

Genetic mapping of *Uba3*^{O.2.2}, a pupal lethal mutation in *Drosophila melanogaster*

Elizabeth Mast^{1*}, Kayla L Bieser^{1§*}, Mary Abraham-Villa², Vanessa Adams², Akinwonuola J Akinlehin², Lynarose Z Aquino¹, Joseph L Austin², Abigail K Austin², Carissa N Beckham³, Ethan J Bengson², Amanda Bieszk², Brianna L Bogard³, Rowan C Brennan², Rebecca M Brnot², Nicholas J Cirone², Mason R Clark², Brianna N Cooper², Dennys Cruz¹, Katlyn A Daprizio¹, Jason DeBoe², Michaela M Dencker², Laura L Donnelly², Leanne Driscoll², Ryan J DuBeau², Sirada W Durso¹, Adam Ejub², Waad Elgosbi², Melanie Estrada², Kaeli Evins⁴, Pearl D Fox², Jacob M France², Maira G Franco Hernandez¹, Lizbeth A Garcia², Olivia Garl², Myeerah R Gorsuch³, Mikayla A Gorzeman-mohr², Madison E Grothouse⁴, Megan E Gubbels², Romina Hakemiamjad¹, Chloé V Harvey², Madeline A Hoeppner², Jessica L Ivanov², Veronica M Johnson², Jessica L Johnson², Ashton Johnson², Kaleigh Johnston², Katie R Keller², Breanna T Kennedy³, Levi R Killian⁴, Marissa Klumb², Olivia L Koehn², Aaron S Koym², Kari J Kress², Regan E Landis³, Kaitlyn N Lewis², Enosh Lim², Ilcen K Lopez², D'Artagnan Lowe², Paula Luengo Carretero², Grace Lunaburg², Samantha L Mallinder², Natalie A Marshall³, Jessica Mathew², Jasmine Mathew², Hailee S Mcmanaway³, Emily N Meegan², Jacob D Meyst³, Meredith J Miller³, Colin K Minogue², Alina A Mohr², Cristhian I Moran¹, Adrian Moran⁴, Morgan D Morris³, Michael D Morrison², Emmily A Moses³, Cade J Mullins³, Citlalli I Neri², Jess M Nichols⁴, Breanna R Nickels³, Akosua M Okai², Chiedu Okonmah², Makenna Paramo², Meagan Paramo², Sydney L Parker³, Neil K Parmar¹, Jacob Paschal², Prem Patel², Deep Patel², Erica B Perkins⁴, Madelyn M Perry⁴, Zachary Perry², Amanda A Pollock², Oxxyris Portalatin¹, Kamron S Proffitt², Jason T Queen², Alexis C Quemeneur², Amelia G Richardson⁴, Kaylee Rosenberger², Allison M Rutherford³, Itchel X Santos-Perez¹, Christy Y Sarti¹, Lacey J Schouweiler¹, Lauren M Sessing², Sara O Setaro², Christopher F Silvestri², Olivia A Smith⁴, Mackenzie J Smith³, Jayson C Sumner³, Rachel R Sutton¹, Lindsay Sweekard², Nicholas B Talbott¹, Peyton A Traxler³, Jenna Truesdell², Aaron F Valenti³, Leif Verace², Pragathi Vijayakumar², William L Wadley¹, Katherine E Walter⁴, Ayanna R Williams², Trey J Wilson³, Makayla A Witbeck³, Trinity M Wobler³, Lucas J Wright², Karolina A Zuczkowska¹, Olivier Devergne², Danielle R Hamill⁴, Hemin P Shah², Jamie Siders³, Elizabeth E Taylor², Alysia D Vrailas-Mortimer⁵, Jacob D Kagey^{6§}

¹Nevada State College

²Northern Illinois University

³Ohio Northern University

⁴Ohio Wesleyan University

⁵Illinois State University

⁶University of Detroit Mercy

§ To whom correspondence should be addressed: kayla.bieser@nsc.edu; kageyja@udmercy.edu

*These authors contributed equally.

Abstract

An EMS mutagenesis screen was conducted in *Drosophila melanogaster* to identify growth control mutants. The multi-institution Fly-CURE consortium phenotypically characterized the O.2.2 mutant using the FLP/FRT system which displayed a mutant lethal phenotype with reduced head development, and darkened ocular tissue. Complementation mapping was conducted to identify the affected gene. A failure to complement was identified in *Uba3*, resulting in the identification of the novel allele, *Uba3*^{O.2.2}. *Uba3* is a known disruptor of the cell cycle and our data are consistent with early larval/embryonic lethality displayed in numerous species.

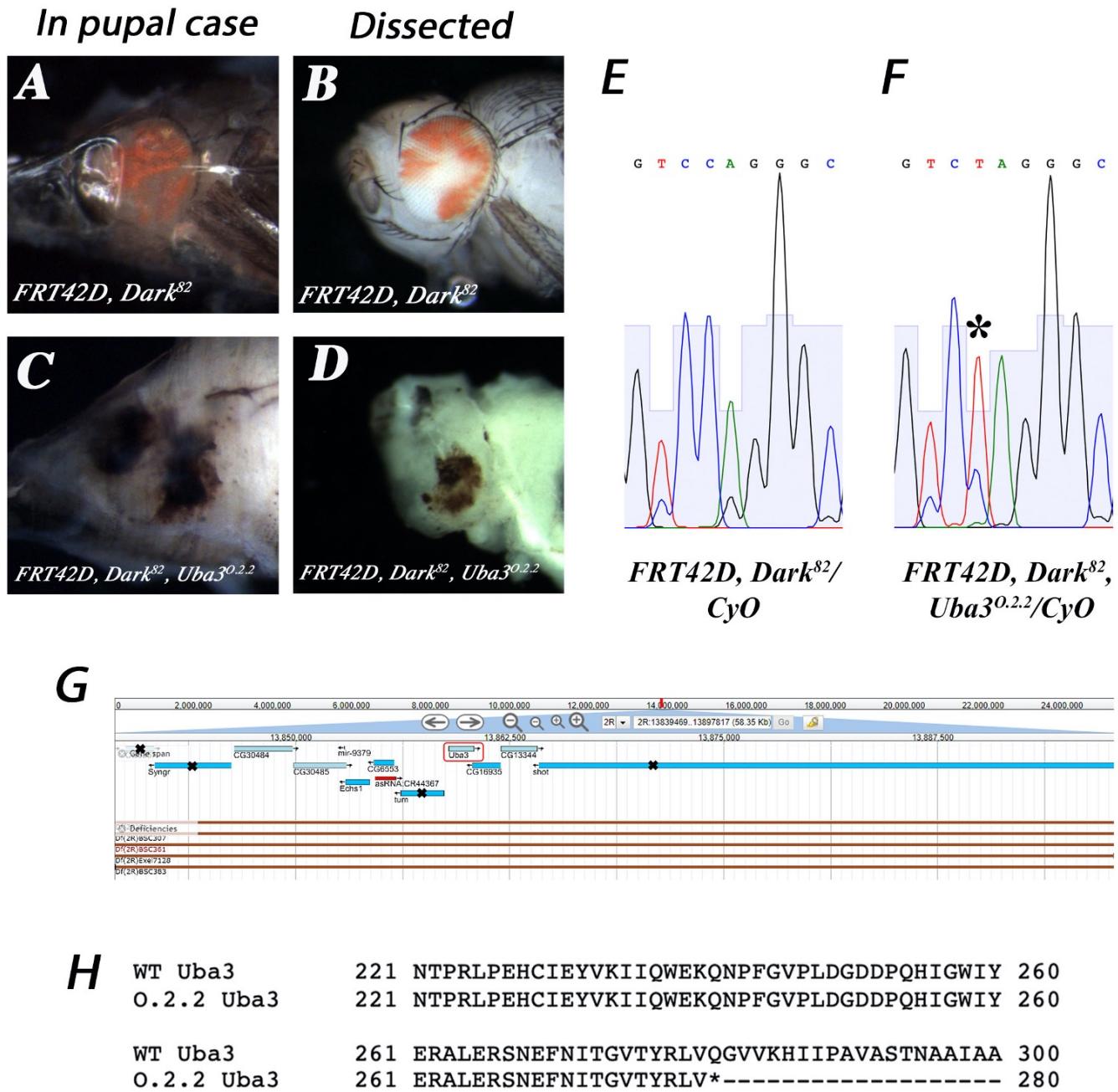


Figure 1. Characterization of the lethal *Uba3*^{O.2.2} mutation by phenotypic analysis and complementation mapping.

FRT42D, Dark⁸² control (A-B) display a mosaic eye of red (mw⁺) (mutant) to white (mw⁻) (wildtype) tissue in a viable pupa. (C-D) *FRT42D, Dark⁸², Uba3^{O.2.2}* mutant was pupal lethal, displaying a reduced head and darkened ocular tissue. (E) Sanger sequencing analysis of *FRT42D, Dark⁸²* control and (F) *FRT42D, Dark⁸², Uba3^{O.2.2}* mutant indicating a heterozygous peak at 2R:13,859,838 (C to T). (G) The smallest region the O.2.2 mutant failed to complement was 2R:13,839,479..13,897,827 as defined by the leftmost breakpoint of Df(2R)BSC383 and the rightmost breakpoint of Df(2R)Exel7128. All genes with an "X" were tested and complemented O.2.2 with only the *Uba3^{SH2028}* failing to complement. (H) Amino acid alignment of *FRT42D, Dark⁸²* control (WT *Uba3*) to *FRT42D, Dark⁸², Uba3^{O.2.2}* mutant (O.2.2 *Uba3*) displaying the nonsense mutation in *Uba3^{O.2.2}*. Image adapted from flybase.org (Gramates et al., 2017).

Description

3/18/2022 - Open Access

Induced ethyl methanesulfonate (EMS) mutations were genetically screened utilizing the FLP/FRT recombinase system to study cell overgrowth phenotypes in mosaic eyes of *Drosophila melanogaster*. EMS is a mutagen typically resulting in single nucleotide substitutions. Homozygosity was induced in the eye utilizing the FLP/FRT mitotic recombination system to study phenotype developments without homozygous lethality throughout the remainder of the specimen. It has been previously reported that genes impacting cell growth or division (such as *Dark*, *Ptc*) trigger apoptosis leading to cell death (Akdemir *et al.* 2006). In the presence of *Dark*⁸², apoptosis is blocked, and the overgrowth phenotype persists in an observable manner (Kagey *et al.* 2012). Male stock *Drosophila* (genotype *FRT42D*, *Dark*⁸², *O.2.2/CyO* and genotype *FRT42D*, *Dark*⁸²/*CyO*) were crossed with females (genotype *FRT42D*; *Ey-Flp*) to promote mitotic recombination, creating offspring exhibiting either the control mosaic eye phenotype or the mutant phenotype (Figures 1A-D). The control cross (Figures 1A and 1B) displayed a typical phenotypic red:white ratio in pharate adults. The *O.2.2* mutant, however, was pupal lethal, displaying an underdeveloped head and black pigmented eye tissue (Figures 1C and 1D).

To identify the gene locus responsible for the *O.2.2* mutation, complementation mapping was conducted by undergraduate researchers at Nevada State College, Northern Illinois University, Albion College, Ohio Northern University, and Ohio Wesleyan University participating in the Fly-CURE consortium. This mapping was conducted during the COVID-19 pandemic which resulted in data collection through in-person, hybrid, and virtual courses. Complementation mapping has been successfully utilized by undergraduates to map the location of mutations driving the phenotypes produced from the FLP/FRT screen (Talley *et al.* 2021). Virgin female *Drosophila* (genotype *FRT42D*, *Dark82*, *O.2.2/CyO*) were crossed with males from each of the 86 deficiency stocks from the Bloomington Stock Center 2R Deficiency Kit (that are distal to the *FRT42D* site). As the *O.2.2* mutation is homozygous lethal, F1 offspring were scored for the presence or absence of straight wings with the absence of straight wings an indicator of a failure to complement (Cook *et al.* 2012) (Table 1). The initial round of mapping resulted in three deficiency stocks failing to complement: *Df(2R)CX1*, *Df(2R)BSC383*, and *Df(2R)BSC307*. Two additional stocks outside of the 2R kit were tested resulting in the smallest region of failure to complement *O.2.2* of 2R:13,839,479..13,897,827 (Figure 1E). Students then selected seven genes within this region for further study (Table 1). Alleles of six of these genes complemented the *O.2.2* mutation (Table 1). We tested two alleles of *Uba3*: *Uba3*^{G8197} and *Uba3*^{SH2028}, which are both homozygous lethal transgenic insertion mutations and *Uba3*^{SH2028} has previously been identified as a null mutation (Du *et al.* 2011). We found that the *Uba3*^{G8197} allele complemented the *O.2.2* mutation while the *Uba3*^{SH2028} mutation failed to complement. We further investigated the *Uba3*^{G8197} stock and found that it was no longer homozygous lethal, suggesting that this stock had lost the mutation in *Uba3*. The data we provided to the BDSC resulted in the removal of the *Uba3*^{G8197} stock. As the *Uba3*^{SH2028} allele failed to complement *O.2.2* (Table 1), students designed primers to different regions of the *Uba3* gene and identified a nonsense mutation at 2R:13,859,838 (Gln281Stop at amino acid 281) leading to a premature stop codon.

Based upon the observed phenotype and genetic mapping in *Drosophila melanogaster*, we conclude that *O.2.2* is a novel allele of *Uba3* (*Uba3*^{O.2.2}), resulting in a mosaic pupal lethal phenotype. *Uba3* encodes for a ubiquitin-like activating enzyme that adds NEDD8 (neural precursor cell expressed, developmentally downregulated 8; E1^{NEDD8}) to proteins in a process called neddylation. Neddylation and the ubiquitin-proteasome system (UPS), which regulate proteolysis in the cell (Du *et al.* 2011, Nalepa *et al.* 2006), are critical for regulation of many developmental processes including multiple pathways for cell cycle progression (Tateishi *et al.* 2001, Nalepa *et al.* 2006). Evidence of *Uba3* knockdown in *Drosophila* and mice result in early larval/embryonic lethality (Du *et al.* 2011, Tateishi *et al.* 2001). The nonsense mutation we report is predicted to eliminate the E2-binding domain (associated with amino acids 354-443), which is a necessary conjugating enzyme in the NEED8 cascade (Huang *et al.* 2005). The elimination of this critical domain is consistent with the lethal phenotype observed in *Uba3*^{O.2.2}.

Due to its role in disrupting the cell cycle, *Uba3* is a plausible early target for disrupting the cell cycle of the E1 activating enzyme in cancer cells. An inhibitor of E1^{NEDD8}, MLN4924, has been utilized *in vitro* to target cancer cells but its effectiveness was reduced in cells with *Uba3* mutations leading to resistance of MLN4924 (Xu *et al.* 2014). More recently, MLN4924, has shown promise in clinical trials for inhibiting growth and migration of cancer cells but a greater mechanistic understanding of the role of *Uba3* in neddylation is still required as there appears to be an interplay between high glucose levels and the upregulation of *Uba3* (Du *et al.* 2021). Future characterizations in *Drosophila* may help to elucidate these complex relationships.

Reagents

w⁻; *FRT42D*, *Dark*⁸²/*CyO* (Akdemir *et al.*, 2006)

w⁻; *FRT42D* *Dark*⁸², *Uba3*^{3O.2.2}/*CyO* (this study)

3/18/2022 - Open Access

w⁻; FRT42D; Ey-Flp (BDSC 8211)Bloomington *Drosophila* Stock Center 2R Deficiency Kit (Cook et al., 2012)

Additional Bloomington Stocks (See Table 1 for complete list of stock numbers)

Acknowledgments: Stocks obtained from the Bloomington *Drosophila* Stock Center (NIH P40OD018537) and Kyoto DGRC were used in this study.

Extended Data

Description: Complementation mapping data. Resource Type: Dataset. File: [O22_Table_1_final.pdf](#). DOI: [10.22002/D1.20065](#)

References

Akdemir F, Farkas R, Chen P, Juhasz G, Medved'ová L, Sass M, Wang L, Wang X, Chittaranjan S, Gorski SM, Rodriguez A, Abrams JM. 2006. Autophagy occurs upstream or parallel to the apoptosome during histolytic cell death. *Development* 133: 1457-65. PubMed ID: [16540507](#)

Cook RK, Christensen SJ, Deal JA, Coburn RA, Deal ME, Gresens JM, Kaufman TC, Cook KR. 2012. The generation of chromosomal deletions to provide extensive coverage and subdivision of the *Drosophila melanogaster* genome. *Genome Biol* 13: R21. PubMed ID: [22445104](#)

Du MG, Peng ZQ, Gai WB, Liu F, Liu W, Chen YJ, Li HC, Zhang X, Liu CH, Zhang LQ, Jiang H, Xie P. 2021. The Absence of PTEN in Breast Cancer Is a Driver of MLN4924 Resistance. *Front Cell Dev Biol* 9: 667435. PubMed ID: [33996822](#)

Du J, Zhang J, Su Y, Liu M, Ospina JK, Yang S, Zhu AJ. 2011. In vivo RNAi screen reveals neddylation genes as novel regulators of Hedgehog signaling. *PLoS One* 6: e24168. PubMed ID: [21931660](#)

Huang DT, Paydar A, Zhuang M, Waddell MB, Holton JM, Schulman BA. 2005. Structural basis for recruitment of Ubc12 by an E2 binding domain in NEDD8's E1. *Mol Cell* 17: 341-50. PubMed ID: [15694336](#)

Kagey JD, Brown JA, Moberg KH. 2012. Regulation of Yorkie activity in *Drosophila* imaginal discs by the Hedgehog receptor gene patched. *Mech Dev* 129: 339-49. PubMed ID: [22705500](#)

Nalepa G, Rolfe M, Harper JW. 2006. Drug discovery in the ubiquitin-proteasome system. *Nat Rev Drug Discov* 5: 596-613. PubMed ID: [16816840](#)

Tateishi K, Omata M, Tanaka K, Chiba T. 2001. The NEDD8 system is essential for cell cycle progression and morphogenetic pathway in mice. *J Cell Biol* 155: 571-9. PubMed ID: [11696557](#)

Talley EM, Watts CT, Aboyer S, Adamson MG, Akoto HA, Altemus H, Avella PJ, Bailey R, Bell ER, Bell KL, Breneman K, Burkhardt JS, Chanley LJ, Cook SS, DesLaurier MT, Dorsey TR, Doyle CJ, Egloff ME, Fasawe AS, Garcia KK, Graves NP, Gray TK, Gustafson EM, Hall MJ, Hayes JD, Holic LJ, Jarvis BA, Klos PS, Kitzmire S, Kuzovko L, Lainez E, McCoy S, Mierendorf JC, Neri NA, Neville CR, Osborn K, Parker K, Parks ME, Peck K, Pitt R, Platta ME, Powell B, Rodriguez K, Ruiz C, Schaefer MN, Shields AB, Smiley JB, Stauffer B, Straub D, Sweeney JL, Termine KM, Thomas B, Toth SD, Veile TR, Walker KS, Webster PN, Woodard BJ, Yoder QL, Young MK, Zeedyk ML, Ziegler LN, Bieser KL, Puthoff DP, Stamm J, Vralias-Mortimer AD, Kagey JD, Merkle JA. 2021. Genetic mapping and phenotypic analysis of *shot^{H.3.2}* in *Drosophila melanogaster*. *MicroPubl Biol* 2021: . PubMed ID: [34278244](#)

Xu GW, Toth JI, da Silva SR, Paiva SL, Lukkarila JL, Hurren R, Maclean N, Sukhai MA, Bhattacharjee RN, Goard CA, Medeiros B, Gunning PT, Dhe-Paganon S, Petroski MD, Schimmer AD. 2014. Mutations in UBA3 confer resistance to the NEDD8-activating enzyme inhibitor MLN4924 in human leukemic cells. *PLoS One* 9: e93530. PubMed ID: [24691136](#)

Funding: Fly-CURE (K. Bieser, J. Kagey, and A. Vralias-Mortimer) is funded by a National Science Foundation IUSE Award (NSF 2021146).

Author Contributions: Elizabeth Mast: data curation, formal analysis, writing - original draft, investigation, writing - review editing. Kayla L Bieser: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project, supervision, validation, writing - original draft, writing - review editing, visualization. Mary Abraham-Villa: data curation, formal analysis, investigation, writing - review editing. Vanessa Adams: data curation, formal analysis, investigation, writing - review editing. Akinwoluola J Akinlehin: data curation, formal analysis, investigation, writing - review editing.

Lynarose Z Aquino: data curation, formal analysis, investigation, writing - review editing. Joseph L Austin: data curation, formal analysis, investigation, writing - review editing. Abigail K Austin: data curation, formal analysis, investigation, writing - review editing. Carissa N Beckham: data curation, formal analysis, investigation, writing - review editing. Ethan J Bengson: data curation, formal analysis, investigation, writing - review editing. Amanda Bieszk: data curation, formal analysis, investigation, writing - review editing. Brianna L Bogard: data curation, formal analysis, investigation, writing - review editing. Rowan C Brennan: data curation, formal analysis, investigation, writing - review editing. Rebecca M Brnot: data curation, formal analysis, investigation, writing - review editing. Nicholas J Cirone: data curation, formal analysis, investigation, writing - review editing. Mason R Clark: data curation, formal analysis, investigation, writing - review editing. Brianna N Cooper: data curation, formal analysis, investigation, writing - review editing. Dennys Cruz: data curation, formal analysis, investigation, writing - review editing. Katlyn A Daprizio: data curation, formal analysis, investigation, writing - review editing. Jason DeBoe: data curation, formal analysis, investigation, writing - review editing. Michaela M Dencker: data curation, formal analysis, investigation, writing - review editing. Laura L Donnelly: data curation, formal analysis, investigation, writing - review editing. Leanne Driscoll: data curation, formal analysis, investigation, writing - review editing. Ryan J DuBeau: data curation, formal analysis, investigation, writing - review editing. Sirada W Durso: data curation, formal analysis, investigation, writing - review editing. Adam Ejub: data curation, formal analysis, investigation, writing - review editing. Waad Elgosbi: data curation, formal analysis, investigation, writing - review editing. Melanie Estrada: data curation, formal analysis, investigation, writing - review editing. Kaeli Evins: data curation, formal analysis, investigation, writing - review editing. Pearl D Fox: data curation, formal analysis, investigation, writing - review editing. Jacob M France: data curation, formal analysis, investigation, writing - review editing. Maira G Franco Hernandez: data curation, formal analysis, investigation, writing - review editing. Olivia Garl: data curation, formal analysis, investigation, writing - review editing. Myeerah R Gorsuch: data curation, formal analysis, investigation, writing - review editing. Mikayla A Gorzeman-mohr: data curation, formal analysis, investigation, writing - review editing. Madison E Grothouse: data curation, formal analysis, investigation, writing - review editing. Megan E Gubbels: data curation, formal analysis, investigation, writing - review editing. Romina Hakemiamjad: data curation, formal analysis, investigation, writing - review editing. Chloé V Harvey: data curation, formal analysis, investigation, writing - review editing. Madeline A Hoeppner: data curation, formal analysis, investigation, writing - review editing. Jessica L Ivanov: data curation, formal analysis, investigation, writing - review editing. Veronica M Johnson: data curation, formal analysis, investigation, writing - review editing. Jessica L Johnson: data curation, formal analysis, investigation, writing - review editing. Ashton Johnson: data curation, formal analysis, investigation, writing - review editing. Kaleigh Johnston: data curation, formal analysis, investigation, writing - review editing. Katie R Keller: data curation, formal analysis, investigation, writing - review editing. Breanna T Kennedy: data curation, formal analysis, investigation, writing - review editing. Levi R Killian: data curation, formal analysis, investigation, writing - review editing. Marissa Klumb: data curation, formal analysis, investigation, writing - review editing. Olivia L Koehn: data curation, formal analysis, investigation, writing - review editing. Kari J Kress: data curation, formal analysis, investigation, writing - review editing. Aaron S Koym: data curation, formal analysis, investigation, writing - review editing. Regan E Landis: data curation, formal analysis, investigation, writing - review editing. Kaitlyn N Lewis: data curation, formal analysis, investigation, writing - review editing. Enosh Lim: data curation, formal analysis, investigation, writing - review editing. Ilcen K Lopez: data curation, formal analysis, investigation, writing - review editing. D'Artagnan Lowe: data curation, formal analysis, investigation, writing - review editing. Paula Luengo Carretero: data curation, formal analysis, investigation, writing - review editing. Grace Lunaburg: data curation, formal analysis, investigation, writing - review editing. Samantha L Mallinder: data curation, formal analysis, investigation, writing - review editing. Natalie A Marshall: data curation, formal analysis, investigation, writing - review editing. Jessica Mathew: data curation, formal analysis, investigation, writing - review editing. Jasmine Mathew: data curation, formal analysis, investigation, writing - review editing. Hailee S Mcmanaway: data curation, formal analysis, investigation, writing - review editing. Emily N Meegan: data curation, formal analysis, investigation, writing - review editing. Jacob D Meyst: data curation, formal analysis, investigation, writing - review editing. Meredith J Miller: data curation, formal analysis, investigation, writing - review editing. Colin K Minogue: data curation, formal analysis, investigation, writing - review editing. Alina A Mohr: data curation, formal analysis, investigation, writing - review editing. Cristhian I Moran: data curation, formal analysis, investigation, writing - review editing. Adrian Moran: data curation, formal analysis, investigation, writing - review editing. Morgan D Morris: data curation, formal analysis, investigation, writing - review editing. Michael D Morrison: data curation, formal analysis, investigation, writing - review editing. Emmily A Moses: data curation, formal analysis, investigation, writing - review editing. Cade J Mullins: data curation, formal analysis, investigation, writing - review editing. Citlalli I Neri: data curation, formal analysis, investigation, writing - review editing. Jess M Nichols: data curation, formal analysis, investigation, writing - review editing. Breanna R Nickels: data curation, formal analysis, investigation, writing - review editing. Akosua M Okai: data curation, formal analysis, investigation, writing - review editing. Chiedu Okonmah: data curation, formal analysis, investigation, writing - review editing. Makena Paramo: data curation, formal analysis, investigation, writing - review editing. Meagan Paramo: data curation, formal analysis, investigation, writing - review editing. Sydney L Parker: data curation, formal

3/18/2022 - Open Access

analysis, investigation, writing - review editing. Neil K Parmar: data curation, formal analysis, investigation, writing - review editing. Jacob Paschal: data curation, formal analysis, investigation, writing - review editing. Prem Patel: data curation, formal analysis, investigation, writing - review editing. Erica B Perkins: data curation, formal analysis, investigation, writing - review editing. Madelyn M Perry: data curation, formal analysis, investigation, writing - review editing. Zachary Perry: data curation, formal analysis, investigation, writing - review editing. Amanda A Pollock: data curation, formal analysis, investigation, writing - review editing. Oxxyris Portalatin: data curation, formal analysis, investigation, writing - review editing. Kamron S Proffitt: data curation, formal analysis, investigation, writing - review editing. Jason T Queen: data curation, formal analysis, investigation, writing - review editing. Alexis C Quemeneur: data curation, formal analysis, investigation, writing - review editing. Amelia G Richardson: data curation, formal analysis, investigation, writing - review editing. Kaylee Rosenberger: data curation, formal analysis, investigation, writing - review editing. Allison M Rutherford: data curation, formal analysis, investigation, writing - review editing. Itchel X Santos-Perez: data curation, formal analysis, investigation, writing - review editing. Christy Y Sarti: data curation, formal analysis, investigation, writing - review editing. Lacey J Schouweiler: data curation, formal analysis, investigation, writing - review editing. Lauren M Sessing: data curation, formal analysis, investigation, writing - review editing. Sara O Setaro: data curation, formal analysis, writing - review editing, investigation. Christopher F Silvestri: data curation, formal analysis, investigation, writing - review editing. Olivia A Smith: data curation, formal analysis, investigation, writing - review editing. Mackenzie J Smith: data curation, formal analysis, investigation, writing - review editing. Jayson C Sumner: data curation, formal analysis, investigation, writing - review editing. Rachel R Sutton: data curation, formal analysis, investigation, writing - review editing. Lindsay Sweekard: data curation, formal analysis, investigation, writing - review editing. Nicholas B Talbott: data curation, formal analysis, investigation, writing - review editing. Peyton A Traxler: data curation, formal analysis, investigation, writing - review editing. Jenna Truesdell: data curation, formal analysis, investigation, writing - review editing. Trey J Wilson: data curation, formal analysis, investigation, writing - review editing. Makayla A Witbeck: data curation, formal analysis, investigation, writing - review editing. Trinity M Wobler: data curation, formal analysis, investigation, writing - review editing. Lucas J Wright: data curation, formal analysis, investigation, writing - review editing. Karolina A Zuczkowska: data curation, formal analysis, investigation, writing - review editing. Olivier Devergne: data curation, formal analysis, investigation, project, validation, writing - review editing, supervision. Danielle R Hamill: data curation, formal analysis, investigation, project, validation, writing - review editing, supervision. Hemin P Shah: data curation, formal analysis, investigation, supervision, project, validation, writing - review editing. Jamie Siders: data curation, formal analysis, investigation, project, supervision, validation, writing - review editing. Elizabeth E Taylor : data curation, formal analysis, investigation, writing - review editing, supervision, validation, project. Alyisia D Vrailas-Mortimer: data curation, formal analysis, investigation, project, validation, funding acquisition, writing - review editing. Jacob D Kagey: conceptualization, data curation, writing - review editing, validation, resources, project, funding acquisition, methodology, investigation, formal analysis, visualization.

Reviewed By: Anonymous

History: Received October 8, 2021 **Revision Received** March 15, 2022 **Accepted** March 16, 2022 **Published** March 18, 2022

Copyright: © 2022 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Mast, E; Bieser, KL; Abraham-Villa, M; Adams, V; Akinlehin, AJ; Aquino, LZ; Austin, JL; Austin, AK; Beckham, CN; Bengson, EJ; Bieszk, A; Bogard, BL; Brennan, RC; Brnot, RM; Cirone, NJ; Clark, MR; Cooper, BN; Cruz, D; Daprizio, KA; DeBoe, J; Dencker, MM; Donnelly, LL; Driscoll, L; DuBeau, RJ; Durso, SW; Ejub, A; Elgosbi, W; Estrada, M; Evins, K; Fox, PD; France, JM; Franco Hernandez, MG; Garcia, LA; Garl, O; Gorsuch, M; Gorzeman-mohr, MA; Grothouse, ME; Gubbels, ME; Hakemiamjad, R; Harvey, CV; Hoeppner, MA; Ivanov, JL; Johnson, VM; Johnson, JL; Johnson, A; Johnston, K; Keller, KR; Kennedy, BT; Killian, LR; Klumb, M; Koehn, OL; Koym, AS; Kress, KJ; Landis, RE; Lewis, KN; Lim, E; Lopez, IK; Lowe, D; Luengo Carretero, P; Lunaburg, G; Mallinder, SL; Marshall, NA; Mathew, J; Mathew, J; Mcmanaway, HS; Meegan, EN; Meyst, JD; Miller, M; Minogue, CK; Mohr, AA; Moran, CI; Moran, A; Morris, MD; Morrison, MD; Moses, EA; Mullins, CJ; Neri, CI; Nichols, JM; Nickels, BR; Okai, AM; Okonmah, C; Paramo, M; Paramo, M; Parker, SL; Parmar, NK; Paschal, J; Patel, P; Patel, D; Perkins, EB; Perry, MM; Perry, Z; Pollock, AA; Portalatin, O; Proffitt, KS; Queen, JT; Quemeneur, AC; Richardson, AG; Rosenberger, K; Rutherford, AM; Santos-Perez, IX; Sarti, CY; Schouweiler, LJ; Sessing, LM; Setaro, SO; Silvestri, CF; Smith, OA; Smith, M; Sumner, JC; Sutton, RR; Sweekard, L; Talbott, NB; Traxler, PA;

3/18/2022 - Open Access

Truesdell, J; Valenti, AF; Verace, L; Vijayakumar, P; Wadley, WL; Walter, KE; Williams, AR; Wilson, TJ; Witbeck, M; Wobler, TM; Wright, LJ; Zuczkowska, KA; Devergne, O; Hamill, DR; Shah, HP; Siders, J; Taylor, EE; Vrailas-Mortimer, AD; Kagey, JD (2022), Genetic mapping of *Uba3*^{O.2.2}, a pupal lethal mutation in *Drosophila melanogaster*. microPublication Biology. [10.17912/micropub.biology.000542](https://doi.org/10.17912/micropub.biology.000542)