear membrane and function in diverse roles including nuclear shape, aging, genome organization, and gene expression [18, 19]. Lamin Dm0 (hereafter referred to as Lamin) is present throughout development in all cells (except mature sperm). Using GST pulldowns with *in vitro* translated and/or bacterially purified proteins, we show that Wash interacts directly with Lamin (Figures 3A-3B). Co-immunoprecipitation experiments using nuclear enriched extracts of 0-12h Drosophila embryos show that this nuclear Wash-Lamin interaction occurs *in vivo* (Figure 3C). To confirm this interaction *in vivo*, we performed a Duolink *in situ* Proximity Ligation Assay (PLA) for Wash and GFP in salivary glands expressing a GFP-Lamin fusion protein. Fluorescent signal was observed and localized exclusively around the DNA confirming the interaction between Wash and GFP-Lamin (Figure 3D-3D’”). Thus, nuclear Wash interacts physically with Lamin.

While Lamin does not exhibit a banding pattern on polytene chromosomes (Figures S2A-S2A”), genome-wide studies have shown that Lamin associates with specific genomic regions termed Lamin-associated domains (LADs) [20, 21]. These regions exhibit low gene

density and are usually associated with repressive chromatin. As Wash associates with discrete regions on polytene chromosomes, we asked whether these regions correspond to LADs. Using DamID chromatin profiling in Kc cells, we identified 593 large continuous Wash-associated domains spread along the Drosophila genome with an average size of 40 Kb (Figures 3E-3F). Wash domains mainly localize with non-transcribed regions during early embryogenesis (Figure 3F). We performed similar DamID profiling for Lamin and identified 616 LADs (Figures 3E-3G). These domains show ~93% overlap with LADs previously described in Kc167 cells using a different microarray platform (385 of 412 LADs identified by [21]) (Figure S2B). Importantly, Wash-associated regions indeed coincide with LADs (Pearson correlation=0.72). Meta-analysis of the Wash DamID signal to LADs boundaries confirms the genomic co-localization between Wash and Lamin (Figures 3H and S2C).

To establish whether Wash domains correspond to functional domains defined by factor binding and epigenetic marks, we performed a meta-analysis of Wash binding to the boundaries of the 5-chromatin types classification (Yellow, Red, Blue, Green & Black) established previously in Kc cells [22]. Wash association with chromatin is mainly enriched in Black chromatin, which is the predominant type of repressive chromatin containing silent or low-expressing genes and co-localizing with Lamin-enriched nuclear envelope. Wash DamID signal is depleted from other chromatin types and coding regions (except Blue (Polycomb chromatin)) (Figure 3I-3J), suggesting that Wash targets transcriptionally silent chromatin regions in a similar fashion to Lamin [21, 22]. Interestingly, Wash also targets expressed developmental-regulated domains, suggesting that Wash binding does not necessarily repress transcription (Figure S2D-S2F).

The alteration of histone modifications and sub-nuclear structures in *wash* mutants strongly suggests that Wash directly impacts chromatin organization. As chromatin accessibility is the main organizing feature that explains chromatin organization [23], we evaluated the effect of Wash knockdown in S2R+ cells on chromatin accessibility using an M.SssI-based approach [24]. Meta-analysis of M.SssI-methylated accessible chromatin of the 9 modENCODE consortium-defined chromatin states revealed higher accessibility in promoters and enhancers than in heterochromatic regions (Figures 4A-4C and S4A-S4H) [25]. Nevertheless, and consistent with its association to BLACK chromatin, Wash knockdown increased chromatin accessibility at the borders of constitutive heterochromatin, but not promoter or heterochromatin-like euchromatin (Figures 4A-4C). MeDIP-qPCR experiments confirmed these structural alterations on constitutive heterochromatin upon Wash knockdown, but not in promoter regions (Figure S3I). Notably, although no effect was observed on promoter, open chromatin, male X-linked genes, or heterochromatin-like euchromatic regions upon Wash knockdown, we observed increased accessibility on enhancers and reduced accessibility on Polycomb, elongating, enhancer, and basal interchromatin regions (Figures S3B-S3H), suggesting that absence of Wash indirectly impacts particular functional domains of the genome.

Similar to Wash, knockdown of Lamin alters nuclear architecture and correlates with alterations on heterochromatin organization of repressive histone modifications (e.g. H3K9m3) [26, 27]. Despite exhibiting a higher global background (Figure S3B), adjusted

chromatin accessibility reveals that, similar to Wash, Lamin depletion causes an increase in accessibility of heterochromatic regions (Figures 4D-4F). Nevertheless, unlike Wash, Lamin knockdown also increased the accessibility of promoter regions (Figure 4D), suggesting that Lamin has more widespread roles in chromatin organization whereas Wash is particularly relevant for heterochromatin architecture, which is consistent with recent reports suggesting that loss of LMNB1 results in decondensation of chromosome territories [28].

Since Wash modifies genome organization by impairing chromatin accessibility of silent regions, we hypothesized that the *wash* mutant should behave as a suppressor of position effect variegation (PEV). Several Drosophila PEV models have been used as *in vivo* reporters of a particular protein's ability to modulate chromatin in different contexts [29]. Using the *brown*-based PEV model [30], we found that lack of *wash* suppresses both bw^{VDE2} (classical) and bw^{D} (non-classical) variegation (less heterochromatic repression; Figures 4H and 4J), which is consistent with our genomic data. Importantly, similar results were obtained using the $lamin^{sz18}$ mutant, however, this lamin-dependent effect is sex-specific in the case of bw^{D} (Figures 4G and 4I). Strikingly, when the $wash^{\Delta 185}$ and $lamin^{sz18}$ alleles were tested in the presence of *white*-based centromeric, heterochromatic, and telomeric PEV [31, 32], we found that both mutants behave as enhancers of centromeric and heterochromatic PEV (Figure 4K-4L and S4A-S4D), while showing no affect on telomeric PEV (Figures S4E-S4H). This is consistent with a previous report for lamin effects on centromeric PEV [33]. It is worth noting that both *brown* and *white* are found in LADs, but while *brown* is located in a gene-rich region flanked by LADs, the *white* gene is embedded in a gene poor region that is part of a LAD (Figures S4I-S4K). To our knowledge this is the first time that the two PEV models have yielded different results when assaying a given mutation, suggesting that these PEV models differentially affect heterochromatic domains.

Classically cytoplasmic proteins are emerging as significant players in the nucleus. Two WAS family members, WASp and WAVE1, have been identified as positive regulators of gene expression [12, 13]. Indeed, before Wash was recognized as a cytoplasmic cytoskeletal factor, it was identified as part of the nuclear TRF2 complex [34] and, more recently, a required regulator of the NURF complex on the c-Myc locus in hematopoesis [35]. Despite this potential role in regulating gene expression, our findings link the nuclear presence of Wash with global nuclear organization, suggesting that Wash is required for the formation, maintenance, and/or movements of specific nuclear domains, organelles, and/or machineries. We also identify Wash as a Lamin-interacting protein and a key component of "BLACK" chromatin. Lamins and lamina-interacting proteins mainly compose the nuclear envelope, which plays key roles in nuclear structure, chromosome organization, DNA repair, transcriptional control, and like its plasma membrane counterpart, is the site for signal sensing, molecule trafficking, and inter/intra-nuclear attachments. Consistent with this, mutations in Lamins and lamina-interacting proteins such as emerin lead to phenotypically diverse diseases including muscular dystrophies, cardiomyopathies, and premature aging syndromes (collectively called laminopathies) [26]. Interestingly, the abnormal wrinkled morphology in both *wash* mutant and knockdown nuclei resembles that observed in diverse laminopathies [14-16], including the Hutchinson-Gilford progeria syndrome. It is possible that Wash dynamically modifies specific compartments of the nuclear envelope to adapt it to

different types of stimuli. It is known that tissue-specific differential expression of lamin isoforms reinforce the nucleus against physical stress by stabilizing the nuclear envelope and chromatin [36, 37]. We have previously shown that, like other WAS family members, Wash requires Arp2/3 for actin nucleation [6, 7]. Recently, both actin and the WAS family-interacting protein Arp2/3 were implicated in nuclear envelope fragmentation in starfish oocytes [38]. Although it is not yet clear whether the Wash-Lamin functional link requires Arp2/3, Wash could potentially be the connection point for coordinating the remodeling of the Lamin-based meshwork along with the cytoplasmic cytoskeleton in response to physical stress. Alternatively, *in vitro* data suggest that Lamin monomers tend to self-assemble into filamentous fibers under particular salt conditions [39, 40], Wash could modulate Lamin self-assembly *in vivo* based on its molecular functions with other structural proteins. Additionally, Wash is involved in endocytosis by modulating vesicle formation and trafficking (cf. [4, 41]), these regulatory activities could be useful at the nuclear envelope for the dynamic remodeling of the lamin meshwork in response to nuclear shape changes required for cells to squeeze through small spaces or when undergoing metastasis. An alternate possibility relies on the fact that actin and actin-binding proteins have been found in several chromatin remodeling factors (cf. [42]): Wash could modulate actin functions and/or its mobility during its association with these chromatin remodelers to maintain a dynamic chromatin structure. Our data highlights Wash as a key regulator of cytoplasmic and nuclear processes by maintaining microfilament (actin), intermediate filament, and microtubule homeostasis, and whose mutations lead to alterations in both cellular compartments. In the future, it will be exciting to further explore Wash's nuclear roles, and to determine if and how closely these roles parallel those in the cytoplasm.

Experimental Procedures

Please refer to the Supplemental Information for detailed experimental procedures.

The raw and normalized datasets and DamID domains can be found in the following GEO dataset: GSE59854.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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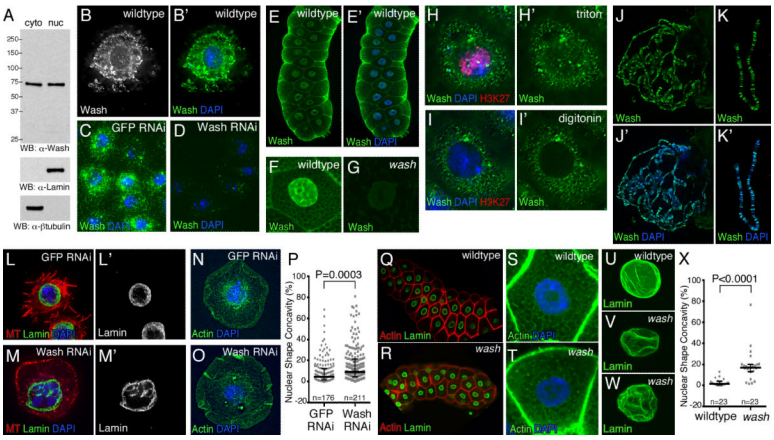

Figure 1.

Wash is in the nucleus and disrupts nuclear morphology. (A) Wash is expressed in both the nucleus and cytoplasm as shown by western blot analysis of nuclear and cytoplasmic Drosophila Kc167 cell extracts. Extract specificity shown by western blot analysis with Lamin (nuclear) and β-tubulin (cytoplasmic). (B-B') Micrographs of immunostained S2R+ cells (single focal plane) showing Wash is both cytoplasmic and nuclear. (C-D) Wash staining in S2R+ cells treated with dsRNA to GFP (control; C) or Wash (D) showing specificity of the Wash antibody. (E-E') Confocal micrograph of larval salivary glands (projection) showing Wash is present in both the cytoplasm and the nucleus. (F-G) Nuclear and cytoplasmic Wash staining in wildtype salivary gland cells (F) and its absence in *wash*$^{\Delta 185}$ mutants (G). (H-I') S2R+ cells treated with 5mg/ml digitonin (to permeabilize only the plasma membrane; I-I') or 0.2% triton X-100 (to permeabilize both the plasma and nuclear membranes; H-H'), then stained for Wash and H3K27me3. Nuclear Wash and H3K27me3 staining are not detected when the nuclear membrane is not permeabilized (I-I'). (J-K') Wash associates with specific regions on third-instar larval polytene chromosomes. (L-O') *wash* knockdown mutants exhibit morphological alterations in nuclear shape. Confocal projections of S2R+ cells treated with dsRNA for GFP (L-L', N-N') or Wash (M-M', O-O') then stained for Lamin (L-M'), microtubules (MT) (L-M), actin (N-O) and DNA (DAPI; L-M, N-O) showing that Wash knockdown disrupts nuclear morphology in addition to cytoplasmic architecture. (P) Quantification of nuclear shape concavity in S2R+ cells treated with dsRNA to GFP (9.8±1.2%, n=176) and Wash (17.0±1.6%, n=211) (P=0.0003). Scatterplot with median ± IQR shown. (Q-V) Confocal projections of wildtype versus *wash*$^{\Delta 185}$ mutant salivary glands stained for actin and Lamin show that Wash affects nuclear morphology without gross cytoplasmic defects. Whole salivary glands (Q-R), salivary gland cells (S-T), and salivary gland nuclei (U-V). (X) Quantification of nuclear shape concavity in wildtype (2.7±0.68%, n=23) and *wash*$^{\Delta 185}$ (18.9±3.2%, n=23) salivary gland nuclei (P<0.0001). Scatterplot with median ± IQR shown. See also Figure S1.

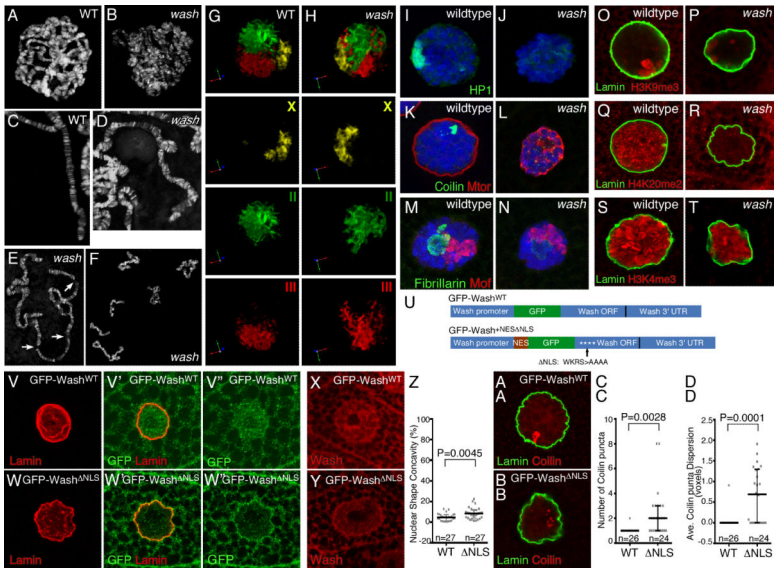

Figure 2.

Wash disrupts nuclear and genome organization. (A-F) Confocal projections of wildtype and *wash*$^{\Delta 185}$ mutant salivary gland polytene chromosomes; *wash*$^{\Delta 185}$ chromosomes show aberrant alignment and banding (D) and are extremely fragile (E-F). (G-H) Fluorescent *in situ* hybridization of chromosome-specific BAC pools hybridized to the X (yellow), second (green) and third (red) chromosome in wildtype and *wash*$^{\Delta 185}$ mutant salivary gland nuclei shows less compact chromosome territories in *wash* mutants. (I-N) Confocal micrographs of wildtype and *wash*$^{\Delta 185}$ mutant salivary gland nuclei stained for DNA and nuclear markers. HP1 (green) chromocenter localization is lost in *wash*$^{\Delta 185}$ nuclei (I-J). Coilin (green) cajal body localization is disrupted in *wash*$^{\Delta 185}$ nuclei while Mtor (red) remains localized to the periphery of salivary gland nuclei (K-L). Fibrillarin (green) localization at the nucleolus is disrupted in *wash*$^{\Delta 185}$ nuclei (M-N). While MOF localizes properly to the X chromosome in both wildtype and *wash*$^{\Delta 185}$ nuclei, it highlights the disruption to chromosome territory compaction observed in *wash* mutants (M-N). (O-T) Confocal projections of histone modifications in wildtype and *wash*$^{\Delta 185}$ salivary gland nuclei. Both repressive histone marks (H3K9me3 (O-P) and H4K20me2 (Q-R)) and the active histone mark H3K4me3 (S-T) are reduced in *wash* nuclei. (U) Schematic diagram of the Wash constructs used to generate the transgenic lines indicating the position of the added NES, GFP fusion, and the substitution mutations (WKRS>AAAA) in the Wash NLS (not drawn to scale). (V-DD) Specific reduction of Wash in the nucleus results in disrupted nuclear shape and sub-nuclear structure/organelle organization. Confocal projections of salivary gland nuclei from *wash*$^{\Delta 185}$ P{*GFP-Wash*^{WT}} (V-V'", X, AA) and *wash*$^{\Delta 185}$ P{*GFP-Wash*$^{+NES\Delta NLS}$} (W-W'", Y, BB) stained for Lamin (V-V', W-W', AA-BB), GFP (V'-V", W'-W"), Wash (X-Y), and Coilin (AA-BB). Quantification of nuclear shape concavity in *wash*$^{\Delta 185}$ P{GFP-Wash^{WT}} (WT: 4.74±0.67%; n=27) or *wash*$^{\Delta 185}$ P{GFP-Wash$^{+NES\Delta NLS}$} (ΔNLS: 8.58±1.09%; n=27) transgenic salivary gland nuclei (P=0.0045) (Z). Scatterplot with median ± IQR shown. Quantification of the number of Coilin puncta in *wash*$^{\Delta 185}$ P{GFP-Wash^{WT}} (WT: 1.04±0.04; n=26) or *wash*$^{\Delta 185}$ P{GFP-Wash$^{+NES\Delta NLS}$} (ΔNLS: 2.38±0.40; n=24) transgenic salivary gland nuclei (P=0.0028) (CC). Quantification of average dispersion of

Coilin puncta in *wash*$^{\Delta 185}$ P{GFP-Wash^{WT}} (WT: 0.04±0.04; n=26) or *wash*$^{\Delta 185}$ P{GFP-Wash$^{+NES\Delta NLS}$} (ΔNLS: 0.69±0.14; n=24) transgenic salivary gland nuclei (P=0.0001) (DD). Boxplot graphs show the median and 25% and 75% percentile measures. The whiskers indicate variability outside the upper and lower quartiles. See also Figure S1 and Movie S1.

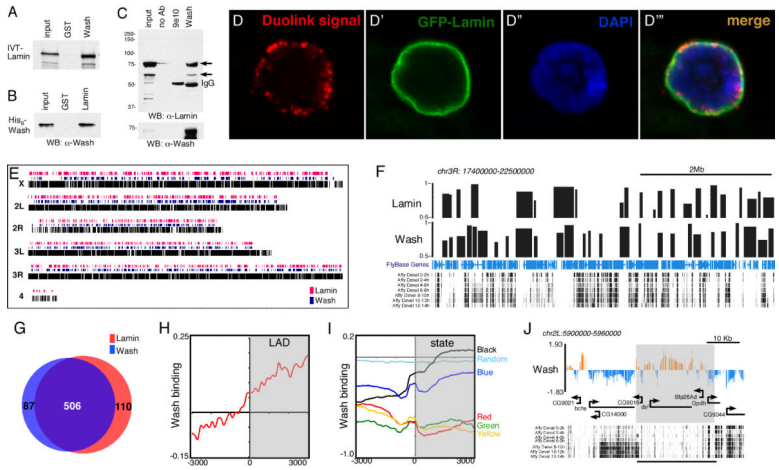

Figure 3.

Wash interacts with lamin and associates with LADs. (A-B) Wash interacts with Lamin directly by GST-pulldown assays using IVT (A) or bacterially expressed proteins (B). (C) Wash and Lamin interact *in vivo*. Western blot of immunoprecipitations from embryo nuclear extracts with Wash, no primary antibody, or with an unrelated antibody (9e10). (D) Duolink Proximity Ligation Assay performed with antibodies recognizing Wash and GFP in GFP-Lamin expressing salivary glands. Duolink signal is only observed if the two antibodies are within 30 nm. (E) Genome-wide chromatin profile of Lamin (red) and Wash (blue) bound regions. (F) Chromosome-3R region aligned to developmental expression showing a significant overlap of LADs with Wash-associated regions at transcriptionally silent regions. Track with squished view of genes is shown in blue. (G) Venn-diagram comparing LADs to Wash associated chromatin regions ($P<1\times10^{-6}$). (H) LADs were aligned at their ends (+/− 3 Kb) and the normalized DamID probe signals were averaged in 150-bp bins for Wash occupancy. (I) DamID-based 5 color chromatin states and random sequences were aligned at their ends and the normalized DamID probe signals were averaged in 150-bp bins for Wash occupancy. YELLOW and RED chromatin contain proteins and histone modifications characteristic of active chromatin. BLUE chromatin contains H3K27me3 and PcG proteins, GREEN chromatin contains HP1 and Su(var)3-9, and BLACK chromatin contains Lamin and histone H1. (J) Wash chromatin profile on a 60Kb region of chromosome 2L showing an inverse correlation with developmental gene expression (similar results were obtained with the RNAseq data for Kc167 cells). See also Figure S2.

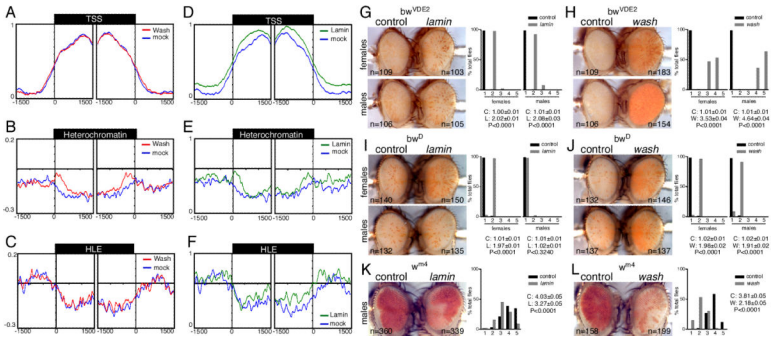

Figure 4.

Wash and Lamin affect chromatin accessibility at heterochromatic regions and Position Effect Variegation. (A-F) Distribution of M.SssI-based chromatin accessibility around active promoter, constitutive heterochromatin and heterochromatin-like euchromatin (HLE) regions. modENCODE Consortium generated chromatin states were aligned at their 5' and 3' (+/− 1.5 Kb) and the normalized probe signals were averaged in 50-bp bins. The y-axis in each plot represents the relative enrichment of M.SssI-methylated DNA for mock and Wash knockdown (A-C) and for mock and Lamin knockdown (D-F) in S2R+ cells. Statistical significance (determined using the two-sample KS test): TSS: Wash, P=0.823, Lamin $P<4\times10^{-3}$; Heterochromatin: Wash, $P<2\times10^{-3}$, Lamin $P<4\times10^{-3}$; HLE: Wash, P= 0.256, Lamin $P<2\times10^{-3}$. (G-J) *wash* and *lamin* suppress *brown*-mediated PEV. Wash and Lamin mediate suppression of the classical PEV allele bw^{VDE2}, a chromosomal rearrangement that juxtaposes the *bw* gene near 2R heterochromatin (G-H). *wash* (male & female) and *lamin* (female only) mediate suppression of the non-classical PEV allele bw^{D}, which inserts heterochromatin into the *bw* gene and silences the homolog (I-J). (K-L) *wash* and *lamin* enhance *white* mediated centromeric PEV (w^{+} gene inserted in proximal X chromosome heterochromatin). Bar plots showing percentage of flies falling into each expression quintile (flies sorted into one of five bins based on the percentage of ommatidia expressing the w^{+} marker or *bw* gene (bin 1 = 0-20% to bin 5 = 80-100%). The median±SEM and P-values are given in each panel. Eyes shown are representative of the average phenotype for each genotype and each pair is an age-matched, sibling pair (G-L). The number of eyes scored (N) is given beside each eye. See also Figures S3 and S4.