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Chloe Friedman April, 15, 2015

Interactions between *domino* and other chromatin protein encoding loci

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Biology

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Abstract
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The Notch signaling pathway plays an integral role in cell signaling. Our lab previously determined genetic interactions between *Drosophila* Notch and a co-activator known as Mastermind (Mam). Our lab also screened for and identified interactions between Mam and transposon-generated random genomic insertions. The screen found loss of function (LOF) *domino*, which encodes a chromatin remodeling protein, strongly enhanced the *Mam* wing phenotype. The lab then constructed a recombinant chromosome containing a Gal4 driver and UAS driven *RNAi* constructs directed against *domino* (*C96-domR*). This chromosome leads to a dominant, partially-penetrant wing nicking phenotype. Domino plays a role in hematopoiesis, cell growth and proliferation, apoptosis, histone exchange during DNA repair, maintenance of stem cells, and autophagy. In this study, we focus on the wider gene network of *domino*. We attempt a rational approach, by testing other chromatin protein encoding loci identified by Van Bemmel *et al.* (2013). With the use of the DNA adenine methyltransferase identification (DamID) system, Van Bemmel *et al.* (2013) identified 42 proteins that had yet to be associated with chromatin, and 70 previously known chromatin proteins. We performed a genetic screen using RNA*i* against 30 of the Van Bemmel loci for modifiers of LOF *domino*. Using a wing-nicking assay, we found 25/30 Van Bemmel loci significantly enhanced or suppressed the *C96-domR* phenotype. Additionally, we found a subset of Van Bemmel loci genetically interact with GOF *domino*. Also, several of the Van Bemmel loci modify LOF *domino* in the eye. Since our lab recently found LOF *domino* leads to cell death along the wing margin, we investigated whether the modification in the *C96-domR* wing nicking phenotype was due to a change in cell death. We found that RNA*i* against Van Bemmel locus, *Caf-1,* synergistically increases cell death with LOF *domino.* The present study validates *C96-domR* is an excellent tool for assaying chromatin function. Further, since the majority of the modifiers in our screen are associated with active chromatin, our study illuminates Domino's role as a gene activator. We confirm that Domino is a protein with pleiotropic functions and widen the existing understanding of Domino's interaction with other chromatin-associated proteins.

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Acknowledgements

I would like to gratefully acknowledge Barry Yedvobnick, Ph.D. for his time, guidance, and support throughout my thesis project. I appreciate his dedication to teaching me about biology, proper experimental design, scientific writing, and presentation techniques.

I would like to thank Kaitlyn Ellis, who has also helped me in many respects during my project.

I am also grateful to my committee members, David Katz, Ph.D. and Roger Deal, Ph.D. Thank you for your valuable comments and support during my project.

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Introduction:

Signaling systems comprise an important mechanism by which cells regulate numerous activities. The Notch signaling pathway is one of the highly conserved, short-range pathways mediating intercellular communication (reviewed by Artavanis-Tsakonas *et al.,* 1999). The quantity of Notch receptors varies between organisms; for example, *Drosophila melanogaster* has one receptor (facilitating genetic studies), whereas mammals have four types (Lai, 2004). The Notch receptor is a large single pass, type I transmembrane protein (Kidd *et al.,* 1986). Its ligands, Delta and Serrate, are also transmembrane proteins (Lai, 2004). Ultimately, the Notch cascade is required for regulation of the expression of multiple genes during development and adult life.

In *Drosophila* development, Notch is involved in specifying cell fate (most notably segregating future neural and epidermal cells), constructing boundaries between two adjacent cell populations, and directing somitogenesis, among many other examples (De La Pompa *et al*., 1997; Conlon and Reaume, 1995; Lai; 2004). Furthermore, in humans, abnormal Notch signaling has been found to be involved in cancer, inherited disorders, cardiac disease, and many other abnormalities (Weng *et al.,* 2004; McDaniell *et al.*, 2006, Lai, 2004; Costa *et al*., 2005; Fischer *et al.,* 2008). Increased Notch signaling is found in 55 to 60% of all T-acute lymphoblastic leukemia/lymphoma (T-ALL) cases (Weng *et al.*, 2004). Notch 1 signaling is required for hypoxia induced cell proliferation, invasion, and chemoresistance in T-ALL cells (Zou *et al.*, 2013). The importance of the Notch pathway has also been demonstrated in cardiac regeneration following injury and cardiomyocyte

proliferation in zebrafish (Zhao *et al.,* 2014). Additionally, components of the Notch signaling pathway are considered possible drug targets. For example, it has been shown in platinum resistant ovarian cancers, inhibition of the Notch pathway with a γ-secretase inhibitor reduces cancer growth (Groeneweg *et al.,* 2014). Additional research is needed, in order to gain a more complete understanding of Notch's role in disease.

The Notch signaling cascade begins with the binding of the Delta ligand and the Notch receptor (Lai, 2004). Following this interaction, there are two proteolytic cleavages of the Notch receptor, resulting in the relocation of the Notch intracellular domain (NICD) (reviewed by Bray, 2006). Once released, the NICD moves into the nucleus and associates with the CSL-family of transcription factors and a co-activator, known as Mastermind (Mam) (Kitagawa *et al.*, 2001). The CSL and Mam components, along with various chromatin-remodeling proteins, including histone acetyltransferases (HATs), and histone deacetylases (HDACs), regulate transcription of specific Notch target genes (reviewed by Baron *et al.,* 2002; Kitagawa *et al.*, 2001). While this pathway is comprised of different proteins in different species, the basic principles of the signaling cascade are quite similar.

In *Drosophila*, Notch signaling plays important roles in formation of imaginal tissues, particularly the wing (Rulifson and Blair, 1995; Shellenbarger and Mohler, 1975; Kim *et al.*, 1996; de Celis and Bray, 1997; Micchelli *et al.,* 1997; Fleming *et al.,* 1997). Furthermore, Notch is involved in vein morphogenesis and dorsal-ventral wing compartment determination (Rulifson and Blair, 1995; de Celis, 1997; Fleming *et al.,* 1997). In the wing margin, Notch positively regulates genes that lead to

differentiation and growth, such as *wingless*, *cut,* and *vestigial* (Helms *et al.,* 1999). During wing patterning, Notch and its ligands are subject to feedback regulation that results in disproportionate distribution of the proteins in neighboring cells and thus limits the amount of Notch signaling in those cells (de Celis and Bray, 1997).

The NICD works in conjunction with Mastermind to associate with specific chromosome sites and RNA Polymerase II during development (Fortini and Artavanis-Tsakonas, 1994, Bettler *et al.,* 1996).

Previous experiments in Dr. Barry Yedvobnick's lab have shown that targeted expression of a truncated version of the Mastermind protein leads to severe wing defects, bristle loss, wing vein thickening, and wing nicking, all of which are typical in loss of function Notch mutations (Helms *et al.,* 1999). These experiments were performed using Gal4-UAS driven expression of the truncated version of *Mam* across the wing margin (Phelps and Brand, 1998) (See Materials and Methods). These results expanded Mastermind's role in the Notch signaling cascade. Additionally, the Yedvobnick lab and collaborators searched for new components of the Notch pathway using genetic modifier screens (Hall *et al.,* 2004; Kankel *et al.,* 2007). Coexpression of *UAS-truncated Mam* along with mutations in other Notch pathway genes, such as *N*, *dx*, and *Su(H*) show enhanced phenotypes, confirming Mam's key role in Notch signaling and establishing Gal4 driven expression of truncated *Mam* as a tool for genetic screening (Brand and Campos-Ortega, 1990; Helms *et al.,* 1999; Xu and Artavanis-Tsakonas*,* 1990; Fortini and Artavanis-Tsakonas, 1994).

The Yedvobnick lab screened for genetic modifiers of truncated *Mam* using random transposon insertions (Hall *et al.,* 2004). Transposons can either lead to overexpression (via *Gal4-UAS*) or knockdown at their site of insertion, and they are therefore an excellent resource for genetic screening. *Drosophila* wing phenotypes associated with *Gal4-UAS* driven expression of both truncated *Mam* and the random transposons sites were scored for modifications, in an effort to identify possible enhancing or suppressing effects. One of the interesting protein-encoding loci found in this experiment was *domino* (Hall *et al.,* 2004; Ruhf *et al.,* 2001). The cross with truncated *Mam* and the *domino* insert showed a strongly enhanced phenotype (Hall *et al*., 2004). Thus, it was determined that *domino* is a genetic modifier of the Notch pathway (Hall *et al.,* 2004). Other studies have also linked Domino to the Notch signaling pathway (Gause *et al.,* 2006; Eissenberg *et al.*, 2005).

Domino, a member of the SWI2/SNF2 class of DNA ATPases, regulates by chromatin modification and nucleosome remodeling (Eisen *et al.,* 1995; Ruhf *et al.,* 2001). There are two homologs of Domino in mammals, SRCAP and p400 (Eissenberg *et al.,* 2005). SRCAP related proteins have been found in organisms ranging from humans, to fish, to flies and yeast, demonstrating it is highly conserved (Johnston *et al.,* 1999; Ruhf *et al.*, 2001; Kobor *et al.*, 2004). In *Drosophila*, there are two isoforms of the protein, known as DOM-A (3202 amino acids) and DOM-B (2498 amino acids) (Ruhf *et al.,* 2001) (Figure 1). *DominoA* and *dominoB* contain 14 and 11 exons respectively, and alternative splicing at exon 11 results in the two isoforms (Ruhf *et al.,* 2001). The expression of DOM-A is limited to the nervous system, including areas of the brain, sensory organs, and some imaginal discs during

development (Ruhf *et al.,* 2001). Expression of DOM-B is more widespread, both throughout development and post embryogenesis, in the brain, imaginal discs, lymph and salivary glands (Ruhf *et al.,* 2001). DOM-B is also expressed in the ovaries, follicle cells, nurse cells, and oocytes in adults (Ruhf *et al*., 2001). Each protein is incorporated into a larger complex, which is present in the nucleus (Ruhf *et al.,* 2001).

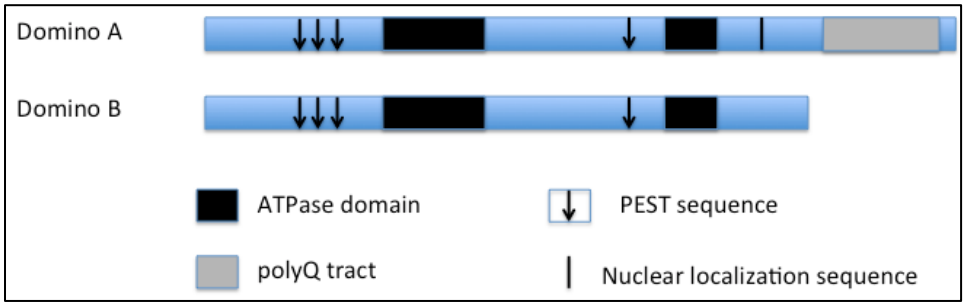

Figure 1: The two isoforms of Domino, DOM-A and DOM-B, and their domains (taken from Ruhf *et al.,* 2001). DOM-A and DOM-B also contain an HSA domain, and DOM-A contains a SANT domain (Eissenberg *et al.*, 2004)

Domino has been associated with hematopoiesis, cell growth and proliferation, histone exchange during DNA repair, apoptosis, maintenance of stem cells, and autophagy (Braun *et al.,* 1997; Ruhf *et al.,* 2001; Lu *et al.*, 2007; Kusch *et al.,* 2004; Prado *et al.,* 2013; Xi and Xie, 2005; Kwon *et al.,* 2013).

In *Drosophila*, Domino has been found to play diverse roles in cell proliferation in hemocytes, neuroblasts, and germline cells (Braun *et al.*, 1997; Ruhf *et al.,* 2001). In flies homozygous for a LOF *domino* mutation, third instar larvae lack imaginal discs and their lymph glands are melanized, an effect due to increased cell death (Braun *et al.,* 1997; Ruhf *et al.,* 2001). Interestingly, other *domino* mutations have been shown to cause lymph gland overgrowth, indicating excess cellular

proliferation (Ruhf *et al.,* 2001). Domino also contributes to cell proliferation through its role in cell viability (Ruhf *et al.,* 2001). Mutations in *domino* have been shown to lead to the arrest of oogenesis in the ovary, and thus lead to female sterility (Ruhf *et al.,* 2001).

Domino further influences cell proliferation through its genetic interaction with the E2F pathway (Lu *et al.,* 2007). E2F family transcription factors regulate proteins involved in both the G1/S phase transition and the activation of DNA replication (reviewed by Nevins, 1998). Domino is recruited to E2F promoters and acts as a negative E2F regulator (Lu *et al.*, 2007). These researchers found that LOF *domino* is sufficient to suppress mutant eye development, characteristic of reduced cyclin E expression, further illustrating Domino's role in cell proliferation (Lu *et al.,* 2007). However, the study also found that depressed expression of *dom* fails to rescue depressed expression of S-phase genes, such as cyclin E (Lu *et al.,* 2007). These complex findings reveal the incomplete understanding of Domino's multifaceted roles in the cell. Regardless, the genetic interaction between *domino* and the E2F pathway highlights the protein's importance in the regulation of cellular proliferation.

Domino is also linked to cell growth through its genetic modifier, *LK6* (Kwon *et al.,* 2013). Lk6 is a kinase responsible for the phosphorylation of eIF4E, a translation initiation factor in *Drosophila* (Arquier *et al.,* 2005). LOF *Lk6* mutations lead to decreased body size and slower development, implicating Lk6's role in cell growth (Arquier *et al.,* 2005). Our lab previously found that LOF *Lk6* mutations enhanced the LOF *domino* wing nicking phenotype (Kwon *et al.,* 2013). This result is

consistent with observations regarding Lk6 and Domino's roles in regulating cell growth.

In addition to regulation of cell growth and proliferation, Domino has been associated with gene silencing through genetic assays on Polycomb (PcG) and Trithorax (TrxG) group mutations. PcG and TrxG are groups of antagonistic homeotic regulators, which are key proteins involved in development (reviewed by Simon, 1995). Since Domino's DNA dependent ATPase domain is homologous to that of another protein known as Brahma (BRM) of the TrxG group, it was hypothesized that Domino may effect homeotic regulators similarly (Tamkun *et al*., 1992; Ruhf *et al.,* 2001). This research demonstrated that Domino has a repressive effect on homeotic genes (Ruhf *et al.,* 2001). Thus, *dom* products act as global transcriptional regulators (Ruhf *et al.,* 2001).

Domino's diverse roles extend to histone exchange during DNA repair (Kusch *et al.,* 2004). At double stranded breaks, many histones, including the H2 variants, H2AX (mammalian) and His2A.V (*Drosophila*), become phosphorylated (Redon *et al.,* 2002). This phosphorylation may act as a marker to attract chromatin-remodeling complexes (reviewed by Allard *et al.,* 2004). Domino associates with the Tip60 histone acetylase complex, which acts as a helicase and an ATPase and has been found to play a role in nucleosome acetylation, DNA repair, and apoptosis (Kusch *et al.,* 2004; Ikura *et al*., 2000). As a part of the dTip60 complex, Domino, along with a histone acetyltransferase, catalyzes the exchange of phospho-His2Av with unmodified His2Av (Kusch *et al.,* 2004). As a part of the multi-functional Tip60

complex, Domino aids in the maintenance of chromatin stability and possibly the regulation of the apoptosis pathway.

More recently, the understanding of Domino's role as a part of the Tip60 complex has been expanded through its interaction with a Bucentaur protein, Yeti (Messina *et. al.,* 2014). Prior to this study, Yeti was known for interacting with microtubule-based motor kinesin-1 (Wisniewski *et al.,* 2003). However, Messina *et al*. (2014) found that in *domino* mutants, Yeti enters the nucleus, but is not deposited onto chromatin. Thus, DOM-A is responsible for recruiting Yeti to chromatin (Messina *et al.*, 2014). Since *Yeti* mutants have reduced chromosomal levels of H2A.V, the study also determined that Yeti plays a role in chromosomal accumulation of H2A.V in polytene chromosomes in *Drosophila* salivary glands (Messina *et al.,* 2014). While the exact nature of the interaction between Yeti and the H2A.V exchanging machinery remains unknown, it is thought that it may be a chaperone or a new subunit of the Tip60 complex (Messina *et. al.,* 2014). Domino's role in Yeti recruitment further contributes its importance in histone exchange as a part of the Tip60 complex.

Domino’s association with His2A.V has also been linked to the maintenance of adult germline (GSC) and somatic cyst stem cells (CySC) in *Drosophila* testis and ovaries (Prado *et al.,* 2013; Xi and Xie, 2005). The presence His2A.V is required for maintenance of stem cells. Research shows that LOF *domino* GSCs have decreased levels of His2A.V in their chromatin (Prado *et al.,* 2013). Thus, Domino appears to be required to localize His2A.V in chromatin in the stem cells of the testis (Prado *et al.,* 2013).

Recently, our lab has linked domino with autophagy (Kwon *et al.,* 2013). Kwon *et al.* (2013) constructed a recombinant chromosome that contains a Gal4 driver and UAS driven RNA*i* constructs directed against *domino*. This chromosome leads to a dominant and partially-penetrant wing nicking phenotype (hereafter the chromosome is referred to as *C96-domR*, see Materials and Methods) (Figure 2). In crosses between *C96-domR* flies and 10 different autophagy pathway RNA*i* strains, the offspring exhibited enhanced or suppressed *C96-domR* wing nicking phenotypes (Kwon *et al.,* 2013).

One genetic modifier is *atg1* (Kwon *et al*., 2013). Depressed *atg1* suppresses the *C96-domR* phenotype, whereas overexpression of *atg1* acts as an enhancer (Kwon *et al.,* 2013). Atg1 has been found to play a role in deactivating S6K, a kinase that promotes cell growth (Lee *et al.,* 2007). In nutrient rich conditions, Atg1 is inhibited and thus, S6K is active in initiating cell growth (Lee *et al.,* 2007). It appears as though mutations in *atg1* may lead to increased levels of S6K, and as a result, rescue growth along the wing margin in the *C96-domR* mutants (Kwon *et al.,* 2013).

Domino has also been linked to autophagy through Wdb, a protein phosphatase (PP2A) regulatory subunit (Kwon *et al.,* 2013; Banreti *et al.*, 2012). Overexpression of *wdb* suppressed the *C96-domR* wing nicking phenotype, and LOF mutations *wdb* enhanced the phenotype (Kwon *et al.,* 2013). *Wdb* has been found to target Atg proteins and thus, positively regulate the autophagy pathway (Banreti *et al*., 2012). Kwon *et al.* (2013) sought to further determine a genetic interaction between other PP2A subunits, CK1, and *domino*. The protein kinase CK1 was selected, since both PP2A and CK1 are Hedgehog pathway proteins that have been

found to act antagonistically (Jia *et al.* 2009). As expected, mutations in *PP2A* and *CK1* had opposite effects on the *C96-domR* phenotype (Kwon *et al.,* 2013). Given the effects of *CK1* on the *C96-domR* phenotype, as well as the knowledge that the Dom sequence has a recognition sequence for CK1, it seems possible that CK1 may phosphorylate Domino (Kwon *et al.*, 2013).

In addition to its role in the Tip60 complex, Domino has been found to be involved in activating gene transcription through other mechanisms (Ruhf *et al.*, 2001; Eissenberg *et al.*, 2005). Eissenberg *et al.* found that Domino and SRCAP (the human *dom* homolog) co-localized with phosphorylated RNA Polymerase II, indicating that Domino is recruited to active transcription sites (2005). Thus, the authors concluded that Domino has multiple roles, both activating and deactivating, which differ depending on the loci at which it is present (Eissenberg *et al*., 2005).

My project will focus on examining the wider gene network of *domino*. Rather than performing an "unbiased" genetic modifier screen (Kwon *et al.,* 2013), I will attempt a "rational" approach, by testing other chromatin protein encoding loci identified by Van Bemmel *et al.* (2013) for a genetic interaction with LOF *domino*. Because knowledge and understanding of chromatin related proteins remains limited, Van Bemmel *et al.* (2013) worked to identify previously uncharacterized chromatin proteins. With the use of a Bayesian network, they were able to draw connections between these new and known chromatin associated proteins and to infer information regarding the functions of the newly identified proteins (Van Bemmel *et al.,* 2013). Van Bemmel *et al.* (2013) were thus able to predict 155 interactions among the 112 chromatin regulators.

Van Bemmel *et al.* (2013) screened 112 proteins and identified 42 proteins that had not been associated with chromatin before, referred to as chromatin component loci (*CC*), and 70 proteins that had been previously associated with chromatin. The 112 proteins were considered chromatin-associated proteins by the use of a technique known as DNA adenine methyltransferase identification (DamID) (Van Bemmel *et al.,* 2013). DamID works by introducing a fusion protein, composed of Dam from *Escherichia coli* and a chromatin protein of interest (van Steensel and Henikoff, 2000). The fusion protein then targets the chromatin-binding site of the selected protein and directs methylation of the DNA. (van Steensel and Henikoff, 2000). This methylation acts as a tag that can be detected in polymerase chain reaction (PCR) or southern blots (van Steensel and Henikoff, 2000).

Van Bemmel *et al.* (2013) selected 112 proteins by 4-tiered criteria: the proteins' availability, expression patterns, increased probability of association with chromatin (containing a domain common to chromatin proteins, like a zinc finger) and lack of prior evidence connecting them to chromatin. Of these 112 candidates, 79 showed detectable methylation when fused to Dam, and 42 of these exhibited quality reproducibility and were ultimately classified as new chromatin proteins (Van Bemmel *et al.*, 2013). Additionally, Van Bemmel *et al.* (2013) constructed a Bayesian network, which enabled them to classify the chromatin proteins into 4 categories including: DNA replication, DNA repair, nuclear pore components, and histone modifications. The functions of the newly identified *CC* loci were inferred based on their proximity to the previously known chromatin proteins (Van Bemmel *et al.,* 2013).

Additionally, Van Bemmel *et al.* (2013) further classified these proteins using previously established color-coded chromatin types by Filion *et al*. (2010). Filion *et al.* (2010) observed the binding patterns of 53 chromatin proteins and characterized 5 types of chromatin based on the profile of the proteins that bound at select sites. The color-coded categories include: Black, which is poorly characterized repressive chromatin, Green, is heterochromatin that is most prominent in pericentric regions, Blue, is polycomb chromatin, Red, which is transcriptionally active chromatin that is mostly comprised of tissue specific chromatin, and Yellow, which is also transcriptionally active chromatin, but usually has a broader expression pattern than Red (Filion *et al*., 2010). The Red and Yellow groups are similar in that they are both euchromatin that produce high levels of mRNA (Filion *et al.,* 2010). However, Filion *et al.* (2010) found that Red chromatin is replicated earlier than yellow, and thus it is thought that DNA replication is initiated in Red chromatin. Additionally, Yellow genes carry H3K36me3, and Red genes do not, Yellow chromatin is linked to more universal cellular processes, and Red is associated with more specific functions (Filion *et al.* 2010). In this study, we use 30 of these chromatin protein loci, for which RNA*i* strains are available, in order to perform loss of function genetic assays with *domino*.

We found 25 of 30 Van Bemmel *et al.* (2013) loci (hereafter referred to as VB) suppressed or enhanced wing nicking, thus signifying LOF *domino* is important for a wide range of chromatin functions. We also show that several of the VB loci genetically interact with gain of function (GOF) *domino*. Additionally, we found that the VB loci also modify LOF *domino* in the eye. Finally, in caspase stainings (a cell

death marker), we found that 34069 (Caf-1) RNA*i* synergistically elicits increased cell death along the wing margin with LOF *domino*. For those VB strains that show phenotypes when crossed to *C96 Gal4* and synergistic enhancement when crossed to *C96-domR*, recombinant chromosomes, *C96-VB loci*, were established.

Materials and Methods:

(i) Construction of *C96-domR* chromosome (prior to this project)

The Yedvobnick lab previously constructed a chromosome (*C96-domR),* which uses the *Gal4-UAS* system (Kwon *et al.,* 2013). This system works via a promoter that drives expression of the Gal4- yeast transcriptional activator, which then activates expression of the *UAS* target gene (in this case, *dom* RNA*i*) (Phelps and Brand, 1998). This system allows for high levels of expression of the selected gene (Phelps and Brand, 1998). The *C96-domR* chromosome contains both the *C96 Gal4* wing margin driver and *UAS dom* RNA*i* transgenes. This chromosome creates a partially penetrant wing nicking phenotype through LOF for *dom* (Kwon *et al.,* 2013).

(ii) Genetic crosses

Female virgin flies with the *C96-domR* chromosome were crossed with males carrying *UAS*-RNA*i* transgenes directed against one of the VB loci. The *C96-Gal4* element drives the expression of both *dom* RNA*i* and RNA*i* against each of the Van Bemmel strains (Figure 2). Flies carrying RNA*i* against the VB loci were obtained from the Bloomington Stock Center. Stock numbers for the VB strains included:

32888, 33361, 33666, 33734, 33974, 34978, 40853, 41937, 42491, 42514, 25993, 26234, 26772, 27085, 31940, 29360, 33394, 31922, 31921, 26231, 33043, 33981, 34580, 33962, 55250, 53697, 34069, 31960, 33725, and 55314.

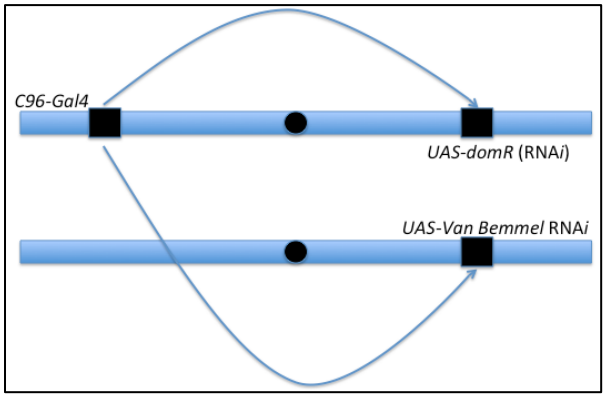

Figure 2: In the test genotype used in this screen, *C96-Gal4* drives the expression of both *UAS-domR* (RNA*i*) and *UAS-Van Bemmel* RNAi

A cross between *C96-domR* and the w^{1118} strain was used as a control. This cross was used to establish the wing nicking penetrance by the Gal4 driven expression of the RNA*i* against *domino*. *C96-Gal4* flies crossed with flies carrying *UAS*-RNA*i* for the VB loci also served as a control. These crosses determine the potential LOF effect of the VB loci without interaction with *dom*. Offspring from all test crosses (Figure 2) were scored for penetrance and severity of wing nicking relative to both control crosses.

The percent of wings exhibiting one or more wing nicks was recorded for experimental and control genotypes. The percentages were normalized in each experiment to the w^{1118} value of 57% or 26%. Crosses with a significantly higher percentage of nicks than the control value were considered enhancers, and strains with a lower percentage were considered suppressors. P-values were obtained from a X^2 test.

Crosses between flies containing *C96 Gal4 + UAS-DomB* and flies with *UAS-*RNA*i* for the VB loci were also performed. *DomB* codes for the normal Domino B protein, which is highly expressed in imaginal discs, and *UAS* driven expression of it along the wing margin has been shown to rescue the *C96-domR* wing nicking phenotype (Ruhf *et al.,* 2001; Kwon *et al.*, 2013). This cross was used to determine if overexpression of *domino* modified the phenotypic effects of LOF for VB loci. Crosses between virgin females with *C96-Gal4* and males with each of the VB RNA*i* strains served as controls. Offspring from all crosses were scored for modification in penetrance and severity of wing nicking. The percentage of wings exhibiting one or more wing nicks was recorded for experimental and control genotypes. P values were calculated using X^2.

We also examined the effects of VB modifiers in the eye. We used Gal4 driven expression of Glass Multimer Reporter (GMR) *domino RNAi* in the eye. Crosses between virgin females containing the recombinant chromosome *GMR-domIR/Sb* and males containing RNA*i* against VB loci were performed. A cross between *GMR-domIR* and w^{1118} was used as a control. Eyes of offspring were observed for roughness, compared to the control eye phenotype, and classified as either enhancers or suppressors.

(iii) Mounting of wings

Wings representative of the average severity of wing nicking for each of the VB RNA*i* strains were mounted onto a slide with Euparol and photographed using a

light microscope (Hall *et al*., 2004). The photographs were put in gray scale and sharpened using Adobe Photoshop.

(iv) Generation of VB loci recombinant chromosomes

Males containing RNA*i* against one of the VB loci were crossed with *C96-Gal4* virgin females. The F1 virgin females were then crossed to w^{1118} males. Wings of male offspring were scored for nicks, curling, and various other defects, indicating the recombination, *C96-VB loci/*w^{1118} on chromosome 3. Finally, positive *C96-VB loci* males were crossed with *Sb/Hu* female virgins, and positive male and female offspring were selected. Thus, we obtained a balanced, *C96-VB loci* strain.

(v) Wing disc stainings

Wing imaginal discs were dissected, stained, and visualized with confocal microscopy by Kaitlyn Ellis, as described in Moberg *et al.* (2005).

Results:

a) *LOF domino exhibits genetic interactions with loss of function for several VB chromatin loci*

In an effort to determine potential genetic interactions between *domino* and the Van Bemmel chromatin loci, we performed a genetic screen of 30 Van Bemmel strains for which RNA*i* was available. Figures 3 and 4 show representative wings from offspring of the crosses between *C96-domR* and VB RNA*i* strains. The w^{1118}

wing (Figures 3a and 4a) does not have any nicks, whereas the *C96-domR x* w^{1118} wing (Figures 3b and 4b) shows the typical *C96-domR* nicking phenotype.

Because the *C96-domR* strain picked up a genetic background modifier, resulting in weaker wing nicking penetrance, the data is divided into two groups, with 20 VB strains in the first, and 10 in the second. The *C96-domR* x w^{1118} wing nicking penetrance in Set 1 was 57% (Figure 3, Tables 1, 3, and 5) and that of Set 2 was 26% (Figure 4, Tables 2, 4, and 6). Offspring resulting from crosses between *C96-domR* and 3 of the VB strains were uniformly smaller in size as compared to the controls (Figure 3 c, f, and h).

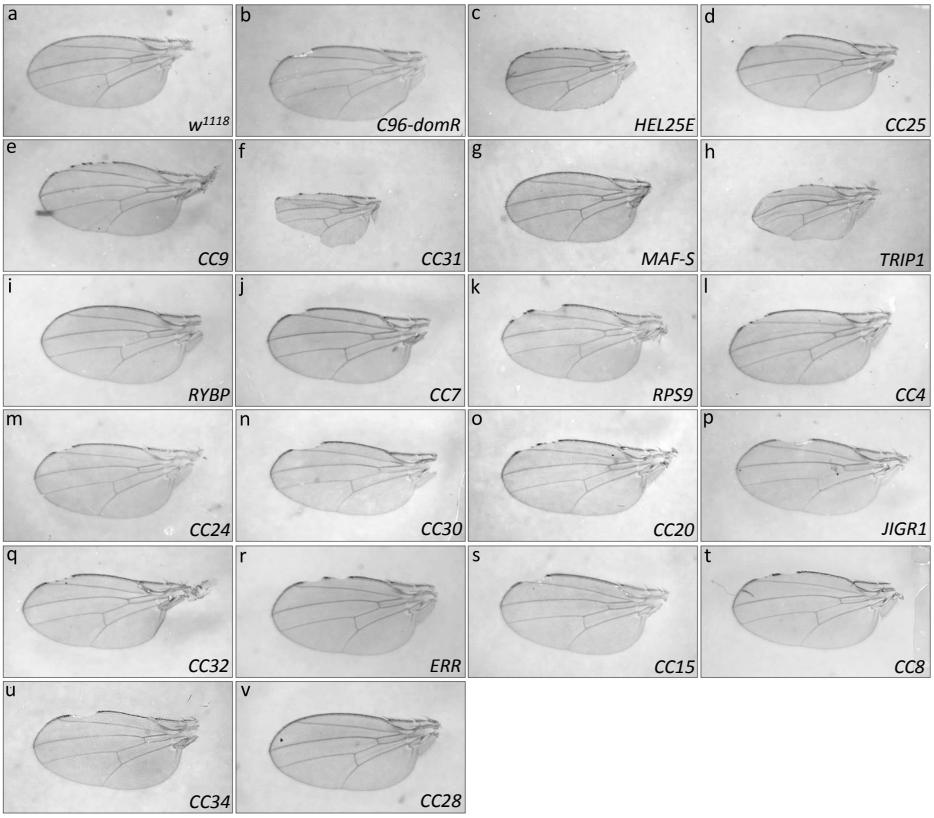

Figure 3: Wing nicking phenotypes for two controls and 20 Van Bemmel *et al*. (2013) strains in Set 1. All wings are from representative males and were imaged at the same magnification. Significantly enhanced nicking frequency was seen in wings shown in panels d, e, f, h, j, k, l, m, o, p, s, and t (Tables 1 and 3).

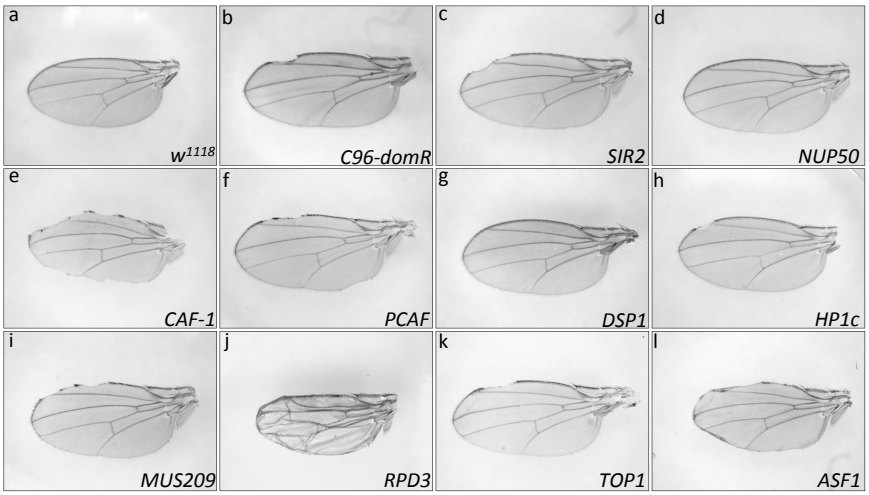

Figure 4: Wing nicking phenotypes for two controls and 10 Van Bemmel *et al*. (2013) strains in Set 2. All wings are from representative males and were imaged at the same magnification. Significantly enhanced nicking frequency was seen in wings shown in panels c, d, e, f, h, i, j, k, and l (Tables 2 and 4).

Additionally, we characterized the genetic interaction between *domino* and the VB loci objectively by scoring wings of the offspring for frequency of wing nicking (See Materials and Methods). Out of the 30 VB loci we tested, 25 of the strains have a significant effect on the *C96-domR* phenotype (Tables 1, 2, 3 and 4) and 5 strains have no effect (Tables 5 and 6). Of the 25 modifiers, 20 are enhancers and 5 are suppressors. We further classified the VB loci based on their effect (or lack thereof) in the *C96-Gal4* control cross. Tables 1 and 2 show the results for the 16 Van Bemmel strains that either enhanced or suppressed the *C96-domR* phenotype and did not have an effect in genotypes with *C96-Gal4* alone.

Strain	Gene Name	Chromatin Type	*C96-domR* Percentage Nicks (Number of Wings Scored)	P value
w^{1118}	N/A	N/A	57.0 (2,145)	N/A
26234 (E)	*CC8*	Red	96.0 (296)	0.0001
31921 (E)	*JIGR1*	Red	81.7 (530)	0.0001
42514 (E)	*CC25*	Green	80.7 (478)	0.0001
31922 (E)	*CC20*	Red	78.8 (286)	0.0001
33394 (E)	*RPS9*	Red	70.9 (270)	0.0001
32888 (E)	*CC24*	Red	70.6 (469)	0.0001
33361 (E)	*CC4*	Green	70.4 (503)	0.0001
33734 (E)	*CC7*	Red	68.8 (492)	0.0001
42491 (E)	*CC9*	Red	67.3 (462)	0.0001
26772 (E)	*CC15*	Red/Yellow	66.3 (695)	0.0001
40853 (S)	*MAF-S*	Red	30 (473)	0.0001
33974 (S)	*RYBP*	Red	29.8 (318)	0.0001
25993 (S)	*CC28*	Yellow	28.2 (360)	0.0001

Table 1: The strain number, gene name and chromatin type (as designated by Van Bemmel *et al.*, 2013), the frequency of nicks as a percentage adjusted to the 57% control, and the number of wings scored. P values were calculated using the X^2 test. Strains are marked with (E) and (S) for enhancer or suppressor, respectively.

Strain	Gene Name	Chromatin Type	*C96-domR* Percentage Nicks (Number of Wings Scored)	P Value
w^{1118}	N/A	N/A	26.0 (2,050)	N/A
33981 (E)	*PCAF*	Red	88.3 (747)	0.0001
33962 (E)	*HP1c*	Green	55.3 (511)	0.0001
34580 (S)	*NUP50*	Red	7.7 (672)	0.0001

Table 2: The strain number, gene name and chromatin type (as designated by Van Bemmel *et al.*, 2013), the frequency of nicks as a percentage adjusted to the 26% control, and the number of wings scored. P values were calculated using the X^2 test. Strains are marked with (E) and (S) for enhancer or suppressor, respectively.

Tables 3 and 4 summarize the results for the 9 strains that had an effect with *C96-Gal4* alone, of which 7 showed modification with *C96-domR*.

Strain	Gene Name	Chromatin Type	*C96-Gal4* Percentage Nicks (Number of Wings Scored)	*C96-domR* Percentage Nicks (Number of Wings Scored)	P value
w^{1118}	N/A	N/A	N/A	57.0	N/A
34978 (E)	*TRIP1*	Red	Lethal	100 (26)	0.0001
41937 (E?)	*CC31*	Red/Yellow	100 (194)	100 (44)	0.0001
33666* (NE)	*HEL25E*	Red	100 (4)	100 (4)	0.0824

Table 3: The strain number, the gene name and chromatin type (as designated by Van Bemmel *et al.*, 2013), the frequency of nicks in the *C96-Gal4* control cross as a percentage, the number of wings scored, the frequency of nicks in the *C96-domR* cross as a percentage adjusted to the 57% control, and the number of wings scored. P values were calculated using the X^2 test. Strains are marked with (E) (S), or (NE) for enhancer, suppressor, or no effect, respectively. * denotes a strain that enhanced wing nicking, but had too few offspring to be considered statistically significant

Strain	Gene Name	Chromatin Type	*C96-Gal4* Percentage Nicks (Number of Wings Scored)	*C96-domR* Percentage Nicks (Number of Wings Scored)	P Value
w^{1118}	N/A	N/A	N/A	26.0 (2,050)	N/A
34069 (E)	*CAF1*	Red	10.2 (570)	100 (473)	0.0001
33043 (E)	*MUS209*	Red	0.9 (344)	80.3 (314)	0.0001
53697 (E)	*SIR2*	Red	61.6 (261)	76.7 (487)	0.0001
55250 (E)	*ASF1*	Red	0 (curling) 246	70.6 (260)	0.0001
55314 (E)	*TOP1*	Red	0 (curling) (206)	37.0 (653)	0.0001
33725 (S)	*RPD3*	Red	11.9 (550)	7.1 (339)	0.0001

Table 4: The strain number, the gene name and chromatin type (as designated by Van Bemmel *et al.*, 2013), the frequency of nicks in the *C96-Gal4* control cross as a percentage, the number of wings scored, the frequency of nicks in the *C96-domR* cross as a percentage adjusted to the 26% control, and the number of wings scored. P values were calculated using the X^2 test. Strains are marked with (E) and (S) for enhancer or suppressor, respectively.

The remaining 5 strains appear to have no effect on the *C96-domR* phenotype, nor do they show an effect of *C96-Gal4* alone (Tables 5 and 6).

Strain	Name	Chromatin type	*C96-domR* Percentage Nicks (Number of wings scored)	P value
w^{1118}	N/A	N/A	57.0 (2,145)	N/A
26231 (NE)	*CC34*	Red/Yellow	59.1 (516)	0.3820
27085 (NE)	*ERR*	Red	59.9 (628)	0.1468
31940 (NE)	*CC30*	Red/Yellow	58.0 (184)	0.7658
29360 (NE)	*CC32*	Red/Yellow	52.8 (334)	0.1219

Table 5: The strain number, gene name and chromatin type (as designated by Van Bemmel *et al.*, 2013), the frequency of nicks as a percentage adjusted to the 57% control, and the number of wings scored. P values were calculated using the X^2 test. Strains are marked with (NE) for no effect.

Strain	Name	Chromatin Type	*C96-domR* Percentage Nicks (Number of wings scored)	P value
w^{1118}	N/A	N/A	26.0 (1327)	N/A
31960 (NE)	*DSP1*	Red	24.8 (594)	0.0878

Table 6: The strain number, gene name and chromatin type (as designated by Van Bemmel *et al.*, 2013), the frequency of nicks as a percentage adjusted to the 26% control, and the number of wings scored. P values were calculated using the X^2 test. Strains are marked with (NE) for no effect.

b) *Gain of function domino (via overexpression of dominoB) modifies the phenotypic effects of LOF domino for a subset of positive VB loci*

Since Domino is involved in a wide range of cellular processes and has been found to act as both an activator and suppressor of gene activity, we sought to determine whether overexpression of *dominoB* would also genetically interact with the VB loci (Ruhf *et al.,* 2001; Kusch *et al.*, 2004; Lu *et al.*, 2007). We chose to test

DominoB since prior studies showed that co-expression of this construct rescues the *C96-domR* wing phenotype, whereas no rescue was observed for DominoA (Kwon *et al.,* 2013). We performed the *C96-Gal4 + UAS-domB x VB* RNA*i* alongside a *C96-Gal4 x VB* RNA*i* control cross. The percentage of wing nicks for the experimental and the control crosses are shown in Tables 7 and 8.

Strain	Gene Name	Chromatin Type	*C96-Gal4* Percentage Nicks (Number of Wings Scored)	*C96-Gal4 + UAS-DomB* Percentage Nicks (Number of Wings Scored)	P value
w^{1118}	N/A	N/A	0 (350)	0 (268)	N/A
34069 (E)	*CAF1*	Red	1.4 (286)	23.4 (384)	0.0001
33043 (E)	*MUS209*	Red	1.1 (190)	11.3 (168)	0.0001
53697 (E)	*SIR2*	Red	13.5 (245)	26.5 (196)	0.0001
33725 (S)	*RPD3*	Red	7.1 (267)	46.2 (251)	0.0001

Table 7: The strain number and its effect in the *C96-domR* cross, the gene name (as designated by Van Bemmel *et al.* (2013), the frequency of nicks in the *C96-Gal4* control cross as a percentage, the number of wings scored, the frequency of nicks in the *C96-Gal4* + *UAS-DomB* cross as a percentage, and the number of wings scored. P values were calculated using a X^2.

Strain	Gene Name	Chromatin Type	*C96-Gal4* Percentage Nicks (Number of Wings Scored)	*C96-Gal4 + UAS-DomB* Percentage Nicks (Number of Wings Scored)
w^{1118}	N/A	N/A	0 (350)	0 (268)
34978 (E)	*TRIP1*	Red	Lethal	100 (6)
41937 (E?)	*CC31*	Red/Yellow	100 (198)	100 (232)
55250 (E)	*ASF1*	Red	0 (curling) (260)	1.9 (208)
55314 (E)	*TOP1*	Red	0 (curling) (338)	0 (135)
33666* (NE)	*HEL25E*	Red	0 (274)	2.6 (312)

Table 8: The strain number (effect in the *C96-domR* cross), the gene name (as designated by Van Bemmel *et al.* (2013), the frequency of nicks in the *C96-Gal4* control cross as a percentage, the number of wings scored, the frequency of nicks in the *C96-Gal4* + *UAS-DomB* cross as a percentage, and the number of wings scored. P values could not be calculated for this set of data. * denotes a strain that enhanced wing nicking, but had too few offspring to be considered statistically significant

We observed a significant effect with 6 of the 9 VB strains tested against *dominoB*. Three of the strains that enhanced *C96-domR* exhibited enhancement of the VB nicking effect via *dominoB* expression (34069, 33043, 53697). One strain (33725) had opposite effects with GOF versus LOF *domino*. 33725 had an enhanced wing nicking with *dominoB*, but suppressed wing nicking with *C96-domR*. One VB strain that led to pupal lethality in combination with *C96-Gal4* produced a small number of offspring, all with nicked wings in combination with *dominoB* (34978); this result is similar to that observed with the *C96-domR* cross (Table 3). The last strain (41937) exhibited 100% nicked wings from the *dominoB* cross, matching the results with *C96-Gal4* and *C96-domR* crosses.

d) *LOF domino in the eye also genetically interacts with VB loci*

In crosses between flies with Gal4 driven expression of *domino* RNA*i* (*GMR-domIR*) and RNA*i* against each of the 30 VB loci in the eye, we found that several modified the rough eye phenotype. The *GMR-domIR* x w^{1118} served as a control and was used as a measure of the baseline eye roughness. Since the phenotype is subtle, Table 9 presents data gathered from Chloe Friedman, Kaitlyn Ellis, and Dr. Barry Yedvobnick independently. Our scores agreed for 17/30 of the VB loci. 9/17 are consistent with the effect found in the *C96-domR* crosses in the wing (Table 9). Further experiments are necessary with additional controls (*GMR-domIR x VB loci*) in order to assay if there is enhancement or just additivity. However, together these results indicate that the VB loci genetically interact with LOF *domino* in an additional tissue, thus supporting our results found in the wing.

Strain Number	Gene Name	*GMR-domIR* phenotype (Chloe Friedman)	*GMR-domIR* phenotype (Kaitlyn Ellis)	*GMR-domIR* phenotype (Dr. Barry Yedvobnick)
w^{1118}	N/A	N/A	N/A	N/A
26234 (E)	*CC8*	E	NE	NE
31921 (E)	*JIGR1*	E, slight	NE	E
42514 (E)	*CC25*	NE	NE	E, slight
31922 (E)	***CC20***	E	E, slight	E
33394 (E)	*RPS9*	E	E	NE
32888 (E)	***CC24***	E	E	E
33361 (E)	*CC4*	NE	NE	E
33734 (E)	***CC7***	NE	NE	NE
42491 (E)	***CC9***	NE	NE	NE
26772 (E)	*CC15*	E	E, slight	NE
34978 (E)	***TRIP1***	None of correct genotype	None of correct genotype	None of correct genotype
41937 (E?)	*CC31*	NE	NE	E, slight
33981 (E)	***PCAF***	E, slight	E	E
33962 (E)	***HP1c***	E	E	E, strong
34069 (E)	*CAF1*	E, slight	NE	NE
33043 (E)	***MUS209***	E	E	E, strong
53697 (E)	***SIR2***	E	E	E, strong
55250 (E)	*ASF1*	NE	No score	S
55314 (E)	*TOP1*	NE	S	S
33666* (NE)	*HEL25E*	NE	E	NE
40853 (S)	***MAF-S***	E	E	E, slight
33974 (S)	*RYBP*	E	E, slight	NE
25993 (S)	***CC28***	NE	NE	NE
34580 (S)	*NUP50*	NE	NE	E, slight
33725 (S)	***RPD3***	E	E	E
26231 (NE)	***CC34***	NE	NE	NE
27085 (NE)	***ERR***	NE	NE	NE
31940 (NE)	***CC30***	NE	NE	NE
29360 (NE)	***CC32***	E	E	E
31960 (NE)	***DSP1***	E	E	E

Table 9: Strain number (effect on the *C96-domR* phenotype), gene name (as designated by Van Bemmel *et al.* (2013)), *GMR-domIR* phenotype as scored by Chloe Friedman, Kailtyn Ellis, and Dr. Barry Yedvobnick independently. Strains for which all 3 scores agreed are in bold. * denotes a strain that enhanced wing nicking in the *C96-domR* cross, but had too few offspring to be considered statistically significant

e) *LOF Caf-1 (34069) with LOF domino synergistically elicits cell death along the wing margin*

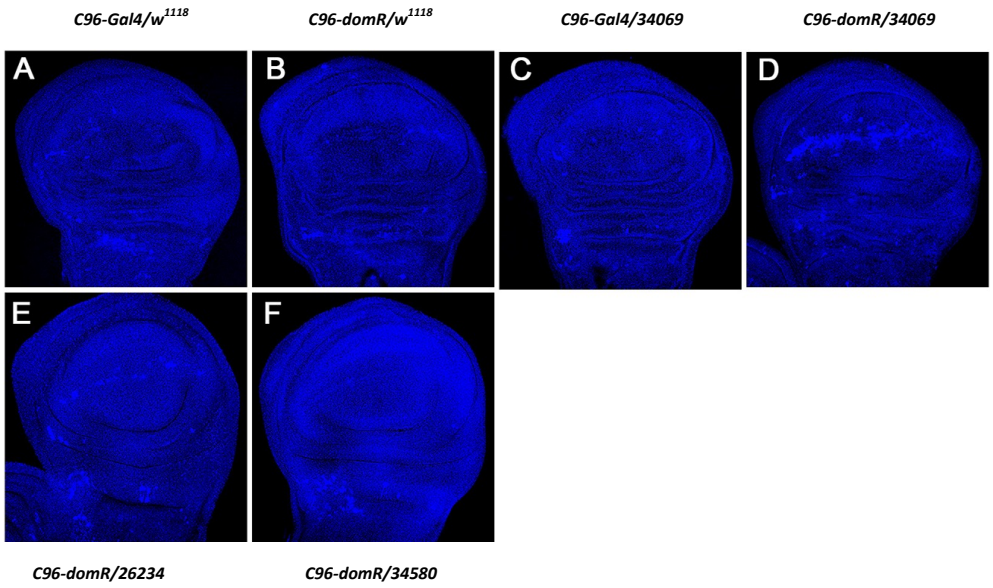

Figure 5: All panels show imaginal disc cleaved caspase stainings for cell death along the wing margin (data from K.E.). Controls are shown in panels A and B. Panels C and D show the experimental *C96-Gal4/34069* and *C96-domR/34069* stained wing discs, respectively. Panel E shows *C96-domR/26234*, and Panel F shows *C96-domR/34580*.

Figure 5 shows the results of imaginal discs stained with the cleaved caspase marker for cell death. Panels A and B are the controls, and show very low levels of staining across the wing margin. Panel C, *C96-Gal4/34069*, exhibits moderate staining along the wing margin, however, Panel D, *C96-domR/34069*, shows a strong synergism with increased staining along the wing margin (Figure 5). Strain 26234 (strong enhancer) appears to slightly increase cell death during enhancement (Figure 5E). Strain 34580 (strong suppressor) appears to show some suppression of cell death during wing nick suppression (Figure 5F). These results suggest that the

enhanced wing nicking frequency seen in the *C96-domR x 34069* cross is due to increased cell death (Table 7, Figure 5).

Discussion

I. *Review of domino and study objectives*

The chromatin remodeling protein, Domino, has a wide range of known roles in the cell. Previously, our lab found *domino* to be a genetic modifier of the Notch signaling pathway (Hall *et al.,* 2004). Additionally, Domino has been associated with hematopoiesis, cell growth and proliferation, apoptosis, histone exchange during DNA repair, maintenance of stem cells, and autophagy (Braun *et al.,* 1997; Ruhf *et al.,* 2001; Lu *et al.*, 2007; Kusch *et al.,* 2004; Prado *et al.,* 2013; Xi and Xie 2005; Kwon *et al.,* 2013). Domino has been found to act as both an activator and a repressor of gene activity. It acts as a negative regulator of the E2F signaling pathway genetically (Lu *et al.,* 2007). Domino has also been found to be a repressor of homeotic genes through its effect on TrxG and PcG group activity (Ruhf *et al.,* 2001). Domino's role in gene activation is largely tied to its function as a component in the Tip60 complex. As the ATPase component of Tip60, Domino plays a role in nucleosome acetylation, as well catalyzing histone exchange (Kusch *et al.,* 2004).

My honors project is focused on exploring genetic interactions between *domino* and other chromatin protein encoding loci. Since much of gene regulation occurs at the transcriptional level, chromatin modifiers are essential for proper gene expression. Thus, Domino, has been implicated in the regulation of cellular processes that when disrupted lead to various diseases. Our study provides

additional insight into Domino's function and builds on possible connections between other chromatin proteins. Although this study was primarily focused on the wing, we did obtain some data that suggests an association of *domino* with VB loci function in the eye tissue as well (Table 9). However, since this data is quite preliminary, and lacks some essential controls, it will not be considered in the discussion.

II. *Discussion of Results:*

Our study demonstrated that 25 out of 30 VB loci genetically interact with LOF *domino*. We found 16 of the strains had an effect with *C96-domR*, but not with *C96-Gal4* expression (Tables 1 and 2). Next, we found that 9 strains had an effect with *C96-Gal4*, and 7 of those had an effect with *C96-domR* (Tables 3 and 4). While initially Domino was known for its activity as a repressor, increasing attention has been given to its role as a gene activator as well (Lu *et al.,* 2007; Ruhf *et al.,* 2001; Kusch *et al.,* 2004). In our screen, of the 25 modifiers, 22 were members of the Red or Yellow chromatin types (Tables 1-4). Since Red and Yellow chromatin are characterized by their role in transcriptional activation, it appears as though our screen largely detected Domino's function as an activator. This was a bit surprising given the well-described role of Domino in gene repression (Ruhf *et al.,* 2001; Lu *et al.,* 2007). As a result, our study further illuminates Domino's important role as an activator of gene transcription.

III. Review of the interactors

We have selected a subset of the interactors with Domino, both those that did and did not have an effect with *C96-Gal4* alone, to review. Among the group of VB loci tested, some of the proteins are well understood, while the functions of others remain largely unknown. The following review aims to provide a brief summary of the literature on a subset of VB loci and use this information to make connections between Domino, the known, and the unknown loci.

a) *Known loci, CAF-1 and TRIP1, exhibited an RNAi phenotype when driven with C96-Gal4 alone, and also modified the C96-domR phenotype*

Chromatin assembly factor 1 (*Caf-1*) was one of the strains that had a slight wing nicking phenotype with *C96-Gal4* (10.2% nicking, Table 4). However, LOF *Caf-1* exhibited strong synergism, a wing nicking frequency of 100%, when crossed with *C96-domR* (Table 4). Caf-1 is a highly studied complex, which contains, Caf1p55, Caf1p180, and Caf1p105, in *Drosophila* (Tyler *et al.,* 2001). In human cells, Caf-1 is required for replication dependent chromatin assembly and has been found to deposit newly synthesized H3 and H4 on replicating DNA (Shibahara and Stillman, 1999). Caf-1 has also been identified as a component of the *Drosophila* RBF, E2F2 and Myb (dREAM/MMB) complex and is required for the complex to carry out one of its roles as a transcriptional repressor of E2F target genes (Beall *et al.*, 2002; DeBruhl *et al.,* 2013; Lewis *et al.,* 2004; Korenjak *et al.,* 2004; Taylor Harding *et al.,* 2004).

Additionally, Caf-1 is important in the regulation of cell death (Collins and Moon, 2003; Lewis *et al.,* 2004). Collins and Moon (2003) found that in *rbf1* mutant cells, Caf-1 is responsible for Posterior Sex comb (Psc) induced cell death. The study found that mutations in *caf1p180* and *caf1p105* result in increased cell death in the eye discs during development; thus, suggesting Caf-1 is required for survival of actively dividing cells (Collins and Moon, 2003, Anderson *et al.,* 2011). This result is consistent with the slight increase in caspase staining we found in the *C96-Gal4/34069* imaginal disc (Figure 5C). It appears as though the enhanced wing nicking frequency we observed in the *C96-domR x 34069* cross is due to increased cell death (Table 4, Figure 5D).

Along with its role in cell growth and proliferation, Caf-1 is involved in the Notch signaling pathway (Yu *et al.,* 2013). Deregulation of *caf1p105* leads to developmental defects that resemble Notch down regulation in the eye and wing. Additionally, Yu *et al.* (2013) found that Caf1p105 regulates the epigenetic modification mark of H4 acetylation in the enhancer region of a Notch target gene, and thus Caf-1 is involved in promoting active chromatin status. Caf-1's epigenetic regulation of the Notch pathway further links it to Domino.

Another one of the interesting interactors in our screen was *Trip1*. When the *Trip1* RNA*i* strain was crossed with *C96-Gal4*, it was lethal (Tables 3 and 8). However, when it was crossed with *C96-domR* and *C96-Gal4 + UAS-domB* lethality was suppressed (Tables 3 and 8). Trip1 is the i subunit of the Eukaryotic translation initiation factor 3 (eIF3) (Flybase; Lasko, 2000). As a component of eIF3, Trip1 is involved in starting the assembly of the pre-initiation complex and translational

control of gene expression (reviewed by Hinnebusch, 2006). In addition to its role in translation, Trip1 was identified in two screens identifying functional networks of microtubule associated proteins, and thus may be implicated in the cell cycle and mitosis (Hughes *et al.,* 2008; Fisher *et al.*, 2008).

More recently, Trip1 has been associated with DNA replication. In a functional analysis, Van Bemmel *et al.* (2013) found that cells expressing RNA*i* against *Trip1* accumulate in G1, and they do not incorporate 5-ethynyl-2'deoxyuridine (EdU), which is a marker of DNA replication (Chehrehasa *et al.,* 2009). This suggests that Trip1 is necessary for entry into S phase (Van Bemmel *et al.,* 2013). This finding identifies Trip1 to be involved in cellular processes other than translation.

The suppression of lethality seen in both the *C96-domR* x *Trip1* RNA*i* and *C96-UAS DomB* crosses is a bit puzzling, since it shows the same rescue effect via LOF or overexpression of Domino. This could have a trivial explanation, for example the titration of the Gal4 protein by both the *UAS-DomB* and the *UAS- RNAi (C96-domR)* constructs, thereby reducing the expression of *UAS-Trip1* RNA*i*. Alternatively, it could reflect the complex interactions of Domino with numerous cellular functions, where LOF versus overexpression impinge on different functions. We favor the latter explanation.

b) *Unknown locus, CC31, had an effect on C96-Gal4 alone and the C96-domR phenotype*

CC31 was one of the highly penetrant and severe genetic interactors that we identified in our screen. Both the *C96-domR* and *C96-Gal4* crosses resulted in 100% wing nicking, and also produced a fly with a uniformly smaller wing than the control (Table 3, Figure 3f). *CC31* codes for a zinc finger protein, known as Motif 1 binding protein (M1BP) (Flybase; Li and Gilmour, 2013). M1BP associates with genes that encode proteins required for cell viability, proliferation, and it is responsible for recruiting RNA Polymerase II (Li and Gilmour, 2013). As a result, is considered a global transcriptional regulator (Li and Gilmour, 2013).

Additionally, M1BP was identified in a screen for proteins involved in the DNA damage G2/M phase checkpoint (Kondo and Perrimon, 2011). The authors induced cell cycle arrest at the G2/M phase with an anti-cancer drug, doxorubicin, which intercalates DNA, disrupts DNA repair, and can lead to the activation of the apoptotic pathway (reviewed by Thron *et al*., 2011; Traganos *et al.,* 1985). Doxorubicin treated cells, with knocked down *M1BP* exhibited cells in mitosis, demonstrating M1BP plays a role in a key cell cycle checkpoint (Kondo and Perrimon, 2011). M1BP has also been associated with the receptor tyrosine kinase (RTK) signaling cascade via mitogen activated protein/extracellular signal regulated kinase (MEK) (Ashton-Beaucage *et al*., 2014; reviewed by McKay and Morrison, 2007). In a recent study, *M1BP* knockdown was among the 4 strongest hits in reducing *mek* transcript levels (Ashton-Beaucage *et al*., 2014). While recently, M1BP has received greater attention; the strong enhancement we observed in our genetic

screen illuminates the cellular importance of this protein and the need for further research.

c) *Known loci, PCAF and RYBP, had an effect on the C96-domR phenotype, and no effect on C96-Gal4*

One of the strong enhancers detected in our screen was *PCAF*, also known as *dGCN5* (Table 2). dGcn5 is a HAT, which has been found to associate with dAda2 proteins (Brownell *et al.,* 1996; Kusch *et al.,* 2003). It is involved in transcriptional activation, through its role in the Spt-Ada-Gcn5-Acetyltransferase (SAGA) type, GCN-5 related histone *N*-acetyltransferases (GNAT) complex (Grant *et al.,* 1997; reviewed by Carrozza *et al.,* 2003). dGcn5 is also one of two acetyltransferases in the Ada2a containing complex (ATAC), which is involved in nucleosome sliding (Suganuma *et al.,* 2008).

As a HAT, dGcn5 is involved in modifying H3 and H4 (Ciuciu *et al.,* 2006; Carre *et al.,* 2005). Carre *et al.* found that the protein is required for metamorphosis, oogenesis, and cellular proliferation in *Drosophila* (2005). LOF *dGcn5* impairs proliferation due to increased apoptosis in imaginal cells during larval instars (Carre *et al.*, 2005). Gcn5 has also been associated with the Notch pathway (Kurooka and Honojo, 2000; Gause *et al.,* 2006). Gcn5 has been found to interact with the NICD in mice (Kurooka and Honojo, 2000). More recently, it was identified for its role in Notch signaling, along with Domino in the Tip60 complex, in *Drosophila* (Gause *et al.,* 2006). This study, along with our observation that RNA*i* against Gcn5 does not have an effect of *C96-Gal4* alone, but strongly enhances the *C96-domR*

phenotype (Table 2), suggests a possible association of Gcn5 and Domino in activating transcription. However, further studies examining molecular pathways are necessary.

One of the suppressors detected in our screen codes for Ring and YY1 Binding Protein (Rybp). In the *C96-domR x Rybp* RNA*i* cross, the wing nicking penetrance was nearly halved (29.8%), as compared to the 57% control (Table 1). This protein is a nucleoporin type C2-C2 zinc finger (Meyer *et al.,* 2000). Rybp is well known for its activity as a transcriptional repressor and regulator of the cell cycle via interaction with PcG genes during embryogenesis and imaginal disc formation (Bejarano *et al.*, 2005; Gonzalez *et al.,* 2008; Martinez *et al.*, 2006). Rybp has also been associated with histone modification via its ubiquitinating activity; it is recruited to the inactivated X chromosome during development (Arrigoni *et al.,* 2006; Napoles *et al.,* 2004). While the mechanisms involving histone ubiquitination remain poorly described, Rybp has been found to monoubiquitinate the H2A histone variant (Arrigoni *et al.,* 2006; Napoles *et al.,* 2004).

Rybp has also been associated with the regulation of apoptosis in *Drosophila*. Gonzalez *et al*. (2008) previously found that overexpression of *Rybp* in wing imaginal discs via the Gal4/UAS system leads to smaller wings in adults. In a follow up study, it was found that the smaller wings are due to increased induction of apoptosis in all imaginal discs via activation of caspase 3 (Gonzalez and Busturia *et al.,* 2009). This induction of apoptosis requires the function of several pro-apoptotic genes, including *reaper, hid, and grim*, as well as other proteins (Gonzalez and Busturia *et al.,* 2009). Additional studies have linked Rybp to the inhibition of

apoptosis (Fereres *et al.*, 2013). As a result, it is possible that the genetic interaction (suppression) we observed in the *C96-domR* x *Rybp* RNA*i*, was due to decreased induction of apoptosis. Further studies on cell death are needed to assess this possibility.

d) *Unknown loci, CC9 and CC20, modified the C96-domR phenotype and showed no effect of C96-Gal4*

CC9 was found to be an enhancer of the *C96-domR* phenotype in our study (Table 1). This gene codes for a protein whose function is largely unknown. It contains a DNA binding domain and a BTB/POZ domain (for *broad complex, tramtrack, and bric a brac/pox virus* and zinc finger) (Flybase). The BTB/POZ domain has been found to be involved in transcriptional repression (Deweindt *et al.,* 1995). In *Drosophila* hemocytes, *CC9* expression was induced by *Ras* overexpression, and thus it may be involved in cellular growth and proliferation (Asha *et al.*, 2003). Our finding, that *CC9* exhibits a statistically significant genetic interaction with *domino*, expands upon the limited understanding of CC9 as a chromatin associated protein.

The final protein we examined was CC20 (strong enhancer), with 78.8% wing nicking in the *C96-domR* cross, as compared to the 57% control (Table 1). This protein has DNA binding activity and contains a BESS motif and a MADF domain (Flybase). Guertin *et al*. (2006) identified *CC20* in a functional genomic screen of Target of Rapamycin (TOR) regulated genes. TOR is a protein kinase that controls cell growth, proliferation and survival and is upregulated in many cancers

(reviewed by Ballou and Lin, 2008). As its name suggests, TOR is inhibited by the drug rapamycin (reviewed by Ballou and Lin, 2008). Knockdown via RNA*i* of *CC20* was found to have effects on both cell size and cell proliferation in cells treated with rapamycin (Guertin *et al.,* 2006). Thus, *CC20* appears to be involved in a cell growth pathway.

e) *The 30 VB loci code for proteins involved in a wide range of cellular processes*

In addition to the 7 proteins reviewed above, we compiled a summary of the domains, motifs, and known biological functions for each of the 30 VB proteins in Table 10.

Strain Number	Gene Name	Alternative Names and Domains/Motif	Biological Function
26234 (E)	*CC8*	MADF domain	Unknown
31921 (E)	*JIGR1*	MADF domain	Cell proliferation and differentiation in hemocytes, eye development, associated with a ubiquitin protease
42514 (E)	*CC25*	BESS motif, MADF domain	Largely unknown, wing hinge patterning
31922 (E)	*CC20*	BESS motif, MADF domain, DNA binding	Cell growth and division
33394 (E)	*RPS9*	(S9) Ribosomal protein S4/S9, rRNA binding	Cell cycle, cell proliferation, protein synthesis
32888 (E)	*CC24*	SOSS-B family DNA binding	DNA repair
33361 (E)	*CC4*	Chromo-domain	Cell cycle and proliferation, associated with telomeres
33734 (E)	*CC7*	Zinc finger C2H2 type/integrase, DNA binding	Unknown
42491 (E)	*CC9*	BTB/POZ like, DNA Binding HTH Domain, Psq-type	Cell growth and proliferation

26772 (E)	*CC15*	(dASCIZ) Zinc finger (C2-H2)	Transcriptional regulation, larval development, wing morphogenesis, mitosis, apoptosis
34978 (E)	*TRIP1*	(eiF3-S2, eiF3i) WD40 repeat	Translation, microtubules, mitosis, DNA replication, may be associated with phagosomes
41937 (E?)	*CC31*	(M1BP) C2H2, zinc finger- AD type	Cell viability, proliferation, transcriptional regulation, DNA damage G2/M phase checkpoint
33981 (E)	*PCAF*	(GCN5, dKAT2) GNAT domain, Bromodomain, PCAF, Acyl-CoA N-acyltransferase, Histone acetylase PCAF	Histone acetylation, H3 and H4 modifier, Notch signaling pathway, apoptosis, cell proliferation, transcriptional regulation, dendrite morphogenesis
33962 (E)	*HP1c*	Chromo shadow domain, methylated histone binding	Associated with telomeric position effect silencing, transcriptional regulation from RNA polymerase II promoter, localizes with kinetochore
34069 (E)	*CAF1*	(p55, Nurf, Nurf55, Caf1p55, RbAp48, dNURF, Nurf 55) WD40/YVTN repeat like containing domain, histone binding protein RBBP4- N terminal	Chromatin assembly (histone acetylation, deacetylation, methylation), cell growth and proliferation, cell death, Notch signaling, DNA replication and repair, nucleosome remodeling, PcG silencing, dendrite morphogenesis, cell survival and patterning during development
33043 (E)	*MUS209*	(dPCNA) Proliferating cell nuclear antigen, DNA binding	DNA replication and repair, mitosis, cell proliferation, wing morphogenesis
53697 (E)	*SIR2*	Deacetylase sirtuin-type domain, DHS like NAD/FAD-binding domain	NAD-dependent histone deacetylase, chromatin silencing by PcG proteins, cell survival and death, life span, Notch signaling, pachytene checkpoint, Huntington's disease
55250 (E)	*ASF1*	Histone chaperone, ASF-1 like	Histone chaperone involved in replication dependent chromatin assembly, silenced chromatin, DNA repair, cell cycle, dendrite morphogenesis, Notch signaling

55314 (E)	*TOP1*	(Topo I, topoI) DNA topoisomerase I, DNA binding, mixed alpha/beta motif, catalytic core, alpha helical subdomain	Cell proliferation, chromosome recombination, replication, repair, development, relaxes DNA supercoiling, RNA splicing, associated with microtubules
33666 (NE)	*HEL25E*	(UAP56, HEL, WM6, jf26) DEAD box helicase family, ATP binding domain, nucleic acid binding, RNA helicase	Promotes open chromatin, mRNA export, localization, and post-translational modification, cell cycle via association with E2F pathway, cell death?, associated with microtubules, alternative splicing
40853 (S)	*MAF-S*	(maf2) Basic leucine zipper domain, Maf-type, transcription factor, Skn-1 like, DNA binding	May be associated with autophagic cell death regulation, age-dependent degenerative processes, cell growth
33974 (S)	*RYBP*	Zinc finger, RanBP2-Type, DNA binding	PcG dependent transcriptional repressor, cell cycle, histone modification, apoptosis
25993 (S)	*CC28*	High mobility box group domain	Largely unknown, apoptosis
34580 (S)	*NUP50*	Ran binding domain, Pleckstrin homology like domain, nuclear pore complex Nup2/50/61	Interacts with highly active transcriptional sites, apoptosis
33725 (S)	*RPD3*	(HDAC1, Su(var326)) Histone deactylase superfamily	Histone deacetylation, PcG mediated silencing, protein biosynthesis, development, dendrite morphogenesis, apoptosis, regulation of heterochromatin structure of telomeres, cell division, cell cycle progression, neurodegenerative disease, Notch signaling, DNA damage (may antagonize Tip60-mediated ATM acetylation), mitochondrial activity
26231 (NE)	*CC34*	MADF domain, AT Hook, DNA binding	Unknown
27085 (NE)	*ERR*	(dERR) Nuclear hormone receptor, ligand-binding, zinc finger, NHR/GATA type, DNA binding	Metabolic processes associated with cell proliferation, developmental growth, associated with mitochondrial biogenesis

31940 (NE)	*CC30*	Zinc finger (C2-H2), BED type	Unknown
29360 (NE)	*CC32*	(Zif) Zinc finger (C2-H2), AD-type	Neuroblast proliferation, lymph gland hemopoiesis
31960 (NE)	*DSP1*	High mobility group box domain, DNA binding domain	Chromatin remodeling involved in regulating homeotic genes, development, cell proliferation?, apoptosis

Table 10: Strain number (effect on *C96-domR* phenotype), gene name (as designated by Van Bemmel *et al.* (2013)), protein alternative names, domains, and motifs, and biological functions. Information on each protein was compiled using Flybase. (http://flybase.org/).

f) *Summary of VB proteins*

The representative subset of interactors reviewed above, along with the information in Table 10, confirms our understanding of Domino as a protein with pleiotropic functions in the cell. Several of the modifiers are involved in transcriptional activation, such as the HAT Gcn5 and Caf-1, while others likely act as repressors, including CC9 and Rybp. Additionally, we found that the 4 strongest enhancers of the *C96-domR* phenotype that had no effect when crossed to *C96-Gal4* alone (*CC8, JIGR1, CC25,* and *CC20*) all contain the Myb/SANT-like Domain in Adf-1 (MADF) domain (Table 10, Flybase). Only one other protein in our study (CC34 (NE)) contained this domain (Table 10, Flybase).

The MADF domain is similar to the SANT domain (a domain found in Domino), and was first identified in the transcriptional activator, Adh transcription factor 1 (Adf-1) (Cutler *et al.,* 1998; England *et al.,* 1990; Eissenberg *et al.,* 2004). Proteins with the MADF domain have been found to act as both activators and repressors (Cutler *et al.,* 1998; England *et al.,* 1990; Bhaskar and Courey, 2002; Maines *et al.*, 2007). A protein with the MADF domain, known as Dorsal interacting

Protein 3 (Dip3), is a co-activator of Dorsal and Twist, which are transcription factors that determine dorsal-ventral cell fate during embryogenesis (Bhaskar and Courey, 2002; reviewed by Rusch and Levine, 1996). It is striking that 4 of the 5 proteins in our study with the MADF domain were in the same class (modified the *C96-domR* phenotype, but show no phenotype with *Gal4* alone), modified in the same direction (enhancement), and were all members of the previously unknown loci identified by Van Bemmel *et al.* (2013). Our results may suggest an important role for the MADF domain in chromatin proteins that function with Domino. However, further research is necessary to investigate this. For example, employing co-immunoprecipitation assays, it would be interesting to determine if Domino is found within multimeric protein complexes containing any of these proteins.

Additionally, the genetic modifiers of *domino* identified in our screen appear to be involved in similar cellular processes. Not surprisingly many of the proteins are involved in the Notch signaling pathway. Additionally, many were found to be involved in cell growth and proliferation. Finally, many are known to be involved in regulation of the cell death pathway.

IV. Concluding remarks

This study builds on our understanding of the chromatin remodeler, Domino. We describe a subset of 25 genes, which code for chromatin associated proteins that genetically interact with *domino*. Our screen identified modifiers that are currently understood to varying degrees: some are well characterized, others are known for their role in other cellular processes and have just recently been found to associate

with chromatin, and yet the function of others remains unknown completely. As 25/30 tested VB loci showed significant genetic interaction with *domino*, this study validates that the *C96-domR* construct is an excellent tool for assaying the status of chromatin function (Kwon *et al.,* 2013). Further, since the VB loci were selected for our analysis based solely on the availability of *UAS-RNAi* strains in a Drosophila stock center, the association of 22/25 with transcriptionally active (Red/Yellow) versus heterchromatic/inactive (Black, Blue, Green) chromatin is noteworthy. The principal conclusion we can reach from this observation is that Domino primarily functions with active chromatin regions. Future studies are needed to investigate the biochemical and functional relationship between *domino* and the identified proteins in this screen. Since chromatin proteins are involved in the regulation of gene expression, and its dysregulation often leads to disease, greater understanding of these proteins is pertinent to human health.

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