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# Investigation of RNA Binding Protein Pumilio as a Genetic Modifier of Mutant CHMP2B in Frontotemporal Dementia (FTD): A Masters Thesis

Xing Du

*University of Massachusetts Medical School*

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INVESTIGATION OF RNA BINDING PROTEIN PUMILIO AS A GENETIC  
MODIFIER OF MUTANT CHMP2B IN FRONTOTEMPORAL DEMENTIA (FTD)

A Masters Thesis Presented

By

XING DU

Submitted to the Faculty of the  
University of Massachusetts Graduate School of Biomedical Sciences, Worcester  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE  
August 14<sup>th</sup>, 2016

NEUROLOGY

INVESTIGATION OF RNA BINDING PROTEIN PUMILIO AS A GENETIC  
MODIFIER OF MUTANT CHMP2B IN FRONTOTEMPORAL DEMENTIA (FTD)

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The signatures of the Master's Thesis Committee signify  
completion and approval as to style and content of the Thesis

Andreas Bergmann, Chair of Committee

Tony Ip, Member of Committee

Fen-Biao Gao, Member of Committee

Anthony Carruthers, Member of Committee

The signature of the Dean of the Graduate School of Biomedical Sciences signifies that  
the student has met all master's degree graduation requirements of the school.

Anthony Carruthers, Ph.D.,  
Dean of the Graduate School of Biomedical Sciences  
Program

Interdisciplinary Graduate Program

Month, Day and Year

August 14<sup>th</sup>, 2016

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I would also like to thank Dr. Dejun Yang who first started the genetic screen in *Drosophila* to identify genes encoding RNA binding proteins that can modify mutant CHMP2B toxicity. Based on his solid work, I was able to continue exploring the underlying mechanism. Dr. Yubing Lu helped me a lot by providing valuable advice during the long trouble-shooting period through out my thesis research.

I would also like to acknowledge my committee of qualifying exam: Dr. Andreas Bergmann, Dr. Sean Ryder, Dr. Mary Munson and Dr. Neal Silverman. Their insightful suggestions contributed a lot to the design and interpretation of my experimental work.

Finally, I owe my very profound gratitude to my wife for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without her. Thank you.

## ABSTRACT

Frontotemporal dementia (FTD) is the second most common early-onset dementia. A rare mutation in *CHMP2B* gene was found to be associated with FTD linked to chromosome 3. Previous studies have shown that mutant CHMP2B could lead to impaired autophagy pathway and altered RNA metabolism. However, it is still unknown what genes mediate the crosstalk between different pathways affected by mutant CHMP2B. Genetic screens designed to identify genes interacting with mutant *CHMP2B* represents a key approach in solving the puzzle. Expression of mutant *CHMP2B* ( $CHMP2B^{\text{intron5}}$ ) in *Drosophila* eyes leads to a neurodegenerative phenotype including melanin deposition and disrupted internal structure of ommatidia. The phenotype is easily quantified by estimating the percentage of black dots on the surface of the eyes. Using this established *Drosophila* model, I searched for genes encoding RNA binding proteins that genetically modify  $CHMP2B^{\text{intron5}}$  toxicity. I found that partial loss of *Pumilio*, a translation repressor, mitigates  $CHMP2B^{\text{intron5}}$  induced toxicity in the fly eyes. Western blot analysis showed that down regulation of *Pumilio* does not significantly decrease  $CHMP2B^{\text{intron5}}$  protein level, indicating indirect regulation involved in suppression of the phenotype. The molecular targets regulated by *Pumilio* and the mechanism underlying  $CHMP2B^{\text{intron5}}$  toxicity suppression by *Pumilio* down-regulation requires further investigation.

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# **LIST OF SYMBOLS, ABBREVIATIONS OR NOMENCLATURE**

FTD: Frontotemporal Dementia

ALS: Amyotrophic Lateral Sclerosis

RBP: RNA Binding Protein

C9ORF72: Chromosome 9 Open Reading Frame 72

TDP-43: TAR DNA Binding Protein-43

FUS: Fused in Sarcoma

CHMP2B: Charged Multi-vesicular Protein 2B

VCP: Valosin-Containing Protein

UBQLN2: Ubiquitin-like Protein Ubiquilin-2

ESCRT: Endosomal Sorting Complex Required for Transport

MVB: Multi-vesicular Body

GMR: Glass Multiple Reporter

UAS: Upstream Activating Sequence

RNAi: RNA interference

siRNA: Double Stranded Small Interfering RNA

## **PREFACE**

The thesis is submitted for the degree of Master of Science at University of Massachusetts Medical School. The research described here is conducted under the supervision of Prof. Fen-Biao Gao in the Department of Neurology, between February 2015 and May 2016. I carried out the genetic screen project that Dr. Dejun Yang initiated and confirmed the genetic interaction between candidate modifiers and CHMP2B<sup>intron5</sup>. I defended my qualifying exam with the screen results on July 2015. Then I continued to work on one of the suppressor genes *Pumilio*. It took me a long time to do troubleshooting and optimization for the western blot. Dr. Yubing Lu offered a lot of help and suggestions during this period. I presented the updated research progress at the IGP seminar on April 2016 and it was well received.

This work is to my best knowledge original, except where acknowledgement and reference were made. Neither this, nor anything substantially similar has been or is being submitted for any other degree, diploma at any other Universities or institutions. The thesis contains less than 5000 words.

Xing Du

July

2016



## Chapter 1.Introduction

### Chapter 1.1 FTD-ALS: RNA metabolism vs. Protein homeostasis

Frontotemporal dementia (FTD) is a progressive neurodegenerative disease with complex genetic basis (1). It is characterized by atrophy of frontal and/or temporal lobe resulting in changes in personality and social behavior (1). FTD overlaps with amyotrophic lateral sclerosis (ALS), a motor neuron degenerative disease with *C9ORF72* intronic GGGGCC repeat expansion being the most common mutation in both diseases (2). Sequestration of RNA binding proteins (RBPs) by repeat expansion leading to disrupted RNA metabolism is one of the possible pathogenic mechanisms for *C9ORF72* FTD/ALS (2). In other FTD cases without *C9ORF72* mutations, aggregation of misfolded RNA binding proteins is also a prevalent hallmark (3). More than 50% of the FTD patients show pathological neuronal inclusions positive for either TDP-43 (45%) or fused in sarcoma (FUS) (9%) (2), and rare mutations in these RNA binding proteins were also found in FTD patients (2). Thus, defective RNA metabolism is a major pathological mechanism in FTD and related disorders. On the other hand, FTD can also be caused by mutations in several genes related to the autophagy pathway, such as the ESCRT-III component *charged multivesicular body protein 2B* (*CHMP2B*), AAA-ATPase member *valosin-containing protein* (*VCP*), ubiquitin-like protein *ubiquilin-2* (*UBQLN2*), and most recently *TBK1* (2, 4). Both *VCP* and *UBQLN2* show TDP-43 pathology (5, 6). Knocking down TDP-43 will decrease mRNA level of major autophagy gene *Atg7* (7). It remains unknown how genetic mutations in distinct molecular

pathways such as RNA metabolism and autophagy can cause the same disease. Nor do we understand whether these molecular pathways interact with each other in FTD.

### **Chapter 1.2 *CHMP2B* mutation associated with FTD-3**

The study of *CHMP2B* mutations can bring insights into the interplay between RNA metabolism and protein homeostasis in FTD-ALS. The Danish *CHMP2B* mutation is caused by a G-to-C transition in the acceptor splice site of exon 6 (8). The mutation leads to replacement of the C-terminal 36 amino acids by either a single valine (*CHMP2B*<sup>intron5</sup>), or 29 amino acids (*CHMP2B*<sup>D10</sup>) (8) (Figure 1.1). *CHMP2B*<sup>D10</sup> is expressed at lower level than *CHMP2B*<sup>intron5</sup> and is not considered the major toxic form of mutant *CHMP2B*(9). *CHMP2B* is a component of the ESCRT-III complex, which is formed transiently on the limiting membrane of early endosomes to promote biogenesis of multivesicular bodies (MVBs) (10). Previous work from our lab and others show that *CHMP2B* functions redundantly with its paralog *CHMP2A* and knocking down *CHMP2B* has no effect on neuronal cell survival (11). *CHMP2B*<sup>intron5</sup> toxicity arises from disrupting interaction with Vps4 and preventing dissociation of ESCRT-III complex from the membrane (11). More importantly, transient overexpression of *CHMP2B*<sup>intron5</sup> causes the accumulation of abnormal autophagosomes in mammalian neurons (11). One genetic screen from our lab identified *Syntaxin 13* as a strong genetic modifier of *CHMP2B*<sup>intron5</sup> and that *Syntaxin 13* plays a novel role in autophagosome maturation, further highlighting the role of autophagy in FTD pathogenesis (12). On the other hand, our lab found that miR-124 but not miR-9 was downregulated in *CHMP2B*<sup>intron5</sup> transgenic mouse model (13). MVBs were also found to associate with components of

miRNA effector complex and regulate miRNA activity (14). However, how  $CHMP2B^{intron5}$  might regulate the miRNA pathway remains to be determined.

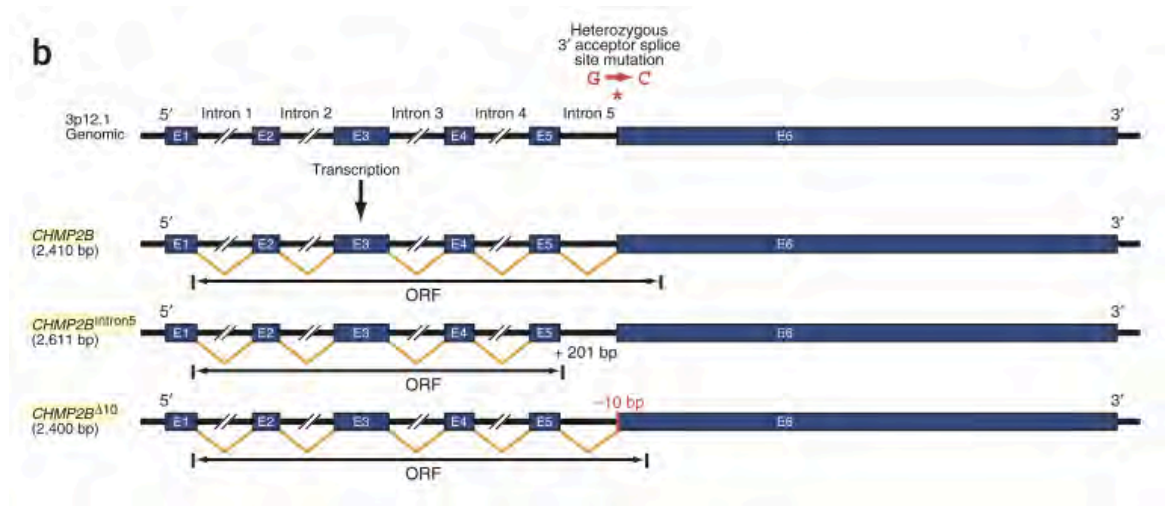


Figure 1.1 Location of Danish  $CHMP2B$  mutation and the resulting transcript (from (8)).

### Chapter 1.3 Animal models of mutant $CHMP2B$ toxicity

Development of animal model is essential to understand the mechanism by which mutant  $CHMP2B$  leads to relevant phenotypes.  $CHMP2B^{intron5}$  is the major form of mutant  $CHMP2B$  protein for its higher stability and toxicity (11). Model systems of  $CHMP2B^{intron5}$  mutation range from *in vitro* models including primary cultured neurons and human cell lines, to *in vivo* models like *Drosophila* and mice. Here we took the advantage of *Drosophila* genetics as a tool to identify genes that interact with  $CHMP2B^{intron5}$ . UAS transgenic flies expressing  $CHMP2B^{WT}$  and  $CHMP2B^{intron5}$  were generated and recombined with GMR-Gal4 to drive the expression specifically in photoreceptor neurons.  $CHMP2B^{intron5}$  expression leads to deposition of melanin on the surface of eyes as well as disrupted internal structure of



ommatidia (15) (Figure 1.2). Accumulation of autophagosomes was also observed in fly eyes by over-expression of CHMP2B<sup>intron5</sup> or knock down of the ESCRT-III subunit Shrub (Figure 1.3) (11).

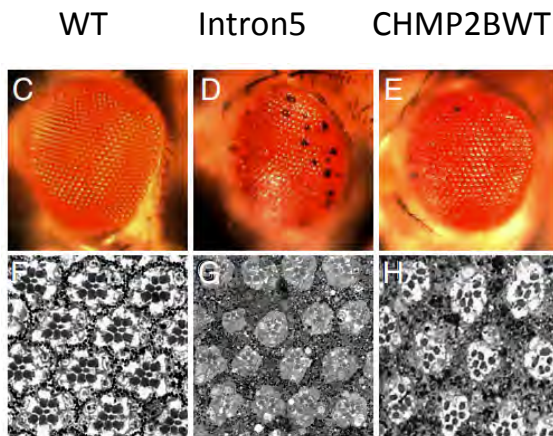


Figure 1.2 CHMP2B<sup>Intron5</sup> expression lead to severe degeneration in *Drosophila* eye. C, F: *GMR-Gal4*; D, G: *GMR-Gal4/UAS-CHMP2B<sup>intron5</sup>*; E, H: *GMR-Gal4/UAS-CHMP2B<sup>WT</sup>* (from (15)).

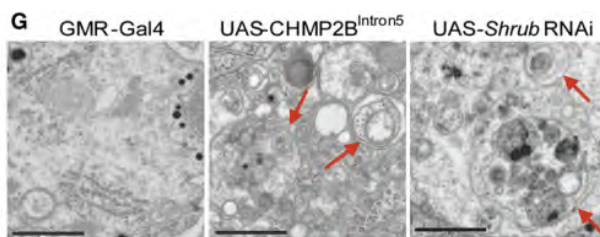


Figure 1.3 Accumulation of autophagosomes in fly eyes (electron microscopy, red arrows). The expression of CHMP2B<sup>intron5</sup> and *shrub* RNAi was driven by the GMR-Gal4. Scale bars for EM images: 1.0 um (from (11) ).

## Chapter 2. Material and methods

### Chapter 2.1 Fly Stocks

*D.melanogaster* strains were raised at 25C on standard food with yeast diet. Lines containing *GMR-Gal4* and *UAS-CHMP2B<sup>intron5</sup>* (or *UAS-CHMP2B<sup>WT</sup>*) were recombined as described before (15). RNAi lines for the genetic screen were ordered from the Vienna Drosophila Resource Center. *Pumilio* mutant line (#3260) was ordered from the Bloomington Drosophila Stock Center. *Pumilio* RNAi line (#v101399) was used to confirm the genetic suppression. *UAS-Pumilio* (on X chromosome) was obtained from the Jan Lab. *Atg5* inverted repeat line (#F003001) was ordered from Fly ORF. The RNAi line targeting GFP was crossed with *GMR-CHMP2B<sup>intron5</sup>* as a UAS control for the genetic screen. To quantify the eye phenotype, we classified it into 3 classes from high (very strong, large area of black dots), medium (considerably strong, medium area of black dots) and low (weak, little black dots). Representative pictures are displayed in Figure 2.1. Genetic interactions were evaluated according to the percentage of each class in F1 progeny.

### Chapter 2.2 Western blots

Rabbit CHMP2B antibody that recognizes both CHMP2B<sup>WT</sup> and CHMP2B<sup>intron5</sup> was reported as described in the previous publication (15). To extract total proteins, ~30 fly heads were homogenized in lysis buffer and ~10µg of protein was separated on 12% SDS gel, blotted onto PVDF membrane and probed with primary antibody against CHMP2B (1:1000,

developed in the Gao lab) or beta-actin (1:1000, cell signaling technology, #4967S) and secondary antibody (1:5000, goat anti-rabbit HRP).

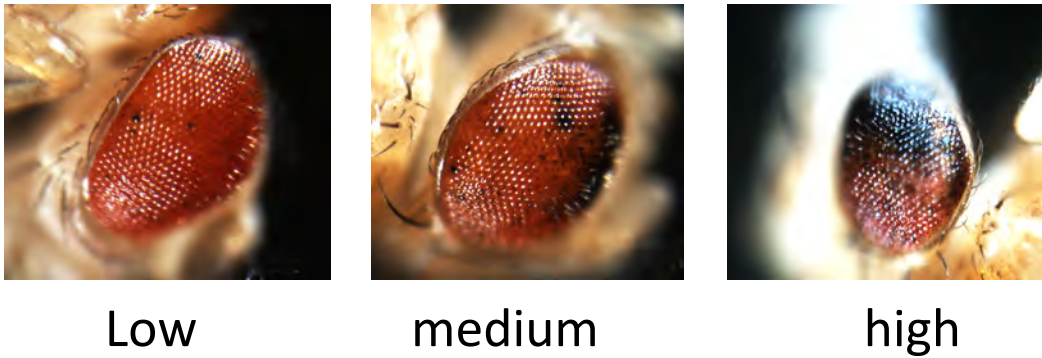


Figure 2.1 Representative images of the *Drosophila* eye phenotype severity in each classification group. Images show eye phenotype induced by CHMP2B<sup>intron5</sup> expression, ranging from low, medium to high.

## Chapter3. Results

### Chapter 3.1 A genetic screen identifies modifiers of CHMP2B<sup>intron5</sup>

CHMP2B<sup>intron5</sup> phenotypes are sensitive to partial loss of other genetic factors (15). To identify genes that genetically interact with mutant *CHMP2B*, genome-wide screens using deficiency lines were conducted which resulted in the discovery of several modifier genes including *Serpin5* (15), *Syntaxin13* (12) and *Rab8* (16). This collection of deficiency lines cover 75% of the *Drosophila* genome(15). Although very useful, deficiency screens lack precision and can create added-up effects. Moreover, haploinsufficiency by 50% downregulation may not be enough for some affected genes to exert an effect. On the other hand, RNA interference, based on sequence complementation between the siRNA and the target RNA, is relatively specific. Its knockdown efficiency of gene expression can reach more than 50%. Therefore, RNAi screens are a complementary way to identify additional genes that could have been missed by deficiency screens. Since our focus is on RNA metabolism affected by mutant *CHMP2B* and RNA binding proteins play important regulatory roles, Dr. Dejun Yang initiated a screen with RNAi lines targeting genes encoding all *Drosophila* RNA binding proteins. Together we identified a number of RNA binding proteins as genetic modifiers of CHMP2B toxicity (Figure 3.1) from a total of 547 RNAi lines covering 338 unique genes encoding RNA binding proteins. RNAi lines of 18 different genes showed potential enhancement effect (more severe eye phenotype than expressing intron5 by itself) and 33 genes showed suppression. We filtered the list by picking only

strong enhancers (more than 85% high phenotype) or suppressors (nearly 100% low phenotype). Furthermore, for some of the candidate modifiers, we crossed GMR-Gal4, UAS-CHMP2B<sup>intron5</sup> with a second RNAi line to confirm the effects of modifier genes. The final list contains 8 enhancers (Figure 3.1, columns 2-9) and 5 suppressors (Figure 3.1, columns 10-14). Therefore, the hit rate is 3.85% (13 out of 338), which is within the typical 1-4% range in similar genetic screens(17, 18). All of the validated modifiers may bring novel insight into the crosstalk between RNA metabolism and the autophagy pathway. Suppressors, whose partial loss of function suppresses CHMP2B toxicity, may further serve as potential therapeutic targets.

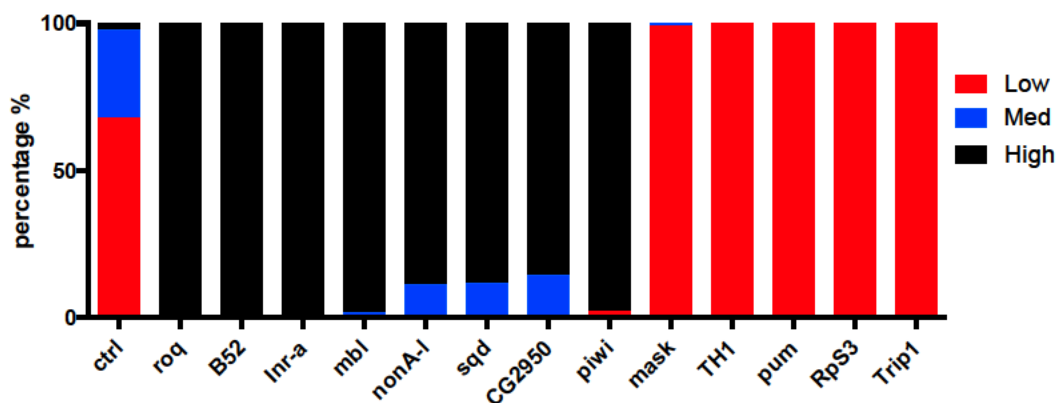


Figure 3.1 Effect of candidate enhancers (8) and suppressors (5) on CHMP2B<sup>intron5</sup> eye phenotype severity. The first column on the left represents control line without modifier.

## Chapter 3.2 Genetic validation of *Pumilio* as suppressor of mutant *CHMP2B* toxicity

We identified *Pumilio* as one of the strong suppressors of FTD-associated *CHMP2B*<sup>intron5</sup> toxicity in fly screen. Two RNAi lines of *Pumilio* were included in the primary screen and both showed a strong suppression of *CHMP2B*<sup>intron5</sup> eye phenotype (Figure 3.1 bar 11 shows one of the them). To confirm the genetic suppression, I also crossed GMR-Gal4, UAS-*CHMP2B*<sup>intron5</sup> flies with *Pumilio* loss of function mutant line. All F1 progeny showed low eye phenotype (Figure 3.2). Consistently, the F1 generation from the cross between GMR-Gal4, UAS-*CHMP2B*<sup>intron5</sup> and *Pumilio* overexpression line is adult lethal (both under 18C and 25C). Even when the overexpression line is crossed with GMR-Gal4 (drives expression mostly in photoreceptor neurons), few of the F1 progenies went through the pupa stage and the enclosed ones exhibit deformed eye shape and colorless eye surface, an indication of the toxicity caused by the overexpression of the RNA binding protein in the eye (Figure 3.2).

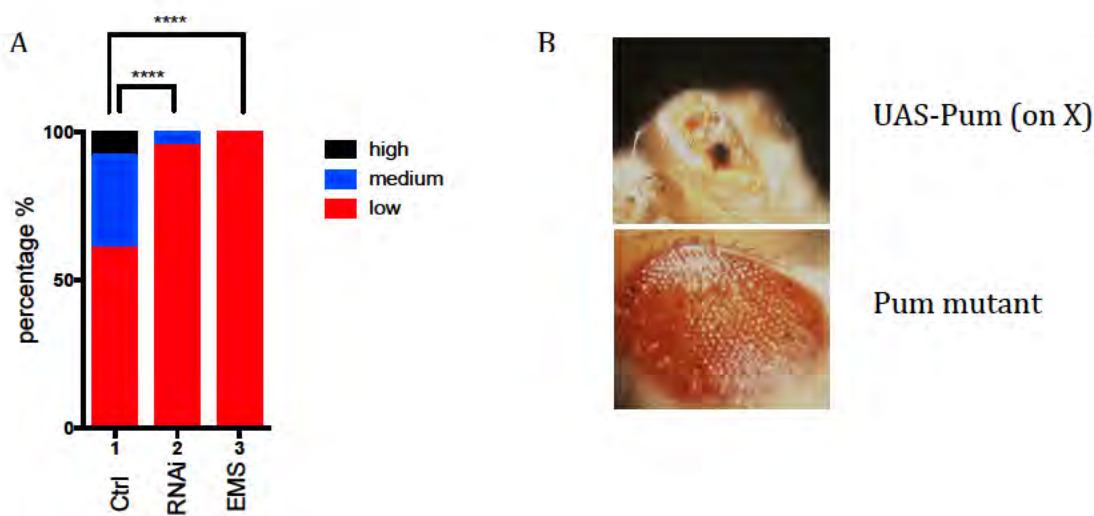


Figure 3.2 A. Both *Pumilio* RNAi and loss of function mutant suppress CHMP2B<sup>intron5</sup> eye phenotype, as summarized in the histogram. The difference between control group and RNAi or mutant group is significant (Chi-square test, p-value < 0.0001). B. Representative image of the eye surface from *Pumilio* overexpression line shows the disrupted eye structure. In contrast, the *Pumilio* mutant line has normal eyes comparable to wild type line.

*Pumilio* is a member of an evolutionally conserved family of RNA binding proteins characterized by Pumilio-Homology domain (Pum-HD) (19). *Pumilio* represses translation of *hunchback* (*hb*) mRNA in the posterior of *Drosophila* embryo and the regulation depends on two bipartite nanos-responsive elements (NRE) in the 3' UTR of *hb* mRNA (20). *Pumilio* binds to the NRE and recruits Nos and Brat, forming a RNA-protein complex that leads to *hb* mRNA deadenylation and translation repression (21). *Pumilio*'s role as translation repressor was also confirmed in post-mitotic neurons, which affected both neuronal development and function. *Pumilio* mutant impairs development of peripheral sensory neurons into multi-dendritic cells and several *Pumilio* alleles were found to affect long-term memory formation in adult flies (22) (23). Its mammalian homolog *Pumilio1* was recently found to regulate ATAXIN1 levels in mice, suggesting that *Pumilio1* haploinsufficiency could contribute to human neurodegeneration (24). *Pumilio1* mutant mice were significantly smaller in body size and developed progressive motor deficits (Figure 3.3)(24). On the other hand, down regulation of *Pumilio* expression was found to suppress neurodegenerative-associated phenotype in our CHMP2B<sup>intron5</sup> fly model. This highlights the complexity of the role played by *Pumilio* in neurodegenerative diseases and calls for further investigation into the mechanism of eye phenotype suppression by *Pumilio* knock down in the *Drosophila* model.



### Chapter 3.3 The expression level of CHMP2B<sup>intron5</sup> is not affected by partial loss of *Pumilio*

Given the canonical function of *Pumilio* as translation regulator, I examined the protein level of CHMP2B<sup>intron5</sup> in *Pumilio* RNAi and mutant lines crossed with GMR-Gal4, UAS-CHMP2B<sup>intron5</sup>. I measured the CHMP2B<sup>intron5</sup> protein level in fly heads of F1 progenies from GMR-Gal4, UAS-CHMP2B<sup>intron5</sup> flies crossed with *w<sup>1118</sup>* flies, *Pumilio* RNAi and *Pumilio* mutant (Figure 3.4). There is no significant difference in CHMP2B<sup>intron5</sup> protein level between control group and RNAi or mutant lines (validated in 3 biological replicates, Figure 3.4). Therefore, *Pumilio* does not directly suppress CHMP2B<sup>intron5</sup> toxicity by regulating its protein level. What the target molecules regulated by *Pumilio* are and what the underlying mechanism for suppression of CHMP2B<sup>intron5</sup> toxicity by *Pumilio* downregulation is, are both pending questions to be answered.

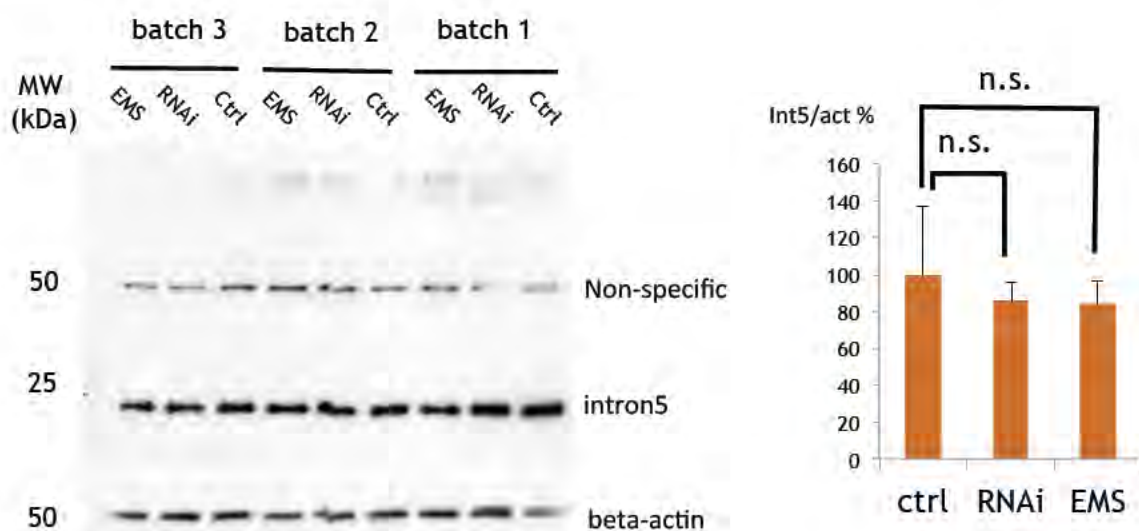




Figure 3.4 Left: Western blot with antibody against CHMP2B-intron5 and beta-actin. Right: Quantification of western blot (intron5 level normalized over beta-actin), no statistically significant difference was detected between control and RNAi or mutant lines (one-way ANOVA).

### Chapter 3.4 *Pumilio* targets and downstream pathways

A previous study identified mRNAs that associated with *Pumilio* using transgenic flies expressing affinity-tagged *Pumilio* by DNA microarrays (25). 714 *Pumilio* targets were found in adult flies and 165 are in embryos with an overlap of 31 genes (25). In adult flies, the target mRNAs of *Pumilio* showed general increases in transcript abundance compared to non-target transcripts, indicating that *Pumilio* might lead to accelerated target mRNA degradation (Figure 4.1) (25). Since the eye phenotype of CHMP2B<sup>intron5</sup> fly is more of a developmental defect, the embryonic target genes of *Pumilio* are of greater interest to us. Autophagy gene *Atg5* is one of the *Pumilio* embryonic targets. We anticipated that the *Atg5* mRNA level would increase in *Pumilio* mutant or knock down flies and down regulation of *Atg5* would enhance CHMP2B<sup>intron5</sup> eye phenotype. Our preliminary data from a genetic interaction experiment supports this scenario (Figure 3.6). The change in *Atg5* transcript level could be further consolidated by quantification of *Atg5* mRNA and protein level in *Pumilio* mutant flies. In addition, the biophysical interaction between *Pumilio* and *Atg5* mRNA could be examined by performing electrophoretic mobility shift assays. Autophagy pathway is one of the pathways affected by CHMP2B<sup>intron5</sup> mutation (11). If a member of the autophagy pathway is confirmed to be downstream target of *Pumilio* regulation upon

CHMP2B<sup>intron5</sup> toxicity, it will advance our current understanding of the cross talk between RNA metabolism and autophagy in FTD-ALS.

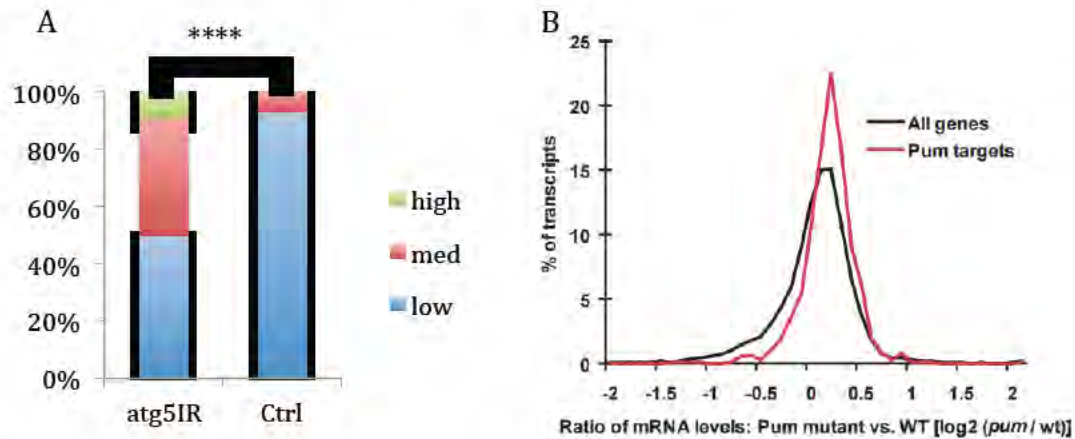


Figure 3.5 A. Atg5-IR (inverted repeats, loss of function) enhances CHMP2B<sup>intron5</sup> toxicity. P-value for Chi-square test is <0.00001. B. Gene expression profile of *Pumilio* mutant flies, *Pumilio* target genes exhibit general increase in transcript abundance relative to non-target genes (from (25)).

## Chapter 4. DISCUSSIONS

Previous studies showed that mutant *CHMP2B* affects RNA metabolism. In this study, we identified several RNA binding proteins as genetic modifiers of *CHMP2B*<sup>intron5</sup> toxicity, indicating that regulation of RNA metabolism could also modify mutant *CHMP2B* phenotype. We further confirmed *Pumilio* as a strong suppressor. The suppression could be due to direct regulation of *CHMP2B*<sup>intron5</sup> protein level, or indirect regulation of downstream pathways. Our data support the latter one. The autophagy related gene *Atg5* is one of the downstream targets of *Pumilio* regulation according to a DNA microarray analysis (25). The genetic interaction between *Atg5* and *CHMP2B*<sup>intron5</sup> indicates that *Pumilio* downregulation may suppress *CHMP2B*<sup>intron5</sup> phenotype by up regulation of *Atg5*. The possibility that *Pumilio* could regulate autophagy pathway would further strengthened the links between RNA metabolism and protein homeostasis, the converging mechanism in FTD-ALS.

However, it remains to be addressed whether the hypothesized regulatory role of *Pumilio* in flies is conserved in mammalian systems. *Pumilio* mutant or RNAi suppresses neurodegenerative phenotype caused by *CHMP2B*<sup>intron5</sup> in fly eyes, whereas partial loss of *Pumilio1* exacerbates SCA1-like neurodegeneration(24). Therefore, it would be necessary to test the interaction between *Pumilio* and *CHMP2B*<sup>intron5</sup> in mammalian systems, both *in vitro* and *in vivo*. We anticipate that in cell lines expressing *CHMP2B*<sup>intron5</sup> and forebrain neurons of transgenic mice expressing *CHMP2B*<sup>intron5</sup> under the control of tetracycline promoter, there will be an increase in expression levels and possible change in subcellular distribution of *Pumilio*. Furthermore, downregulation of *Pumilio* may be developed into a therapeutic strategy for FTD with *CHMP2B* mutations.

## APPENDICES

Table 1. List of candidate RNAi lines for genetic screen

Screen #	CG #	VDRC #	Targeted Gene Name	
1	CG3373	1278GD	Hmu	Hemomucin
2	CG13425	2912GD	bl	bancal
3	CG5393	4289GD	apt	apontic
4	CG43113	5158GD	orb2	also CG43782
5	CG6203	8933GD	Fmr1	
6	CG31716	10850GD	Cnot4	Cnot 4 homologue
7	CG6222	10853GD	su(s)	suppressor of sable
8	CG5099	11784GD	msi	musashi
9	CG1101	12031GD	Ref1	RNA and export factor binding protein 1
10	CG10293	13756GD	how	held out wings
11	CG7879	15260GD	CG7879	
12	CG10377	16040GD	Hrb27C	Heterogeneous nuclear ribonucleoprotein at 27C
13	CG11738	16289GD	l(1)G0004	lethal (1) G0004
14	CG10711	16846GD	Vps36	Vacuolar protein sorting 36
15	CG32721	17038GD	NELF-B	
16	CG11726	17544GD	CG11726	
17	CG11726	17545GD	CG11726	
18	CG14506	17684GD	CG14506	
19	CG1691	20321GD	Imp	IGF-II mRNA-binding protein
20	CG2910	20942GD	nito	spenito
21	CG2931	20946GD	CG2931	
22	CG5994	21009GD	Nelf-E	Negative elongation factor E
23	CG17136	21083GD	Rbp1	RNA-binding protein 1
24	CG3162	21379GD	LS2	Large Subunit 2
25	CG4760	21536GD	bol	boule
26	CG3949	21752GD	hoip	hoi-polloi
27	CG4051	21779GD	egl	egalitarian
28	CG34354	21949GD	CG34354	
29	CG5263	22044GD	smg	smaug
30	CG5655	22186GD	Rsf1	Repressor splicing factor 1
31	CG5808	22199GD	CG5808	

32	CG6937	22315GD	CG6937	
33	CG10302	22837GD	bsf	bicoid stability factor
34	CG10466	23368GD	CG10466	
35	CG8021	23675GD	CG8021	
36	CG1316	23851GD	CG1316	
37	CG5728	24696GD	CG5728	
38	CG3312	24742GD	Rnp4F	RNA-binding protein 4F
39	CG14628	24889GD	CG14628	
40	CG6049	25497GD	barc	barricade
41	CG3294	26110GD	CG3294	
42	CG3594	26246GD	Eap	Exu-associated protein
43	CG3691	26270GD	Pof	Painting of fourth
44	CG3613	26332GD	qkr58E-1	quaking related 58E-1
45	CG4119	26395GD	CG4119	
46	CG4266	26472GD	CG4266	
47	CG4806	26633GD	CG4806	
48	CG9346	27013GD	CG9346	
49	CG5654	27472GD	yps	ypsilon schachtel
50	CG5720	27487GD	Nab2	
51	CG6605	27683GD	BicD	Bicaudal D
52	CG7804	28068GD	CG7804	
53	CG9218	28117GD	sm	smooth
54	CG5422	28649GD	Rox8	
55	CG8636	28937GD	CG8636	
56	CG32562	28996GD	xmas-2	
57	CG9654	29116GD	Rbp4	RNA-binding protein 4
58	CG9983	29523GD	Hrb98DE	Heterogeneous nuclear ribonucleoprotein at 98DE
59	CG10203	31203GD	x16	
60	CG10948	31388GD	CG10948	
61	CG13298	31777GD	CG13298	
62	CG1340	32192GD	CG1340	
63	CG16901	32395GD	sqd	squid
64	CG17031	32829GD	Ref2	RNA and export factor binding protein 2
65	CG17838	33011GD	Syp	Syncrip
66	CG1954	33434GD	Pkc98E	Protein C kinase 98E
67	CG31000	33735GD	heph	hephaestus
68	CG32062	34046GD	A2bp1	Ataxin-2 binding protein 1

69	CG4429	34301GD	Rbp2	RNA-binding protein 2
70	CG33522	34426GD	scaf6	
71	CG3808	34713GD	CG3808	
72	CG5215	34969GD	Zn72D	Zinc-finger protein at 72D
73	CG8781	36024GD	tsu	tsunagi
74	CG5170	37583GD	Dp1	Dodeca-satellite-binding protein 1
75	CG5821	37850GD	qkr58E-2	quaking related 58E-2
76	CG32423	37863GD	shep	alan shepard
77	CG10327	38377GD	TBPH	TAR DNA-binding protein-43 homolog
78	CG14641	38790GD	CG14641	
79	CG32707	38988GD	APC4	Anaphase Promoting Complex subunit 4
80	CG14718	39702GD	CG14718	
81	CG11820	39725GD	PQBP1	Poly-glutamine tract binding protein 1
82	CG6227	40351GD	CG6227	
83	CG3780	40471GD	Spx	Spliceosomal protein on the X
84	CG5442	40590GD	SC35	
85	CG4824	42004GD	BicC	Bicaudal C
86	CG8614	42146GD	Neos	Neosin
87	CG8980	42175GD	NiPp1	Nuclear inhibitor of Protein phosphatase 1
88	CG9984	42217GD	TH1	
89	CG11886	42971GD	Slbp	Stem-loop binding protein
90	CG5213	43450GD	CG5213	
91	CG5589	44322GD	CG5589	
92	CG9373	44658GD	rump	rumpelstiltskin
93	CG11454	45116GD	CG11454	
94	CG3689	45278GD	CG3689	
95	CG9143	46330GD	CG9143	
96	CG14230	46902GD	CG14230	
97	CG10279	46908GD	Rm62	
98	CG14414	48176GD	CG14414	
99	CG4896	48197GD	CG4896	
100	CG4396	48891GD	fne	found in neurons
101	CG1034	48966GD	bcd	bicoid
102	CG4887	49022GD	CG4887	
103	CG5893	49549GD	D	Dichaete

104	CG42276	50870GD	Pde9	Phosphodiesterase 9
105	CG12749	51759GD	Hrb87F	Heterogeneous nuclear ribonucleoprotein at 87F
106	CG16788	51851GD	RnpS1	RNA-binding protein S1
107	CG4612	52497GD	CG4612	
108	CG3460	52672GD	Nmd3	Nonsense-mediated mRNA 3
109	CG9373	100001KK	rump	rumpelstiltskin
110	CG9984	100009KK	TH1	
111	CG33522	100199KK	scaf6	
112	CG10203	100226KK	x16	
113	CG3373	100286KK	Hmu	Hemomucin
114	CG3606	100291KK	caz	cabeza
115	CG11820	100381KK	PQBP1	Poly-glutamine tract binding protein 1
116	CG3691	100546KK	Pof	Painting of fourth
117	CG5422	100563KK	Rox8	
118	CG4211	100723KK	nonA	no on or off transient A
119	CG12749	100732KK	Hrb87F	Heterogeneous nuclear ribonucleoprotein at 87F
120	CG10293	100775KK	how	held out wings
121	CG11886	100785KK	Slbp	Stem-loop binding protein
122	CG10128	100805KK	tra2	transformer 2
123	CG4429	100817KK	Rbp2	RNA-binding protein 2
124	CG3151	101412KK	Rbp9	RNA-binding protein 9
125	CG4760	101435KK	bol	boule
126	CG4396	101508KK	fne	found in neurons
127	CG8376	101511KK	ap	apterous
128	CG31184	101528KK	LSm3	
129	CG10128	101548KK	tra2	transformer 2
130	CG10377	101555KK	Hrb27C	Heterogeneous nuclear ribonucleoprotein at 27C
131	CG10328	101567KK	nonA-l	nonA-like
132	CG4070	101765KK	Tis11	Tis11 homolog
133	CG3056	101781KK	ssx	sister-of-Sex-lethal
134	CG6354	102159KK	Rb97D	Ribonuclear protein at 97D
135	CG3429	102368KK	swa	swallow
136	CG12478	102442KK	bru-3	bruno-3
137	CG34354	102597KK	CG34354	
138	CG1340	102825KK	CG1340	
139	CG9809	103355KK	Spargel	

140	CG43081	103427KK	vas	vasa
141	CG5393	103665KK	apt	apontic
142	CG6937	103728KK	CG6937	
143	CG5808	103789KK	CG5808	
144	CG14414	103952KK	CG14414	
145	CG14230	104096KK	CG14230	
146	CG4051	104141KK	egl	egalitarian
147	CG32562	104156KK	xmas-2	
148	CG1034	104160KK	bcd	bicoid
149	CG4528	104334KK	snf	sans fille
150	CG9888	104372KK	Fib	Fibrillarlin
151	CG10327	104401KK	TBPH	TAR DNA-binding protein-43 homolog
152	CG43113	104441KK	orb2	also CG43782
153	CG1101	104471KK	Ref1	RNA and export factor binding protein 1
154	CG10466	104715KK	CG10466	
155	CG43065	104949KK	bru-2	bruno-2
156	CG5442	104978KK	SC35	
157	CG1316	105148KK	CG1316	
158	CG13425	105271KK	bl	bancal
159	CG4887	105322KK	CG4887	
160	CG8636	105325KK	CG8636	
161	CG3689	105499KK	CG3689	
162	CG14718	105543KK	CG14718	
163	CG17031	105585KK	Ref2	RNA and export factor binding protein 2
164	CG3460	105619KK	Nmd3	Nonsense-mediated mRNA 3
165	CG13298	105704KK	CG13298	
166	CG14838	105707KK	CG14838	
167	CG9143	105825KK	CG9143	
168	CG14506	105907KK	CG14506	
169	CG5215	105954KK	Zn72D	Zinc-finger protein at 72D
170	CG5170	106047KK	Dp1	Dodeca-satellite-binding protein 1
171	CG5874	106245KK	Nelf-A	Negative elongation factor A
172	CG7903	106475KK	CG7903	
173	CG3949	106496KK	hoip	hoi-polloi
174	CG42458	106608KK	CG42458	
175	CG5753	106645KK	stau	staufen



176	CG4119	106696KK	CG4119	
177	CG3594	106734KK	Eap	Exu-associated protein
178	CG4896	106762KK	CG4896	
179	CG5821	106944KK	qkr58E-2	quaking related 58E-2
180	CG10881	106972KK	CG10881	
181	CG30122	106984KK	CG30122	
182	CG6049	107013KK	barc	barricade
183	CG3312	107063KK	Rnp4F	RNA-binding protein 4F
184	CG7185	107147KK	CG7185	
185	CG5893	107194KK	D	Dichaete
186	CG3780	107304KK	Spx	Spliceosomal protein on the X
187	CG8781	107385KK	tsu	tsunagi
188	CG10711	107417KK	Vps36	Vacuolar protein sorting 36
189	CG8205	107575KK	fus	fusilli
190	CG1794	107888KK	Mmp2	Matrix metalloproteinase 2
191	CG16788	107953KK	RnpS1	RNA-binding protein S1
192	CG6695	107965KK	CG6695	
193	CG42458	108072KK	CG42458	
194	CG6605	108084KK	BicD	Bicaudal D
195	CG9218	108351KK	sm	smooth
196	CG5589	108642KK	CG5589	
197	CG3808	108653KK	CG3808	
198	CG4886	108734KK	cyp33	cyclophilin-33
199	CG8980	108859KK	NiPp1	Nuclear inhibitor of Protein phosphatase 1
200	CG8614	108950KK	Neos	Neosin
201	CG2910	109436KK	nito	spenito
202	CG7804	109689KK	CG7804	
203	CG43065	109739KK	bru-2	bruno-2
204	CG9412	109911KK	rin	rasputin
205	CG17136	110008KK	Rbp1	RNA-binding protein 1
206	CG5263	110207KK	smg	smaug
207	CG11094	110306KK	dsx	doublesex
208	CG10948	110357KK	CG10948	
209	CG31716	110472KK	Cnot4	Cnot 4 homologue
210	CG14641	110507KK	CG14641	
211	CG32062	110518KK	A2bp1	Ataxin-2 binding protein 1
212	CG5720	110710KK	Nab2	
213	CG31000	110749KK	heph	hephaestus

214	CG6227	110778KK	CG6227	
215	CG7082	2554GD	papi	
216	CG11274	6439GD	SRm160	
217	CG1101	12031GD	Ref1	RNA and export factor binding protein 1
218	CG2522	14877GD	Gtp-bp	GTP-binding protein
219	CG11505	15286GD	CG11505	
220	CG10384	16045GD	CG10384	
221	CG11109	17528GD	CG11109	
222	CG11123	18142GD	CG11123	
223	CG1582	19617GD	CG1582	
224	CG12924	20280GD	Lsm11	
225	CG18787	20697GD	CG18787	
226	CG18789	20699GD	CG18789	
227	CG18823	20708GD	CG18823	
228	CG5434	21614GD	Srp72	Signal recognition particle protein 72
229	CG2950	21763GD	CG2950	
230	CG4547	21867GD	Atx-1	Ataxin 1
231	CG4816	21951GD	qkr54B	quaking related 54B
232	CG4849	21962GD	CG4849	
233	CG5347	22089GD	CG5347	
234	CG7035	22331GD	Cbp80	cap binding protein 80
235	CG18591	23569GD	SmE	Small ribonucleoprotein particle protein SmE
236	CG16807	23843GD	roq	roquin
237	CG33526	25787GD	PNUTS	
238	CG31957	25926GD	CG31957	
239	CG5316	25953GD	CG5316	
240	CG3584	26242GD	qkr58E-3	quaking related 58E-3
241	CG4810	26637GD	CG4810	
242	CG34334	27020GD	CG34334	
243	CG9596	27026GD	CG9596	
244	CG10803	27318GD	CG10803	
245	CG7437	28024GD	mub	mushroom-body expressed
246	CG13124	29649GD	CG13124	
247	CG1249	31946GD	SmD2	Small ribonucleoprotein particle protein SmD2
248	CG2253	33057GD	Upf2	
249	CG6779	37741GD	RpS3	Ribosomal protein S3

250	CG2100	38037GD	CG2100	
251	CG31155	39134GD	Rpb7	
252	CG9705	40665GD	CG9705	
253	CG40351	40682GD	Set1	
254	CG9004	40727GD	CG9004	
255	CG18410	41669GD	Ude	Uracil-DNA degrading factor
256	CG5434	43978GD	Srp72	Signal recognition particle protein 72
257	CG9484	44675GD	hyd	hyperplastic discs
258	CG12131	45744GD	Adam	
259	CG31992	45772GD	gw	gawky
260	CG9755	45815GD	pum	pumilio
261	CG4659	46199GD	Srp54k	Signal recognition particle protein 54k
262	CG14100	46739GD	CG14100	
263	CG8241	47782GD	pea	peanuts
264	CG17768	49674GD	CG17768	
265	CG9099	49895GD	CG9099	
266	CG1677	50195GD	CG1677	
267	CG4279	50653GD	LSm1	
268	CG13690	100017KK	CG13690	
269	CG31256	100122KK	Brf	
270	CG8635	100278KK	CG8635	
271	CG6011	100287KK	Prp18	
272	CG31426	100304KK	ligatin	
273	CG31155	100309KK	Rpb7	
274	CG16725	100392KK	Smn	survival motor neuron
275	CG3508	100500KK	Hexim	HEXIM ortholog
276	CG1249	100690KK	SmD2	Small ribonucleoprotein particle protein SmD2
277	CG4816	100702KK	qkr54B	quaking related 54B
278	CG8443	100709KK	clu	clueless
279	CG11274	100751KK	SRm160	
280	CG13124	100966KK	CG13124	
281	CG8276	101090KK	bin3	bicoid-interacting protein 3
282	CG11123	101297KK	CG11123	
283	CG5641	101343KK	CG5641	
284	CG31140	101347KK	CG31140	
285	CG10851	101740KK	B52	
286	CG9099	101746KK	CG9099	

287	CG9677	103559KK	Int6	Int6 homologue
288	CG8427	103560KK	SmD3	Small ribonucleoprotein particle protein SmD3
289	CG31992	103581KK	gw	gawky
290	CG6694	103643KK	ZC3H3	
291	CG10228	103710KK	Inr-a	Inverse regulator a
292	CG1664	103715KK	sbr	small bristles
293	CG33158	103751KK	CG33158	
294	CG6412	103791KK	CG6412	
295	CG12938	103819KK	Lsm10	
296	CG8395	103922KK	Rrp42	
297	CG1898	104327KK	HBS1	
298	CG4810	104342KK	CG4810	
299	CG5941	104349KK	CG5941	
300	CG3642	104351KK	Clp	Clipper
301	CG9705	104426KK	CG9705	
302	CG1101	104471KK	Ref1	RNA and export factor binding protein 1
303	CG4620	104665KK	unk	unkempt
304	CG3019	104716KK	su(w <sup>a</sup> )	suppressor of white-apricot
305	CG3249	105107KK	spoon	spoonbill
306	CG8778	105442KK	CG8778	
307	CG33197	105486KK	mbl	
308	CG7437	105495KK	mub	mushroom-body expressed
309	CG42257	105744KK	Snp	Snipper
310	CG17737	105763KK	CG17737	
311	CG31601	105860KK	CG31601	
312	CG1987	105883KK	Rbp1-like	
313	CG10418	105940KK	CG10418	
314	CG6094	106144 KK	CG6094	
315	CG9596	106324KK	CG9596	
316	CG5193	106688KK	TfIIB	Transcription factor IIB
317	CG15440	106754KK	CG15440	
318	CG4457	106756KK	Srp19	Signal recognition particle protein 19
319	CG9425	106833KK	CG9425	
320	CG33526	106862KK	PNUTS	
321	CG17018	106964KK	CG17018	
322	CG1685	106974KK	pen	penguin

323	CG2100	107002KK	CG2100	
324	CG5941	107297KK	CG5941	
325	CG34362	107503KK	CG34362	
326	CG4567	107709KK	ico	iconoclast
327	CG12750	107717KK	ncm	nucampholin
328	CG42670	107912KK	ps	pasilla
329	CG42551	107986KK	larp	La related protein
330	CG6413	108013KK	Dis3	
331	CG18591	108126KK	SmE	Small ribonucleoprotein particle protein SmE
332	CG11337	108158KK	CG11337	
333	CG8335	108169KK	CG8335	
334	CG18596	108183KK	CG18596	
335	CG14100	108449KK	CG14100	
336	CG8194	108790KK	RNaseX2 5	Ribonuclease X25
337	CG5649	108801KK	kin17	
338	CG9107	109500KK	CG9107	
339	CG12085	109796KK	pUf68	poly U binding factor 68kD
340	CG1987	109844KK	Rbp1- like	
341	CG34334	110116KK	CG34334	
342	CG6999	110143KK	CG6999	
343	CG8273	110165KK	CG8273	
344	CG5347	110427KK	CG5347	
345	CG11360	110476KK	CG11360	
346	CG10110	110571KK	Cpsf160	Cleavage and polyadenylation specificity factor 160
347	CG7035	110673KK	Cbp80	cap binding protein 80
348	CG42382	10463GD	CG42382	
349	CG40351	10833GD	Set1	
350	CG8194	13018GD	RNaseX2 5	Ribonuclease X25
351	CG5836	13426GD	SF1	Splicing factor 1
352	CG6011	13760GD	Prp18	
353	CG8335	15507GD	CG8335	
354	CG10473	16052GD	Acn	Acinus
355	CG11337	16422GD	CG11337	
356	CG12129	17065GD	CG12129	
357	CG12750	17304GD	ncm	nucampholin

358	CG42551	17366GD	larp	La related protein
359	CG10110	18009GD	Cpsf160	Cleavage and polyadenylation specificity factor 160
360	CG5705	19855GD	CG5705	
361	CG12085	20144GD	pUf68	poly U binding factor 68kD
362	CG16941	20338GD	CG16941	
363	CG31426	21340GD	ligatin	
364	CG31601	21378GD	CG31601	
365	CG4043	21776GD	Rrp46	
366	CG4076	21784GD	Nufip	
367	CG4709	21923GD	CG4709	
368	CG5263	22044GD	smg	smaug
369	CG5454	22132GD	snRNP-U1-C	small ribonucleoprotein particle U1 subunit C
370	CG6169	22272GD	Dcp2	Decapping protein 2
371	CG10630	22846GD	blanks	
372	CG5417	23422GD	Srp14	Signal recognition particle protein 14
373	CG8778	23621GD	CG8778	
374	CG9344	23689GD	CG9344	
375	CG14648	23918GD	lost	
376	CG8635	24131GD	CG8635	
377	CG8395	24305GD	Rrp42	
378	CG30176	25592GD	wibg	within bgcn
379	CG3019	25598GD	su(w <sup>a</sup> )	suppressor of white-apricot
380	CG32364	26044GD	CG32364	
381	CG8963	26917GD	CG8963	
382	CG31140	27193GD	CG31140	
383	CG6422	27615GD	CG6422	
384	CG6987	27776GD	SF2	
385	CG4279	28793GD	LSm1	
386	CG8273	28887GD	CG8273	
387	CG8882	28976GD	Trip1	
388	CG8912	28989GD	Psi	P-element somatic inhibitor
389	CG17737	29216GD	CG17737	
390	CG7006	29512GD	CG7006	
391	CG1957	29575GD	Cpsf100	Cleavage and polyadenylation specificity factor 100
392	CG3927	29674GD	CG3927	
393	CG6342	30153GD	Irp-1B	Iron regulatory protein 1B

394	CG10753	31342GD	SmD1	Small ribonucleoprotein particle protein SmD1
395	CG10837	31364GD	eIF-4B	Eukaryotic initiation factor 4B
396	CG1664	32691GD	sbr	small bristles
397	CG17018	32810GD	CG17018	
398	CG7184	34373GD	Mkrn1	Makorin 1
399	CG31950	34750GD	CG31950	
400	CG4567	34875GD	ico	iconoclast
401	CG7878	35288GD	CG7878	
402	CG8427	35933GD	SmD3	Small ribonucleoprotein particle protein SmD3
403	CG7066	36572GD	Sbp2	SECIS-binding protein 2
404	CG10228	38365GD	Inr-a	Inverse regulator a
405	CG33100	38399GD	4EHP	eIF4E-Homologous Protein
406	CG12938	38754GD	Lsm10	
407	CG10851	38860GD	B52	
408	CG5352	40587GD	SmB	Small ribonucleoprotein particle protein SmB
409	CG8443	42136GD	clu	clueless
410	CG15014	43615GD	CG15014	
411	CG4817	44344GD	Ssrp	Structure specific recognition protein
412	CG9323	44984GD	CG9323	
413	CG5605	45027GD	eRF1	eukaryotic release factor 1
414	CG16725	45447GD	Smn	survival motor neuron
415	CG9609	47605GD	CG9609	
416	CG33158	47737GD	CG33158	
417	CG3249	48005GD	spoon	spoonbill
418	CG5641	48200GD	CG5641	
419	CG6094	48721GD	CG6094	
420	CG30100	48945GD	CG30100	
421	CG32708	49325GD	CG32708	
422	CG18497	49542GD	spen	split ends
423	CG18259	50094GD	CG18259	
424	CG10418	50245GD	CG10418	
425	CG12357	50876GD	Cbp20	cap binding protein 20
426	CG33714	51019GD	CG33714	
427	CG4602	51088GD	Srp54	
428	CG16940	51854GD	CG16940	
429	CG4043	100277KK	Rrp46	

430	CG8268	100768KK	Srp9	Signal recognition particle protein 9
431	CG7138	101288KK	r2d2	
432	CG7184	101694KK	Mkrl1	Makorin 1
433	CG14891	102118KK	CG14891	
434	CG12493	102360KK	CG12493	
435	CG34362	102523KK	CG34362	
436	CG5334	102765KK	CG5334	
437	CG8882	103141KK	Trip1	
438	CG5064	104867KK	Srp68	Signal recognition particle protein 68
439	CG10384	104941KK	CG10384	
440	CG6169	105130KK	Dcp2	Decapping protein 2
441	CG8912	105135KK	Psi	P-element somatic inhibitor
442	CG4900	105583KK	Irp-1A	Iron regulatory protein 1A
443	CG12413	105672KK	CG12413	
444	CG8268	105682KK	Srp9	Signal recognition particle protein 9
445	CG11505	105949KK	CG11505	
446	CG10084	105950KK	swm	second mitotic wave missing
447	CG10306	105991KK	CG10306	
448	CG6382	106240KK	Elf	Ef1 $\alpha$ -like factor
449	CG10868	106257KK	orb	oo18 RNA-binding protein
450	CG6779	106321KK	RpS3	Ribosomal protein S3
451	CG10803	106599KK	CG10803	
452	CG6610	106830KK	CG6610	
453	CG12357	107112KK	Cbp20	cap binding protein 20
454	CG16941	107162KK	CG16941	
455	CG42768	107183KK	Msp-300	Muscle-specific protein 300
456	CG13163	107373KK	CG13163	
457	CG16792	107644KK	SmF	Small ribonucleoprotein particle protein SmF
458	CG32708	107827KK	CG32708	
459	CG7006	108205KK	CG7006	
460	CG12924	108336KK	Lsm11	
461	CG5316	108346KK	CG5316	
462	CG4547	108396KK	Atx-1	Ataxin 1
463	CG4849	108597KK	CG4849	
464	CG42670	108666KK	ps	pasilla
465	CG15014	108789KK	CG15014	



466	CG18497	108828KK	spen	split ends
467	CG5637	108900KK	nos	nanos
468	CG5454	108933KK	snRNP-U1-C	small ribonucleoprotein particle U1 subunit C
469	CG42768	109023KK	Msp-300	Muscle-specific protein 300
470	CG32706	109212KK	CG32706	
471	CG1677	109697KK	CG1677	
472	CG6961	109951KK	CG6961	
473	CG18787	109955KK	CG18787	
474	CG18823	110023KK	CG18823	
475	CG5263	110207KK	smg	smaug
476	CG18811	110272KK	Capr	Caprin
477	CG4659	110284KK	Srp54k	Signal recognition particle protein 54k
478	CG9323	110410KK	CG9323	
479	CG15481	110412KK	Ski6	
480	CG32344	110441KK	CG32344	
481	CG8241	110452KK	pea	peanuts
482	CG5352	110713KK	SmB	Small ribonucleoprotein particle protein SmB
483	CG14648	110736KK	lost	
484	CG31957	110758KK	CG31957	
485	CG4620	4267GD	unk	unkempt
486	CG1147	9605GD	NPFR1	neuropeptide F receptor
487	CG10993	16166GD	CG10993	
488	CG32344	21675GD	CG32344	
489	CG6122	22235GD	piwi	
490	CG6143	22245GD	Pep	Protein on ecdysone puffs
491	CG13277	23862GD	LSm7	
492	CG13690	24648GD	CG13690	
493	CG3642	26259GD	Clp	Clipper
494	CG3688	26269GD	l(2)35Bd	lethal (2) 35Bd
495	CG3931	26310GD	Rrp4	
496	CG7138	26727GD	r2d2	
497	CG10889	27330GD	CG10889	
498	CG5064	27351GD	Srp68	Signal recognition particle protein 68
499	CG18178	29264GD	CG18178	
500	CG16785	30214GD	fz3	frizzled 3
501	CG42569	31635GD	Larp7	La related protein 7

502	CG13472	32193GD	CG13472	
503	CG15481	32612GD	Ski6	
504	CG17540	32948GD	Spf45	
505	CG33106	33396GD	mask	multiple ankyrin repeats single KH domain
506	CG1898	33419GD	HBS1	
507	CG42257	33706GD	Snp	Snipper
508	CG3508	34632GD	Hexim	HEXIM ortholog
509	CG17768	34752GD	CG17768	
510	CG6413	35090GD	Dis3	
511	CG6961	35152GD	CG6961	
512	CG10084	38336GD	swm	second mitotic wave missing
513	CG11360	38491GD	CG11360	
514	CG6712	39012GD	CG6712	
515	CG9742	39255GD	SmG	Small ribonucleoprotein particle protein SmG
516	CG13163	39785GD	CG13163	
517	CG18596	41675GD	CG18596	
518	CG6999	41828GD	CG6999	
519	CG4021	41974GD	CG4021	
520	CG4973	42016GD	CG4973	
521	CG30100	43236GD	CG30100	
522	CG6412	44327GD	CG6412	
523	CG31156	44479GD	CG31156	
524	CG18811	45295GD	Capr	Caprin
525	CG5642	46592GD	CG5642	
526	CG6745	46746GD	CG6745	
527	CG12493	49836GD	CG12493	
528	CG4021	52380GD	CG4021	
529	CG16940	100322KK	CG16940	
530	CG7971	101384KK	CG7971	
531	CG9755	101399KK	pum	pumilio
532	CG5417	101444KK	Srp14	Signal recognition particle protein 14
533	CG3931	102375KK	Rrp4	
534	CG10473	102407KK	Acn	Acinus
535	CG33106	103411KK	mask	multiple ankyrin repeats single KH domain
536	CG7082	103708KK	papi	
537	CG9609	105776KK	CG9609	

538	CG7066	106169KK	Sbp2	SECIS-binding protein 2
539	CG6422	106251KK	CG6422	
540	CG4709	106252KK	CG4709	
541	CG5642	107267KK	CG5642	
542	CG31156	107269KK	CG31156	
543	CG1147	107663KK	NPFR1	neuropeptide F receptor
544	CG5705	108376KK	CG5705	
545	CG42382	109862KK	CG42382	
546	CG12131	110514KK	Adam	
547	CG8963	110576KK	CG8963	

## BIBLIOGRAPHY

1. Ferrari R, Hernandez DG, Nalls MA, Rohrer JD, Ramasamy A, Kwok JB, Dobson-Stone C, Brooks WS, Schofield PR, Halliday GM, Hodges JR, Piguet O, Bartley L, Thompson E, Haan E, Hernandez I, Ruiz A, Boada M, Borroni B, Padovani A, Cruchaga C, Cairns NJ, Benussi L, Binetti G, Ghidoni R, Forloni G, Galimberti D, Fenoglio C, Serpente M, Scarpini E, Clarimon J, Lleo A, Blesa R, Waldo ML, Nilsson K, Nilsson C, Mackenzie IR, Hsiung GY, Mann DM, Grafman J, Morris CM, Attems J, Griffiths TD, McKeith IG, Thomas AJ, Pietrini P, Huey ED, Wassermann EM, Baborie A, Jaros E, Tierney MC, Pastor P, Razquin C, Ortega-Cubero S, Alonso E, Pernecky R, Diehl-Schmid J, Alexopoulos P, Kurz A, Rainero I, Rubino E, Pinessi L, Rogaeva E, St George-Hyslop P, Rossi G, Tagliavini F, Giaccone G, Rowe JB, Schlachetzki JC, Uphill J, Collinge J, Mead S, Danek A, Van Deerlin VM, Grossman M, Trojanowski JQ, van der Zee J, Deschamps W, Van Langenhove T, Cruts M, Van Broeckhoven C, Cappa SF, Le Ber I, Hannequin D, Golfier V, Vercelletto M, Brice A, Nacmias B, Sorbi S, Bagnoli S, Piaceri I, Nielsen JE, Hjermand LE, Riemenschneider M, Mayhaus M, Ibach B, Gasparoni G, Pichler S, Gu W, Rossor MN, Fox NC, Warren JD, Spillantini MG, Morris HR, Rizzu P, Heutink P, Snowden JS, Rollinson S, Richardson A, Gerhard A, Bruni AC, Maletta R, Frangipane F, Cupidi C, Bernardi L, Anfossi M, Gallo M, Conidi ME, Smirne N, Rademakers R, Baker M, Dickson DW, Graff-Radford NR, Petersen RC, Knopman D, Josephs KA, Boeve BF, Parisi JE, Seeley WW, Miller BL, Karydas AM, Rosen H, van Swieten JC, Dopper EG, Seelaar H, Pijnenburg YA, Scheltens P, Logroscino G, Capozzo R, Novelli V, Puca AA, Franceschi M, Postiglione A, Milan G, Sorrentino P, Kristiansen M, Chiang HH, Graff C, Pasquier F, Rollin A, Deramecourt V, Lebert F, Kapogiannis D, Ferrucci L, Pickering-Brown S, Singleton AB, Hardy J, Momeni P. Frontotemporal dementia and its subtypes: a genome-wide association study. *Lancet Neurol.* 2014;13(7):686-99. doi: 10.1016/S1474-4422(14)70065-1. PubMed PMID: 24943344; PMCID: PMC4112126.
2. Ling SC, Polymenidou M, Cleveland DW. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron.* 2013;79(3):416-38. doi: 10.1016/j.neuron.2013.07.033. PubMed PMID: 23931993; PMCID: PMC4411085.
3. Janssens J, Wils H, Kleinberger G, Joris G, Cuijt I, Ceuterick-de Groote C, Van Broeckhoven C, Kumar-Singh S. Overexpression of ALS-associated p.M337V human TDP-43 in mice worsens disease features compared to wild-type human TDP-43 mice. *Mol Neurobiol.* 2013;48(1):22-35. doi: 10.1007/s12035-013-8427-5. PubMed PMID: 23475610; PMCID: PMC3718993.
4. Freischmidt A, Wieland T, Richter B, Ruf W, Schaeffer V, Muller K, Marroquin N, Nordin F, Hubers A, Weydt P, Pinto S, Press R, Millecamps S, Molko N, Bernard E, Desnuelle C, Soriani MH, Dorst J, Graf E, Nordstrom U, Feiler MS, Putz S, Boeckers TM, Meyer T, Winkler AS, Winkelmann J, de Carvalho M, Thal DR, Otto M, Brannstrom T, Volk AE, Kursula P, Danzer KM, Lichtner P, Dikic I, Meitinger T, Ludolph AC, Strom TM, Andersen PM, Weishaupt JH. Haploinsufficiency of TBK1 causes familial

- ALS and fronto-temporal dementia. *Nat Neurosci.* 2015;18(5):631-6. doi: 10.1038/nn.4000. PubMed PMID: 25803835.
5. Gitcho MA, Strider J, Carter D, Taylor-Reinwald L, Forman MS, Goate AM, Cairns NJ. VCP mutations causing frontotemporal lobar degeneration disrupt localization of TDP-43 and induce cell death. *J Biol Chem.* 2009;284(18):12384-98. doi: 10.1074/jbc.M900992200. PubMed PMID: 19237541; PMCID: PMC2673306.
  6. Cassel JA, Reitz AB. Ubiquilin-2 (UBQLN2) binds with high affinity to the C-terminal region of TDP-43 and modulates TDP-43 levels in H4 cells: characterization of inhibition by nucleic acids and 4-aminoquinolines. *Biochim Biophys Acta.* 2013;1834(6):964-71. doi: 10.1016/j.bbapap.2013.03.020. PubMed PMID: 23541532; PMCID: PMC3960997.
  7. Bose JK, Huang CC, Shen CK. Regulation of autophagy by neuropathological protein TDP-43. *J Biol Chem.* 2011;286(52):44441-8. doi: 10.1074/jbc.M111.237115. PubMed PMID: 22052911; PMCID: PMC3247982.
  8. Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, Nielsen JE, Hodges JR, Spillantini MG, Thusgaard T, Brandner S, Brun A, Rossor MN, Gade A, Johannsen P, Sorensen SA, Gydesen S, Fisher EM, Collinge J. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nat Genet.* 2005;37(8):806-8. doi: 10.1038/ng1609. PubMed PMID: 16041373.
  9. Urwin H, Authier A, Nielsen JE, Metcalf D, Powell C, Froud K, Malcolm DS, Holm I, Johannsen P, Brown J, Fisher EM, van der Zee J, Bruyland M, Consortium FR, Van Broeckhoven C, Collinge J, Brandner S, Futter C, Isaacs AM. Disruption of endocytic trafficking in frontotemporal dementia with CHMP2B mutations. *Hum Mol Genet.* 2010;19(11):2228-38. doi: 10.1093/hmg/ddq100. PubMed PMID: 20223751; PMCID: PMC2865375.
  10. Adell MA, Teis D. Assembly and disassembly of the ESCRT-III membrane scission complex. *FEBS Lett.* 2011;585(20):3191-6. doi: 10.1016/j.febslet.2011.09.001. PubMed PMID: 21924267; PMCID: PMC3192940.
  11. Lee JA, Beigneux A, Ahmad ST, Young SG, Gao FB. ESCRT-III dysfunction causes autophagosome accumulation and neurodegeneration. *Curr Biol.* 2007;17(18):1561-7. doi: 10.1016/j.cub.2007.07.029. PubMed PMID: 17683935.
  12. Lu Y, Zhang Z, Sun D, Sweeney ST, Gao FB. Syntaxin 13, a genetic modifier of mutant CHMP2B in frontotemporal dementia, is required for autophagosome maturation. *Mol Cell.* 2013;52(2):264-71. doi: 10.1016/j.molcel.2013.08.041. PubMed PMID: 24095276; PMCID: PMC3825790.
  13. Gascon E, Lynch K, Ruan H, Almeida S, Verheyden JM, Seeley WW, Dickson DW, Petrucelli L, Sun D, Jiao J, Zhou H, Jakovcevski M, Akbarian S, Yao WD, Gao FB. Alterations in microRNA-124 and AMPA receptors contribute to social behavioral deficits in frontotemporal dementia. *Nat Med.* 2014;20(12):1444-51. doi: 10.1038/nm.3717. PubMed PMID: 25401692; PMCID: PMC4257887.
  14. Gibbins DJ, Ciaudo C, Erhardt M, Voinnet O. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. *Nat Cell Biol.* 2009;11(9):1143-9. doi: 10.1038/ncb1929. PubMed PMID: 19684575.

15. Ahmad ST, Sweeney ST, Lee JA, Sweeney NT, Gao FB. Genetic screen identifies *serpin5* as a regulator of the toll pathway and CHMP2B toxicity associated with frontotemporal dementia. *Proc Natl Acad Sci U S A*. 2009;106(29):12168-73. doi: 10.1073/pnas.0903134106. PubMed PMID: 19581577; PMCID: PMC2715505.
16. West RJ, Lu Y, Marie B, Gao FB, Sweeney ST. Rab8, POSH, and TAK1 regulate synaptic growth in a *Drosophila* model of frontotemporal dementia. *J Cell Biol*. 2015;208(7):931-47. doi: 10.1083/jcb.201404066. PubMed PMID: 25800055; PMCID: PMC4384727.
17. Chai A, Pennetta G. Insights into ALS pathomechanisms: from flies to humans. *Fly (Austin)*. 2015;9(2):91-8. doi: 10.1080/19336934.2015.1114694. PubMed PMID: 26594942; PMCID: PMC4826116.
18. Sanhueza M, Chai A, Smith C, McCray BA, Simpson TI, Taylor JP, Pennetta G. Network analyses reveal novel aspects of ALS pathogenesis. *PLoS Genet*. 2015;11(3):e1005107. doi: 10.1371/journal.pgen.1005107. PubMed PMID: 25826266; PMCID: PMC4380362.
19. Wang X, McLachlan J, Zamore PD, Hall TM. Modular recognition of RNA by a human pumilio-homology domain. *Cell*. 2002;110(4):501-12. PubMed PMID: 12202039.
20. Wharton RP, Sonoda J, Lee T, Patterson M, Murata Y. The Pumilio RNA-binding domain is also a translational regulator. *Mol Cell*. 1998;1(6):863-72. PubMed PMID: 9660969.
21. Sonoda J, Wharton RP. *Drosophila* Brain Tumor is a translational repressor. *Genes Dev*. 2001;15(6):762-73. doi: 10.1101/gad.870801. PubMed PMID: 11274060; PMCID: PMC312658.
22. Ye B, Petritsch C, Clark IE, Gavis ER, Jan LY, Jan YN. Nanos and Pumilio are essential for dendrite morphogenesis in *Drosophila* peripheral neurons. *Curr Biol*. 2004;14(4):314-21. doi: 10.1016/j.cub.2004.01.052. PubMed PMID: 14972682.
23. Dubnau J, Chiang AS, Grady L, Barditch J, Gossweiler S, McNeil J, Smith P, Buldoc F, Scott R, Certa U, Broger C, Tully T. The *staufer/pumilio* pathway is involved in *Drosophila* long-term memory. *Curr Biol*. 2003;13(4):286-96. PubMed PMID: 12593794.
24. Gennarino VA, Singh RK, White JJ, De Maio A, Han K, Kim JY, Jafar-Nejad P, di Ronza A, Kang H, Sayegh LS, Cooper TA, Orr HT, Sillitoe RV, Zoghbi HY. Pumilio1 haploinsufficiency leads to SCA1-like neurodegeneration by increasing wild-type Ataxin1 levels. *Cell*. 2015;160(6):1087-98. doi: 10.1016/j.cell.2015.02.012. PubMed PMID: 25768905; PMCID: PMC4383046.
25. Gerber AP, Luschnig S, Krasnow MA, Brown PO, Herschlag D. Genome-wide identification of mRNAs associated with the translational regulator PUMILIO in *Drosophila melanogaster*. *Proc Natl Acad Sci U S A*. 2006;103(12):4487-92. doi: 10.1073/pnas.0509260103. PubMed PMID: 16537387; PMCID: PMC1400586.