A peer-reviewed version of this preprint was published in PeerJ on 15 September 2016.

M'Angale PG, Staveley BE. 2016. *Bcl-2* homologue *Debcl* enhances *α-synuclein*-induced phenotypes in *Drosophila*. PeerJ 4:e2461 https://doi.org/10.7717/peerj.2461

Bcl-2 homologue *debcl* enhances *α-synuclein*-induced phenotypes in Drosophila

Peter G M'Angale, Brian E Staveley

Background Parkinson disease (PD) is a debilitating movement disorder that afflicts 1 to 2% of the population over 50 years of age. The common hallmark for both sporadic and familial forms of PD is mitochondrial dysfunction. Mammals have at least twenty proapoptotic and antiapoptotic Bcl-2 family members, in contrast, only two *Bcl-2* family genes have been identified in *Drosophila melanogaster,* the proapoptotic mitochondrial localized *debcl* and the antiapoptotic *Buffy.* The expression of *α-synuclein*, the first gene identified to contribute to inherited forms of PD, in the dopaminergic neurons (DA) of flies has provided a robust and well-studied Drosophila model of PD complete with the loss of neurons and accompanying motor defects. The altered expression of *debcl* in the DA neurons and neuron-rich eye and along with the expression of *α-synuclein* offers an opportunity to highlight the role of debcl in mitochondrial-dependent neuronal degeneration and death. **Results** The directed overexpression of *debcl* using the *Ddc-Gal4* transgene in the dopaminergic neurons of Drosophila resulted in flies with severely decreased survival and a premature age-dependent loss in climbing ability. The inhibition of *debcl* resulted in enhanced survival and improved climbing ability whereas the overexpression of *debcl* in the *α-synuclein*-induced Drosophila model of PD resulted in more severe phenotypes. In addition, the co-expression of *debcl* along with *Buffy* partially counteracts the *debcl*-induced phenotypes, to improve the lifespan and the associated loss of locomotor ability observed. In complementary experiments, the overexpression of *debcl* along with the expression of *α-synuclein* in the eye, enhanced the eye ablation that results from the overexpression of *debcl*. The co-expression of *Buffy* along with *debcl* overexpression results in the rescue of the moderate developmental eye defects. The co-expression of *Buffy* along with inhibition of *debcl* partially restores the eye to a roughened eye phenotype. **Discussion** The overexpression of *debcl* in DA neurons produces flies with shortened lifespan and impaired locomotor ability, phenotypes that are strongly associated with models of PD in Drosophila. The co-expression of *debcl* along with *α-synuclein* enhanced the Parkinson disease-like phenotypes. The co-expression of debcl along with Buffy suppresses these phenotypes. Complementary experiments in the Drosophila eye show similar trends during development. Taken all together these results suggest a role

for *debcl* in neurodegenerative disorders.

Bcl-2 homologue *debcl* enhances *α-synuclein*-induced phenotypes in Drosophila

P. Githure M'Angale[1], Brian E. Staveley[1]

[1] Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland & Labrador, Canada

Corresponding Author:

Brian Staveley[1]

232 Elizabeth Avenue, St. John's, Newfoundland & Labrador, A1B 3X9, Canada

Email address: bestave@mun.ca

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2025v1 | CC-BY 4.0 Open Access | rec: 4 May 2016, publ: 4 May 2016

Abstract

Background

Parkinson disease (PD) is a debilitating movement disorder that afflicts 1 to 2% of the population over 50 years of age. The common hallmark for both sporadic and familial forms of PD is mitochondrial dysfunction. Mammals have at least twenty proapoptotic and antiapoptotic Bcl-2 family members, in contrast, only two *Bcl-2* family genes have been identified in *Drosophila melanogaster,* the proapoptotic mitochondrial localized *debcl* and the antiapoptotic *Buffy.* The expression of *α-synuclein*, the first gene identified to contribute to inherited forms of PD, in the dopaminergic neurons (DA) of flies has provided a robust and well-studied Drosophila model of PD complete with the loss of neurons and accompanying motor defects. The altered expression of *debcl* in the DA neurons and neuron-rich eye and along with the expression of *α-synuclein* offers an opportunity to highlight the role of debcl in mitochondrial-dependent neuronal degeneration and death.

Results

The directed overexpression of *debcl* using the *Ddc-Gal4* transgene in the dopaminergic neurons of Drosophila resulted in flies with severely decreased survival and a premature age-dependent loss in climbing ability. The inhibition of *debcl* resulted in enhanced survival and improved climbing ability whereas the overexpression of *debcl* in the *α-synuclein*-induced Drosophila model of PD resulted in more severe phenotypes. In addition, the co-expression of *debcl* along with *Buffy* partially counteracts the *debcl*-induced phenotypes, to improve the lifespan and the associated loss of locomotor ability observed. In complementary experiments, the overexpression of *debcl* along with the expression of *α-synuclein* in the eye, enhanced the eye ablation that results from the overexpression of *debcl*. The co-expression of *Buffy* along with *debcl* overexpression results in the rescue of the moderate developmental eye defects. The co-

expression of *Buffy* along with inhibition of *debcl* partially restores the eye to a roughened eye phenotype.

Discussion

The overexpression of *debcl* in DA neurons produces flies with shortened lifespan and impaired locomotor ability, phenotypes that are strongly associated with models of PD in Drosophila. The co-expression of *debcl* along with *α-synuclein* enhanced the Parkinson disease-like phenotypes. The co-expression of debcl along with Buffy suppresses these phenotypes. Complementary experiments in the Drosophila eye show similar trends during development. Taken all together these results suggest a role for *debcl* in neurodegenerative disorders.

Introduction

Parkinson disease (PD) is a human movement disorder that is strongly associated with the selective and profound degeneration and loss of dopaminergic (DA) neurons to result in a set of marked clinical features (Forno 1996). The neuropathological hallmarks exhibited by PD patients include the presence of Lewy Bodies (LB) which are intracytoplasmic proteinaceous inclusions composed of α-synuclein and ubiquitin among other proteins (Forno 1996; Leroy et al. 1998; Polymeropoulos et al. 1997). This atypical protein aggregation and accumulation is believed to lead to cellular toxicity and contribute to the pathogenesis of PD. Additional pathological mechanisms that are associated with PD include aberrant protein aggregation and mitochondrial damage (Gupta et al. 2008; Jörg 2007; Whitworth 2011). Familial forms of PD have highlighted the genetic basis of PD and the study of the associated gene loci in model organisms offers great understanding of the disease aetiology and pathology (Ambegaokar et al. 2010; Gasser 2009; Guo 2012). The gene encoding α-synuclein, a small soluble protein of largely unknown function predominantly found in neural tissues, was first to be identified as responsible for inherited PD

(Polymeropoulos et al. 1997). Mitochondrial dysfunction due to the accumulation of α-synuclein has been implicated as one of the mechanisms leading to PD (Chinta et al. 2010; Choubey et al. 2011; Esteves et al. 2011; Zhu et al. 2011). The association of α-synuclein with components of the mitochondria is thought to lead to oxidative stress, apoptosis, autophagy and eventually, neurodegeneration. The first Drosophila model of PD utilized a human *α-synuclein* transgene to induce the PD-like symptoms (Feany & Bender 2000). This model system is very successful and widely applied, as it displays the age-dependent loss of locomotor function, the degeneration of DA neurons and LB-like inclusions, features that are present in human PD (Auluck et al. 2002; Botella et al. 2009; Buttner et al. 2014; Feany & Bender 2000; Kong et al. 2015; Staveley 2014; Webb et al. 2003; Zhu et al. 2016). Drosophila has available tissue specific gene enhancers such as *TH-Gal4*, *elav-Gal4* and *Ddc-Gal4*, which are used to model PD in flies in combination with the powerful bipartite UAS/Gal4 (Brand & Perrimon 1993) system. Of importance is the correlation between DA neuron loss and the age-dependent loss of locomotor function (Park et al. 2007; Staveley 2014) which validates the implication that age-dependent loss of locomotor function is as a result of DA neuron degeneration.

The *Bcl-2* family of genes are crucial controllers of apoptosis in animals and are functionally composed of proapoptotic and antiapoptotic members (Adams & Cory 1998; Cory & Adams 2002; Fu & Fan 2002; Siddiqui et al. 2015). In mammals, this multigene family has about 20 members, the antiapoptotic proteins protect the mitochondria from disruption by the proapoptotic proteins (Colin et al. 2009; Cory & Adams 2002; Martinou & Youle 2011; Suen et al. 2008; Tsujimoto 2002). The antiapoptotic members possess four Bcl-2 homology (BH) domains while the proapoptotic members have three to four BH domains. The proapoptotic proteins initiate apoptosis by the permeabilization of the outer mitochondrial membrane which results in the

release of apoptogenic factors into the cytosol (Delbridge & Strasser 2015; Doerflinger et al. 2015; Li & Dewson 2015; Lopez & Tait 2015). The antiapoptotic members protect the mitochondria from permeabilization by the proapoptotic members and block the release of apoptogenic factors such as cytochrome c, apoptosis inducing factor (AIF) among others from being released from the inner mitochondrial membrane into the cytosol.

Drosophila melanogaster possesses many of the apoptotic pathway proteins that participate in the intrinsic and extrinsic cell death pathways (Kornbluth & White 2005; Richardson & Kumar 2002). The *Bcl-2* family member homologues in Drosophila are limited to the single antiapoptotic *Buffy* (Quinn et al. 2003), and the sole proapoptotic *death executioner Bcl-2 homologue, debcl* (Brachmann et al. 2000; Colussi et al. 2000; Igaki et al. 2000; Quinn et al. 2003; Zhang et al. 2000). Debcl has a strong similarity with the mammalian mitochondria outer membrane permeabilization protein Bok/Mtd.

The importance of *debcl* is demonstrated by the different promoters contained in its genomic regions including the 5' nuclear transcription factor Y (NF-Y) which has been shown to be important for gene promoter activity (Ly et al. 2013). The tumour suppressor gene *Retinoblastoma* (*Rbf1* in Drosophila) induces a *debcl*-and *Drp1*-dependent mitochondrial cell death (Clavier et al. 2015). *Rbf1* induces cell death by reducing the expression of the sole debcl antagonist Buffy (Clavier et al. 2014). The *Rbf1*-induced apoptosis is dependent on *debcl*-dependent mitochondrial ROS production and essentially debcl is required downstream of Buffy for apoptosis to occur. The *debcl*-induced ROS production appears to be through Glycerophosphate oxidase 1 participation to increase mitochondria ROS accumulation (Colin et al. 2015). The organic solute carrier partner 1/ oxidored nitrodomain-containing protein 1 (OSCP1/NOR1), a known tumour suppressor induces apoptosis by the down-regulation of the

Buffy gene and the up-regulation of the *debcl* gene (Huu et al. 2015). Debcl is not required for most developmental cell death, but has been shown to play a role in embryonic cell death (Galindo et al. 2009) and stress-induced apoptosis (Sevrioukov et al. 2007). Antiapoptotic Buffy antagonizes *debcl*-induced apoptosis by physical interaction (Quinn et al. 2003), probably at the mitochondria where debcl localizes (Doumanis et al. 2007). The presence of a MOM-targeting motif in debcl indicates it possibly has a role in mitochondrial cell death pathway.

The role of the mitochondria in PD pathogenesis makes the *α-synuclein*-induced model of PD (Feany & Bender 2000) a very attractive model for the investigation of the role of Bcl-2 proteins. Here, we investigate the potential enhancement or suppression of the *α-synuclein*-induced PD phenotypes by the inhibition and overexpression of the pro-apoptotic Bcl-2 homologue *debcl*.

Materials & methods

Drosophila media and culture

Stocks and crosses were maintained on standard cornmeal/molasses/yeast/agar media treated with propionic acid and methylparaben. Stocks were sustained on solid media for two to three weeks before being transferred onto new media to re-culture. Stocks were kept at room temperature (22°C ± 2°C) while crosses and experiments were carried out at 25°C and 29°C.

Drosophila stocks and derivative lines

UAS-Buffy (Quinn et al. 2003) was a gift from Dr. Leonie Quinn of University of Melbourne, *UAS-α-synuclein* (Feany & Bender 2000) by Dr. M. Feany of Harvard Medical School and *Ddc-Gal4* (Li et al. 2000) by Dr. J. Hirsch of University of Virginia. y^1 v^1*; P{y[+t7.7] v[+t1.8]=TRiP.JF02429}attP2* hereby referred to as *debcl-RNAi*, *GMR-Gal4* (Freeman 1996) and *UAS-lacZ* were sourced from the Bloomington Drosophila Stock Center at Indiana University. The *UAS-α-synuclein/CyO; Ddc-Gal4/TM3; UAS-α-synuclein/CyO; GMR-Gal4;*

UAS-Buffy/CyO; Ddc-Gal4 and *UAS-Buffy/CyO; GMR-Gal4* derivative lines were generated using standard homologous recombination methods and were used for overexpression of either *α-synuclein* or *Buffy* in DA neurons using the *Ddc-Gal4* transgene or in the developing eye using the *GMR* response elements. PCR reactions and gel electrophoresis were used for analysis of recombination events.

Ageing assay

Several single vial matings were made and a cohort of critical class male flies was collected upon eclosion. At least two hundred flies were aged per genotype at a density of 20 or fewer flies per vial to avoid crowding on fresh media which was replenished every other day. Flies were observed and scored every two days for the presence of deceased adults. Flies were considered dead when they did not display movement upon agitation (Staveley et al. 1990). Longevity data was analysed using the GraphPad Prism version 5.04 and survival curves were compared using the log-rank (Mantel-Cox) test. Significance was determined at 95%, at a P-value less than or equal to 0.05 with Bonferroni correction.

Climbing assay

A batch of male flies was collected upon eclosion and scored for their ability to climb (Todd & Staveley 2004). Every 7 days, 50 males from every genotype were assayed for their ability to climb 10 centimetres in 10 seconds in a clean climbing apparatus in 10 repetitions. Analysis was performed using GraphPad Prism version 5.04 and climbing curves were fitted using non-linear regression and compared using 95% confidence interval with a 0.05 P-value.

Scanning electron microscopy of the drosophila eye

Several single vial crosses were made at 29°C and adult male flies collected upon eclosion and aged for three days before being frozen at -80°C. Whole flies were mounted on scanning electron

microscope stubs, desiccated overnight and photographed with a FEI Mineral Liberation Analyzer 650F scanning electron microscope.

Results

Debcl is similar to the human proapoptotic *Bcl-2 ovarian killer (Bok)*

Bioinformatic analysis of the protein sequences encoded by the *debcl* and *Bok* genes reveal 37% identity and 55% similarity. The debcl protein consists of 300 amino acids and indicates the existence of the BH1, BH2, BH3, BH4 and TM domains, similar to the 212 amino acids human Bok (Figure 1). An ELM resource search for functional sites (Dinkel et al. 2016) indicates the presence of a transmembrane domain (membrane anchor region), an inhibitor of apoptosis binding motif (IBM), a PDZ domain, an ER retention motif, an Atg8 binding motif, a nuclear receptor box motif, and a ubiquitination motif. There is a number of BH3-homology region binding sites in the central region of the protein as determined by an NCBI conserved domain search (Marchler-Bauer et al. 2015). Although the two proteins Bok and debcl have been determined to be antiapoptotic, both show the presence of a BH4 domain, the homology domain that is most often associated with pro-survival proteins.

Directed misexpression of *debcl* in DA neurons alters lifespan and locomotor ability

The inhibition of *debcl* in the DA neurons by RNA interference results in a lifespan with a median survival of 64 days that is similar to 62 days for the controls expressing the benign *lacZ* transgene as determined by a Log-rank (Mantel-Cox) test (Figure 2A). The locomotor ability showed a slight improvement when nonlinear fitting of the climbing curves was performed, with significant differences at 95% confidence intervals (Figure 2B). This suggests that the inhibition of the proapoptotic debcl confers a small advantage for the normal functioning of DA neurons.

When *debcl* is overexpressed in DA neurons, the survival criteria of these flies differ greatly (Figure 2A), with *debcl*-overexpressing flies having a median lifespan of 48 days compared to 62 days for the controls expressing the benign *lacZ* transgene as indicated by a Log-rank (Mantel-Cox) test. The overexpression of *debcl* in DA neurons severely impairs climbing ability as determined by the nonlinear fitting of the curve with 95% CI (Figure 2B). This suggests that the overexpression of *debcl* in DA neurons interferes with the normal functioning of these flies and results in compromised "healthspan".

The overexpression of the pro-survival *Buffy* rescues the *debcl*-induced phenotypes

The overexpression of *Buffy* and *debcl* in DA neurons results in a longer lifespan and improved locomotor ability (Figure 2). The median lifespan of these flies was 64 days when compared to *Buffy* and *lacZ* overexpressing controls at 72 days. The median survival of *debcl-RNAi* flies was 68 days as determined by a Log-rank (Mantel-Cox) test (Figure 2C). The climbing ability of these flies was also much improved as determined by comparing the climbing indices at 95% CI (Figure 2D). Taken together these results suggest that *Buffy* antagonizes the *debcl*-induced phenotypes of shortened lifespan and poor climbing ability to markedly improve "healthspan".

Altered expression of *debcl* influences the *α–synuclein*-induced phenotypes

The inhibition of *debcl* by RNAi along with the expression of *α-synuclein* under the direction of the *Ddc-Gal4* transgene results in increased lifespan and healthier climbing ability compared to the control (Figure 3). The *debcl-RNAi* along with *α-synuclein*-expressing flies had a median lifespan of 67 days, while that of *α-synuclein*-expressing controls was 60 days as determined by a Log-rank (Mantel-Cox) test (Figure 3A). The climbing ability of these flies was slightly improved than of the *α-synuclein*-expressing controls as indicated by the nonlinear fitting of the climbing curves and compared the 95% CI (Figure 3B). These results show that the inhibition of

the proapoptotic debcl confers a significant advantage to flies under the influence of the neurotoxic effects of the human transgene *α-synuclein*.

The overexpression of *debcl* along with *α-synuclein* in DA neurons results in decreased median lifespan of 44 days, compared to 60 days for the control flies as determined by a Log-rank (Mantel-Cox) test (Figure 3A). The climbing curves indicate that there was a significant reduction in the climbing ability of the flies with overexpression of *debcl* (Figure 3B) and thus, enhancing the phenotypes observed when *α-synuclein* is expressed in DA neurons. This suggests that the overexpression of *debcl* further increases the toxic effects of the expression of *α-synuclein*.

Overexpression of *debcl* enhances the *α-synuclein*-induced developmental eye defects

The overexpression of *debcl* in the Drosophila eye results in severe ablation of the eye due to apoptosis (Colussi et al. 2000; Igaki et al. 2000) while expression of *α-synuclein* in the eye results in developmental defects. When *debcl* is overexpressed in the eye, developmental defects resulting from *Gal4* (Kramer & Staveley 2003) (Figure 5 I), inhibition of *debcl* (Figure 5 II), and overexpression of debcl (Figure 5 III) are enhanced. The inhibition of *debcl* along with *α-synuclein* expression (Figure 5 IV) and the co-expression of *debcl* and *α-synuclein* (Figure 5 V) result in enhanced phenotypes. The disruption of the ommatidial array due to fusion of the ommatidia and smaller eye is severely enhanced by the overexpression of *debcl* together with *α-synuclein*. The ommatidial disarray that results from inhibition of *debcl* are completely rescued by overexpression of the pro-survival *Buffy* (Figure 5 VI), while the ablated eye resulting from *debcl* overexpression is partially rescued upon *Buffy* overexpression, restoring the ablation to a mildly severe rough eye phenotype (Figure 5 VII). These results suggest that overexpression of

debcl along with expression of *α-synuclein* enhances the *debcl*-induced eye ablation, while the overexpression of *debcl* together with *Buffy* partially rescues the eye phenotype.

Discussion

Since mitochondrial dysfunction is central to the pathology of both sporadic and familial forms of PD (Subramaniam & Chesselet 2013), it was important to highlight the role and consequences of the altered expression of the proapoptotic mitochondrial gene *debcl* in this process. The overexpression of *debcl* in Drosophila and other systems, including mammalian, has been demonstrated to lead to apoptosis (Brachmann et al. 2000; Colussi et al. 2000; Galindo et al. 2009; Igaki et al. 2000; Senoo-Matsuda et al. 2005; Sevrioukov et al. 2007; Zhang et al. 2000). The recapitulation of PD-like symptoms in *Drosophila melanogaster,* especially the age-dependent loss of climbing ability, has led to investigation of genes that could suppress these phenotypes (Auluck et al. 2002; Feany & Bender 2000; Haywood & Staveley 2004). Our results show that the overexpression of *debcl* results in a severely shortened lifespan followed by premature loss in climbing ability; phenotypes that are reminiscent of PD-like symptoms in model organisms. Thus our work shows the intricate balance between life and death decisions in the sensitive dopamine producing neurons. It seems that excess amounts of debcl protein are sufficient to upset the survival mechanisms and lead to degeneration and death of DA neurons. The importance of *debcl*-induced apoptosis is exhibited by the strict control in its gene product by the tumour suppressors *Rbf1* (Clavier et al. 2015), *OSCP1/NOR1* (Huu et al. 2015), and *NF-Y* (Ly et al. 2013). Furthermore, it has a motif for ubiquitination, probably by the *TrCP* homologue *slimb* that targets it for destruction by the proteasome (Colin et al. 2014). The inhibition of *debcl* had a converse result, with flies that had a longer lifespan and healthy climbing ability. It is possible that the suppression of *debcl* tips the balance towards the survival pathways controlled

by the antiapoptotic *Buffy*. Our results indicate that overexpression of *debcl* appears to be a novel model of PD as a result of neuronal apoptosis.

The *α-synuclein*-induced model of PD in Drosophila shows little difference in lifespan between the control and wild type, A53T and A30P *α-synuclein* flies (Feany & Bender 2000). In our study, the overexpression of *debcl* in the DA neurons resulted in a marked decrease in lifespan. This is in part due to toxic effects as a result of the expression of *α-synuclein*, and additionally, due to *debcl*-induced apoptosis. The *debcl*-induced apoptosis is mediated by other factors including; the mitochondrial fission protein *Drp1* (Clavier et al. 2015) that interacts with debcl to induce mitochondrial fragmentation; *Glycerophosphate oxidase-1* (Colin et al. 2015) that increases mitochondrial ROS accumulation; and possibly through the initiation of autophagy, since both *α-synuclein* expression (Xilouri & Stefanis 2015) and *debcl* (Hou et al. 2008) overexpression are implicated in this process. This worsening of phenotypes was also observed when *debcl* was overexpressed with *α-synuclein* in the eye. The inhibition of *debcl* in the DA neurons resulted in a marked increase in survival and improved locomotor ability. This inhibition of *debcl* is sufficient to negate its apoptotic role and thus promote cell survival through the opposing antiapoptotic Buffy.

Locomotor dysfunction is one of the major symptoms of PD. The demonstration of an age-dependent loss of climbing ability is pivotal to highlighting the effects of degeneration and death of DA neurons, ultimately as a consequence of altered gene expression as opposed to cellular senescence (Rodriguez et al. 2015). The overexpression of *debcl* in the DA neurons produced a climbing index significantly different from that of control flies with the loss of climbing ability in an age-dependent manner and likely due to *debcl*-induced neuronal degeneration. The degree of locomotor dysfunction seemed to be similar to that observed when *α-synuclein* is

overexpressed in DA neurons. Taken together, these results would indicate a detrimental effect in overexpression of debcl in DA neurons that result in a novel model of PD in flies.

In contrast, the inhibition of *debcl* in the same neurons results in a remarkable improvement in climbing ability when compared to the controls. The inhibition of *debcl* in the DA neurons of the *α-synuclein*-induced PD model significantly increased lifespan and climbing ability, indicating that reduced levels of *debcl* are sufficient to alter the healthspan of DA neurons. The *debcl*-induced apoptosis relies on downstream effectors that either induces ROS accumulation (Colin et al. 2015) or the fragmentation of the mitochondria (Clavier et al. 2015). As the down-regulation of *Buffy* or up-regulation of *debcl* results in apoptosis (Huu et al. 2015), the cellular advantage of *debcl* inhibition may be indirect through the de-repression of the *Buffy* gene product that confers survival advantages. The directed expression of *Buffy* along with *debcl* results in an improved "healthspan" compared to the *debcl*-induced phenotypes and corroborate other studies that show the overexpression of the pro-survival Buffy confers survival advantages through increased survival and improved climbing ability under conditions of stress (M'Angale & Staveley, in press). Our study suggests that the overexpression of *Buffy* is similar to an up-regulation that ultimately blocks *debcl*-induced apoptosis, similar to results obtained when its regulation by Rbf1 or dE2F2 is altered to repress it transcriptionally (Clavier et al. 2014; Clavier et al. 2015). This suppression of *Buffy* is sufficient to induce *debcl*-dependent apoptosis, in addition to the promotion of debcl activity by dNF-Y (Ly et al. 2013). The co-overexpression of *debcl* and *Buffy* in the eye resulted in a partial rescue of the *debcl*-induced phenotypes. Therefore, overexpression of the pro-survival *Buffy* suppresses the *debcl*-dependent phenotypes.

Conclusions

Directed inhibition of *debcl* results in improved survivorship and extended climbing ability whereas the directed expression of *debcl* results in reduced lifespan and impaired locomotor function. These phenotypes are rescued upon co-expression with the pro-survival *Buffy*. The overexpression of *debcl* enhances the effects of *α-synuclein* expression. *Buffy* counteracts *debcl*-induced phenotypes, and represents a potential target to enhance neuronal survival in response to the detrimental effects of *debcl*-induced apoptosis.

References

Adams JM, and Cory S. 1998. The Bcl-2 protein family: arbiters of cell survival. *Science* 281:1322-1326.

Ambegaokar SS, Roy B, and Jackson GR. 2010. Neurodegenerative models in Drosophila: polyglutamine disorders, Parkinson disease, and amyotrophic lateral sclerosis. *Neurobiology of disease* 40:29-39.

Auluck PK, Chan HY, Trojanowski JQ, Lee VM, and Bonini NM. 2002. Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science* 295:865-868.

Botella JAA, Bayersdorfer F, Gmeiner F, and Schneuwly S. 2009. Modelling Parkinson's disease in Drosophila. *Neuromolecular Medicine* 11:268-280.

Brachmann CB, Jassim OW, Wachsmuth BD, and Cagan RL. 2000. The Drosophila bcl-2 family member dBorg-1 functions in the apoptotic response to UV-irradiation. *Current Biology* 10:547-550.

Brand AH, and Perrimon N. 1993. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118:401-415.

Buttner S, Broeskamp F, Sommer C, Markaki M, Habernig L, Alavian-Ghavanini A, Carmona-Gutierrez D, Eisenberg T, Michael E, Kroemer G, Tavernarakis N, Sigrist SJ, and Madeo F. 2014. Spermidine protects against alpha-synuclein neurotoxicity. *Cell Cycle* 13:3903-3908.

Chinta SJ, Mallajosyula JK, Rane A, and Andersen JK. 2010. Mitochondrial alpha-synuclein accumulation impairs complex I function in dopaminergic neurons and results in increased mitophagy in vivo. *Neurosci Lett* 486:235-239.

Choubey V, Safiulina D, Vaarmann A, Cagalinec M, Wareski P, Kuum M, Zharkovsky A, and Kaasik A. 2011. Mutant A53T alpha-synuclein induces neuronal death by increasing mitochondrial autophagy. *The Journal of biological chemistry* 286:10814-10824.

Clavier A, Baillet A, Rincheval-Arnold A, Coleno-Costes A, Lasbleiz C, Mignotte B, and Guenal I. 2014. The pro-apoptotic activity of Drosophila Rbf1 involves dE2F2-dependent downregulation of diap1 and buffy mRNA. *Cell death & disease* 5:e1405.

338 Clavier A, Ruby V, Rincheval-Arnold A, Mignotte B, and Guénal I. 2015. The Drosophila
339 retinoblastoma protein, Rbf1, induces a Debcl- and Drp1-dependent mitochondrial
340 apoptosis. *Journal of Cell Science* 128:3239-3249.
341 Colin J, Garibal J, Clavier A, Rincheval-Arnold A, Gaumer S, Mignotte B, and Guenal I. 2014.
342 The drosophila Bcl-2 family protein Debcl is targeted to the proteasome by the beta-TrCP
343 homologue slimb. *Apoptosis* 19:1444-1456.
344 Colin J, Garibal J, Clavier A, Szuplewski S, Risler Y, Milet C, Gaumer S, Guenal I, and
345 Mignotte B. 2015. Screening of suppressors of bax-induced cell death identifies
346 glycerophosphate oxidase-1 as a mediator of debcl-induced apoptosis in Drosophila.
347 *Genes Cancer* 6:241-253.
348 Colin J, Gaumer S, Guenal I, and Mignotte B. 2009. Mitochondria, Bcl-2 family proteins and
349 apoptosomes: of worms, flies and men. *Front Biosci (Landmark Ed)* 14:4127-4137.
350 Colussi PA, Quinn LM, Huang DC, Coombe M, Read SH, Richardson H, and Kumar S. 2000.
351 Debcl, a proapoptotic Bcl-2 homologue, is a component of the Drosophila melanogaster
352 cell death machinery. *Journal of Cell Biology* 148:703-714.
353 Cory S, and Adams JM. 2002. The Bcl2 family: regulators of the cellular life-or-death switch.
354 *Nat Rev Cancer* 2:647-656.
355 Delbridge AR, and Strasser A. 2015. The BCL-2 protein family, BH3-mimetics and cancer
356 therapy. *Cell Death and Differentiation* 22:1071-1080.
357 Dinkel H, Van Roey K, Michael S, Kumar M, Uyar B, Altenberg B, Milchevskaya V, Schneider
358 M, Kuhn H, Behrendt A, Dahl SL, Damerell V, Diebel S, Kalman S, Klein S, Knudsen
359 AC, Mader C, Merrill S, Staudt A, Thiel V, Welti L, Davey NE, Diella F, and Gibson TJ.
360 2016. ELM 2016-data update and new functionality of the eukaryotic linear motif
361 resource. *Nucleic acids research* 44:D294-300.
362 Doerflinger M, Glab JA, and Puthalakath H. 2015. BH3-only proteins: a 20-year stock-take. *The*
363 *FEBS journal* 282:1006-1016.
364 Doumanis J, Dorstyn L, and Kumar S. 2007. Molecular determinants of the subcellular
365 localization of the Drosophila Bcl-2 homologues DEBCL and BUFFY. *Cell Death and*
366 *Differentiation* 14:907-915.
367 Esteves AR, Arduino DM, Silva DF, Oliveira CR, and Cardoso SM. 2011. Mitochondrial
368 Dysfunction: The Road to Alpha-Synuclein Oligomerization in PD. *Parkinson's disease*
369 2011:693761.
370 Feany MB, and Bender WW. 2000. A Drosophila model of Parkinson's disease. *Nature* 404:394-
371 398.
372 Forno LS. 1996. Neuropathology of Parkinson's disease. *Journal of Neuropathology &*
373 *Experimental Neurology* 55:259-272.
374 Freeman M. 1996. Reiterative use of the EGF receptor triggers differentiation of all cell types in
375 the Drosophila eye. *Cell* 87:651-660.
376 Fu YF, and Fan TJ. 2002. Bcl-2 family proteins and apoptosis. *Sheng Wu Hua Xue Yu Sheng Wu*
377 *Wu Li Xue Bao (Shanghai)* 34:389-394.
378 Galindo KA, Lu WJ, Park JH, and Abrams JM. 2009. The Bax/Bak ortholog in Drosophila,
379 Debcl, exerts limited control over programmed cell death. *Development* 136:275-283.
380 Gasser T. 2009. Molecular pathogenesis of Parkinson disease: insights from genetic studies.
381 *Expert Reviews in Molecular Medicine* 11:null-null.

382 Goujon M, McWilliam H, Li W, Valentin F, Squizzato S, Paern J, and Lopez R. 2010. A new
383 bioinformatics analysis tools framework at EMBL–EBI. *Nucleic acids research*
384 38:W695-W699.
385 Guo M. 2012. Drosophila as a model to study mitochondrial dysfunction in Parkinson's disease.
386 *Cold Spring Harbor perspectives in medicine* 2.
387 Gupta A, Dawson VL, and Dawson TM. 2008. What causes cell death in Parkinson's disease?
388 *Annals of Neurology*.
389 Haywood AF, and Staveley BE. 2004. Parkin counteracts symptoms in a Drosophila model of
390 Parkinson's disease. *BMC Neuroscience* 5:14.
391 Hou YC, Chittaranjan S, Barbosa SG, McCall K, and Gorski SM. 2008. Effector caspase Dcp-1
392 and IAP protein Bruce regulate starvation-induced autophagy during Drosophila
393 melanogaster oogenesis. *Journal of Cell Biology* 182:1127-1139.
394 Huu NT, Yoshida H, and Yamaguchi M. 2015. Tumor suppressor gene OSCP1/NOR1 regulates
395 apoptosis, proliferation, differentiation, and ROS generation during eye development of
396 Drosophila melanogaster. *The FEBS journal*.
397 Igaki T, Kanuka H, Inohara N, Sawamoto K, Nunez G, Okano H, and Miura M. 2000. Drob-1, a
398 Drosophila member of the Bcl-2/CED-9 family that promotes cell death. *Proceedings of*
399 *the National Academy of Sciences of the United States of America* 97:662-667.
400 Jörg BS. 2007. Mechanisms of neurodegeneration in idiopathic Parkinson's disease.
401 *Parkinsonism & Related Disorders* 13.
402 Kong Y, Liang X, Liu L, Zhang D, Wan C, Gan Z, and Yuan L. 2015. High Throughput
403 Sequencing Identifies MicroRNAs Mediating alpha-Synuclein Toxicity by Targeting
404 Neuroactive-Ligand Receptor Interaction Pathway in Early Stage of Drosophila
405 Parkinson's Disease Model. *PLoS One* 10:e0137432.
406 Kornbluth S, and White K. 2005. Apoptosis in Drosophila: neither fish nor fowl (nor man, nor
407 worm). *Journal of Cell Science* 118:1779-1787.
408 Kramer JM, and Staveley BE. 2003. GAL4 causes developmental defects and apoptosis when
409 expressed in the developing eye of Drosophila melanogaster. *Genet Mol Res* 2:43-47.
410 Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, Brownstein MJ,
411 Jonnalagada S, Chernova T, Dehejia A, Lavedan C, Gasser T, Steinbach PJ, Wilkinson
412 KD, and Polymeropoulos MH. 1998. The ubiquitin pathway in Parkinson's disease.
413 *Nature* 395:451-452.
414 Li H, Chaney S, Roberts IJ, Forte M, and Hirsh J. 2000. Ectopic G-protein expression in
415 dopamine and serotonin neurons blocks cocaine sensitization in Drosophila melanogaster.
416 *Current Biology* 10:211-214.
417 Li MX, and Dewson G. 2015. Mitochondria and apoptosis: emerging concepts. *F1000Prime Rep*
418 7:42.
419 Lopez J, and Tait SW. 2015. Mitochondrial apoptosis: killing cancer using the enemy within.
420 *British Journal of Cancer* 112:957-962.
421 Ly LL, Suyari O, Yoshioka Y, Tue NT, Yoshida H, and Yamaguchi M. 2013. dNF-YB plays
422 dual roles in cell death and cell differentiation during Drosophila eye development. *Gene*
423 520:106-118.
424 Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F, Geer LY, Geer RC, He J,
425 Gwadz M, Hurwitz DI, Lanczycki CJ, Lu F, Marchler GH, Song JS, Thanki N, Wang Z,
426 Yamashita RA, Zhang D, Zheng C, and Bryant SH. 2015. CDD: NCBI's conserved
427 domain database. *Nucleic acids research* 43:D222-226.

428 Martinou JC, and Youle RJ. 2011. Mitochondria in apoptosis: Bcl-2 family members and
429 mitochondrial dynamics. *Dev Cell* 21:92-101.
430 Park SS, Schulz EM, and Lee D. 2007. Disruption of dopamine homeostasis underlies selective
431 neurodegeneration mediated by alpha-synuclein. *European Journal of Neuroscience*
432 26:3104-3112.
433 Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H,
434 Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A,
435 Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, and
436 Nussbaum RL. 1997. Mutation in the alpha-synuclein gene identified in families with
437 Parkinson's disease. *Science (New York, NY)* 276:2045-2047.
438 Quinn L, Coombe M, Mills K, Daish T, Colussi P, Kumar S, and Richardson H. 2003. Buffy, a
439 Drosophila Bcl-2 protein, has anti-apoptotic and cell cycle inhibitory functions. *EMBO*
440 *Journal* 22:3568-3579.
441 Richardson H, and Kumar S. 2002. Death to flies: Drosophila as a model system to study
442 programmed cell death. *Journal of Immunological Methods* 265:21-38.
443 Rodriguez M, Rodriguez-Sabate C, Morales I, Sanchez A, and Sabate M. 2015. Parkinson's
444 disease as a result of aging. *Aging Cell* 14:293-308.
445 Senoo-Matsuda N, Igaki T, and Miura M. 2005. Bax-like protein Drob-1 protects neurons from
446 expanded polyglutamine-induced toxicity in Drosophila. *EMBO Journal* 24:2700-2713.
447 Sevrioukov EA, Burr J, Huang EW, Assi HH, Monserrate JP, Purves DC, Wu JN, Song EJ, and
448 Brachmann CB. 2007. Drosophila Bcl-2 proteins participate in stress-induced apoptosis,
449 but are not required for normal development. *Genesis* 45:184-193.
450 Siddiqui WA, Ahad A, and Ahsan H. 2015. The mystery of BCL2 family: Bcl-2 proteins and
451 apoptosis: an update. *Archives of Toxicology* 89:289-317.
452 Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert
453 M, Söding J, Thompson JD, and Higgins DG. 2011. Fast, scalable generation of
454 high-quality protein multiple sequence alignments using Clustal Omega. *Molecular*
455 *Systems Biology* 7.
456 Staveley BE. 2014. Drosophila Models of Parkinson Disease. In: LeDoux MS, ed. *Movement*
457 *Disorders: Genetics and Models*. Second ed: Elsevier Science, 345-354.
458 Staveley BE, Phillips JP, and Hilliker AJ. 1990. Phenotypic consequences of copper-zinc
459 superoxide dismutase overexpression in Drosophila melanogaster. *Genome* 33:867-872.
460 Subramaniam SR, and Chesselet MF. 2013. Mitochondrial dysfunction and oxidative stress in
461 Parkinson's disease. *Progress in Neurobiology* 106-107:17-32.
462 Suen DF, Norris KL, and Youle RJ. 2008. Mitochondrial dynamics and apoptosis. *Genes &*
463 *Development* 22:1577-1590.
464 Todd AM, and Staveley BE. 2004. novel assay and analysis for measuring climbing ability in
465 *Drosophila*. *Drosophila Information Services* 87:101-107.
466 Tsujimoto Y. 2002. Bcl-2 family of proteins: life-or-death switch in mitochondria. *Biosci Rep*
467 22:47-58.
468 Webb JL, Ravikumar B, Atkins J, Skepper JN, and Rubinsztein DC. 2003. Alpha-Synuclein is
469 degraded by both autophagy and the proteasome. *The Journal of biological chemistry*
470 278:25009-25013.
471 Whitworth AJ. 2011. Drosophila models of Parkinson's disease. *Advances in Genetics* 73:1-50.

Xilouri M, and Stefanis L. 2015. Chaperone mediated autophagy to the rescue: A new-fangled target for the treatment of neurodegenerative diseases. *Molecular and cellular neurosciences* 66:29-36.

Zhang H, Huang Q, Ke N, Matsuyama S, Hammock B, Godzik A, and Reed JC. 2000. Drosophila pro-apoptotic Bcl-2/Bax homologue reveals evolutionary conservation of cell death mechanisms. *The Journal of biological chemistry* 275:27303-27306.

Zhu Y, Duan C, Lu L, Gao H, Zhao C, Yu S, Ueda K, Chan P, and Yang H. 2011. alpha-Synuclein overexpression impairs mitochondrial function by associating with adenylate translocator. *Int J Biochem Cell Biol* 43:732-741.

Zhu ZJ, Wu KC, Yung WH, Qian ZM, and Ke Y. 2016. Differential interaction between iron and mutant alpha-synuclein causes distinctive Parkinsonian phenotypes in Drosophila. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*.

Figures

Figure 1 - Debcl is related to human Bcl-2 ovarian killer (Bok)

(A). When debcl protein is aligned with human Bok the Bcl-2 homology (BH) domains show strong conservation. Clustal Omega multiple sequence alignment (Goujon et al. 2010; Sievers et al. 2011) of *Drosophila melanogaster* debcl protein (Dmel is *Drosophila melanogaster* NP_788278.1) with the human Bok (Hsap is *Homo sapiens* NP_115904.1), mouse Bok (Mmus is *Mus musculus* NP_058058.1) and mosquito Bok (Agam is *Anopheles gambiae* NP_309956.4) showing the highlighted conserved BH domains and the TM helices. The domains were identified using NCBI Conserved Domain Database Search (CDD) (Marchler-Bauer et al. 2015) and ELM resource search for functional sites (Dinkel et al. 2016). "*" indicate the residues that are identical, ":" indicate the conserved substitutions, "." indicate the semi-conserved substitutions. Colours show the chemical nature of amino acids. Red is small hydrophobic (including aromatic), Blue is acidic, Magenta is basic, and Green is basic with hydroxyl or amine groups.

Figure 2 – *Debcl*-induced phenotypes are rescued by the pro-survival Buffy

A) The directed inhibition of *debcl* in the DA neurons driven by *Ddc-Gal4* results in a slightly increased median survival compared to the control flies overexpressing *UAS-lacZ*, while the

overexpression of *debcl* results in severely reduced survival. The genotypes are *UAS-lacZ/ Ddc-Gal4; UAS-debcl-RNAi/ Ddc-Gal4* and *UAS-debcl/ Ddc-Gal4*. Longevity is shown as percent survival ($P < 0.01$, determined by log-rank and $n \geq 200$). B) The inhibition of *debcl* results in improved climbing ability whereas the overexpression of debcl results in a highly compromised climbing ability as determined by non-linear fitting of the climbing curves and comparing at 95% confidence intervals. The genotypes are *UAS-lacZ/ Ddc-Gal4; UAS-debcl-RNAi/ Ddc-Gal4* and *UAS-debcl/ Ddc-Gal4*. Error bars indicate the standard error of the mean (SEM) and $n=50$. C) The overexpression of *Buffy* along with the overexpression of *debcl* or *debcl-RNAi* restores lifespan and D) significantly improves the climbing ability of these flies. The genotypes are *UAS-Buffy; Ddc-Gal4/ UAS-lacZ, UAS-Buffy; Ddc-Gal4/ UAS-debcl-RNAi* and *UAS-Buffy; Ddc-Gal4/ UAS-debcl*. Longevity was determined by log-rank (Mantel-Cox) test and $n \geq 200$ while climbing ability curves were fitted non-linearly and compared with 95% CI.

Figure 3 – Overexpression of *debcl* enhances the *α-synuclein*-induced phenotypes

A) Directed overexpression of *debcl* in the DA neurons severely decreases longevity whereas its inhibition shows an improvement in lifespan. Genotypes are *UAS-α-synuclein; Ddc-Gal4/UAS-lacZ; UAS-α-synuclein; Ddc-Gal4/ UAS-debcl-RNAi;* and *UAS-α-synuclein; Ddc-Gal4/ UAS-debcl.* Longevity is shown as percent survival ($P < 0.01$, determined by log-rank and $n \geq 200$). B) The co-expression of *debcl* in the *α-synuclein* model of PD enhanced the age-dependent loss in climbing ability. The directed inhibition of *debcl* in the DA neurons improved the climbing ability over time compared to the control. The genotypes are *UAS-α-synuclein; Ddc-Gal4/UAS-lacZ, UAS-α-synuclein; Ddc-Gal4/ UAS-debcl-RNAi,* and *UAS-α-synuclein; Ddc-Gal4/ UAS-debcl.* Analysis of the climbing curves and significance was determined by comparing the 95% confidence intervals. Error bars indicate the SEM and $n=50$.

Figure 4 – *Buffy* partially rescues the *debcl*-induced developmental eye defects

Scanning electron micrographs when *debcl* is overexpressed or inhibited in the eye with the eye-specific *GMR-Gal4* transgene; (I) *GMR-Gal4/ UAS-lacZ;* (II) *GMR-Gal4/ UAS-debcl-RNAi;* (III) *GMR-Gal4/ UAS-debcl,* when co-expressed with *α-synuclein*; (IV) *UAS-α-synuclein; GMR-Gal4 / UAS-lacZ;* V) *UAS-α-synuclein; GMR-Gal4 / UAS-debcl-RNAi* VI) *UAS- α-synuclein; GMR-Gal4/ UAS-debcl;* and when co-expressed with *Buffy* VII) *UAS-Buffy; GMR-Gal4/ UAS-lacZ* VIII) *UAS-Buffy; GMR-Gal4/ UAS-debcl-RNAi* and IX) *UAS-Buffy; GMR-Gal4/ UAS-debcl*.

Figure 1(on next page)

Figure 1 - Debcl is related to human Bcl-2 ovarian killer (Bok)

(A). When debcl protein is aligned with human Bok the Bcl-2 homology (BH) domains show strong conservation. Clustal Omega multiple sequence alignment (Goujon et al. 2010 ; Sievers et al. 2011) of *Drosophila melanogaster* debcl protein (Dmel is *Drosophila melanogaster* NP_788278.1) with the human Bok (Hsap is *Homo sapiens* NP_115904.1), mouse Bok (Mmus is *Mus musculus* NP_058058.1) and mosquito Bok (Agam is *Anopheles gambiae* NP_309956.4) showing the highlighted conserved BH domains and the TM helices. The domains were identified using NCBI Conserved Domain Database Search (CDD) (Marchler-Bauer et al. 2015) and ELM resource search for functional sites (Dinkel et al. 2016) . "*" indicate the residues that are identical, ":" indicate the conserved substitutions, "." indicate the semi-conserved substitutions. Colours show the chemical nature of amino acids. Red is small hydrophobic (including aromatic), Blue is acidic, Magenta is basic, and Green is basic with hydroxyl or amine groups.

1

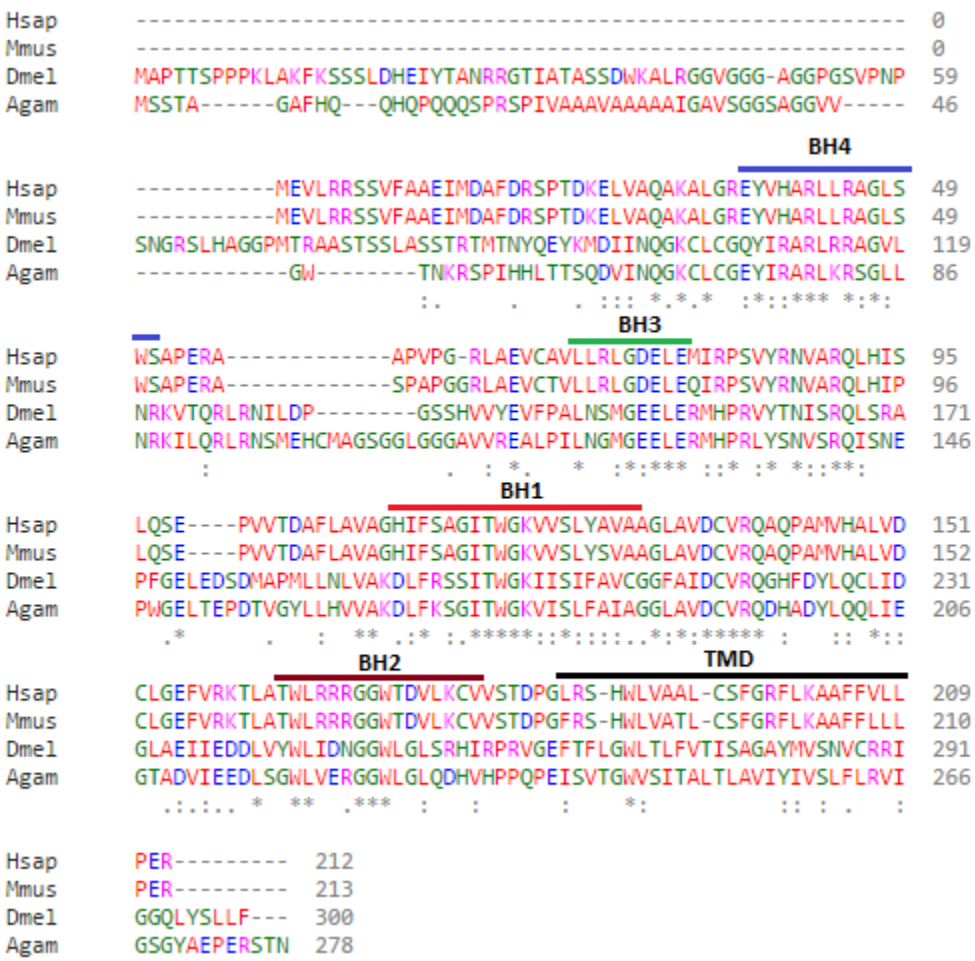

BH4
BH3
BH1
BH2
TMD
Hsap ---------- 0
Mmus ---------- 0
Dmel MAPTTSPPPKLAKFKSSSLDHEIYTANRRGTIATASSDWKALRGGVGGG-AGGPGSVPNP 59
Agam MSSTA------GAFHQ---QHQPQQQSPRSPIVAAAVAAAAAIGAVSGGSAGGVV----- 46
Hsap ----------MEVLRRSSVFAAEIMDAFDRSPTDKELVAQAKALGREYVHARLLRAGLS 49
Mmus ----------MEVLRRSSVFAAEIMDAFDRSPTDKELVAQAKALGREYVHARLLRAGLS 49
Dmel SNGRSLHAGGPMTRAASTSSLASSTRTMTNYQEYKMDIINQGKCLCGQYIRARLRRAGVL 119
Agam ------------GW--------TNKRSPIHHLTTSQDVINQGKCLCGEYIRARLKRSGLL 86
Hsap WSAPERA-------------APVPG-RLAEVCAVLLRLGDELEMIRPSVYRNVARQLHIS 95
Mmus WSAPERA-------------SPAPGGRLAEVCTVLLRLGDELEQIRPSVYRNVARQLHIP 96
Dmel NRKVTQRLRNILDP--------GSSHVVYEVFPALNSMGEELERMHPRVYTNISRQLSRA 171
Agam NRKILQRLRNSMEHCMAGSGGLGGGAVVREALPILNGMGEELERMHPRLYSNVSRQISNE 146
Hsap LQSE----PVVTDAFLAVAGHIFSAGITWGKVVSLYAVAAGLAVDCVRQAQPAMVHALVD 151
Mmus LQSE----PVVTDAFLAVAGHIFSAGITWGKVVSLYSVAAGLAVDCVRQAQPAMVHALVD 152
Dmel PFGELEDSDMAPMLLNLVAKDLFRSSITWGKIISIFAVCGGFAIDCVRQGHFDYLQCLID 231
Agam PWGELTEPDTVGYLLHVVAKDLFKSGITWGKVISLFAIAGGLAVDCVRQDHADYLQQLIE 206
Hsap CLGEFVRKTLATWLRRRGGWTDVLKCVVSTDPGLRS-HWLVAAL-CSFGRFLKAAFFVLL 209
Mmus CLGEFVRKTLATWLRRRGGWTDVLKCVVSTDPGFRS-HWLVATL-CSFGRFLKAAFFLLL 210
Dmel GLAEIIEDDLVYWLIDNGGWLGLSRHIRPRVGEFTFLGWLTLFVTISAGAYMVSNVCRRI 291
Agam GTADVIEEDLSGWLVERGGWLGLQDHVHPPQPEISVTGWVSITALTLAVIYIVSLFLRVI 266
Hsap PER--------- 212
Mmus PER--------- 213
Dmel GGQLYSLLF--- 300
Agam GSGYAEPERSTN 278

Figure 2(on next page)

Figure 2 – *Debcl*-induced phenotypes are rescued by the pro-survival Buffy

A) The directed inhibition of *debcl* in the DA neurons driven by *Ddc-Gal4* results in a slightly increased median survival compared to the control flies overexpressing *UAS-lacZ*, while the overexpression of *debcl* results in severely reduced survival. The genotypes are *UAS-lacZ/ Ddc-Gal4; UAS-debcl-RNAi/ Ddc-Gal4* and *UAS-debcl/ Ddc-Gal4.* Longevity is shown as percent survival ($P < 0.01$, determined by log-rank and $n \geq 200$). B) The inhibition of *debcl* results in improved climbing ability whereas the overexpression of debcl results in a highly compromised climbing ability as determined by non-linear fitting of the climbing curves and comparing at 95% confidence intervals. The genotypes are *UAS-lacZ/ Ddc-Gal4; UAS-debcl-RNAi/ Ddc-Gal4* and *UAS-debcl/ Ddc-Gal4.* Error bars indicate the standard error of the mean (SEM) and $n=50$. C) The overexpression of *Buffy* along with the overexpression of *debcl* or *debcl-RNAi* restores lifespan and D) significantly improves the climbing ability of these flies. The genotypes are *UAS-Buffy; Ddc-Gal4/ UAS-lacZ, UAS-Buffy; Ddc-Gal4/ UAS-debcl-RNAi* and *UAS-Buffy; Ddc-Gal4/ UAS-debcl*. Longevity was determined by log-rank (Mantel-Cox) test and $n \geq 200$ while climbing ability curves were fitted non-linearly and compared with 95% CI.

2

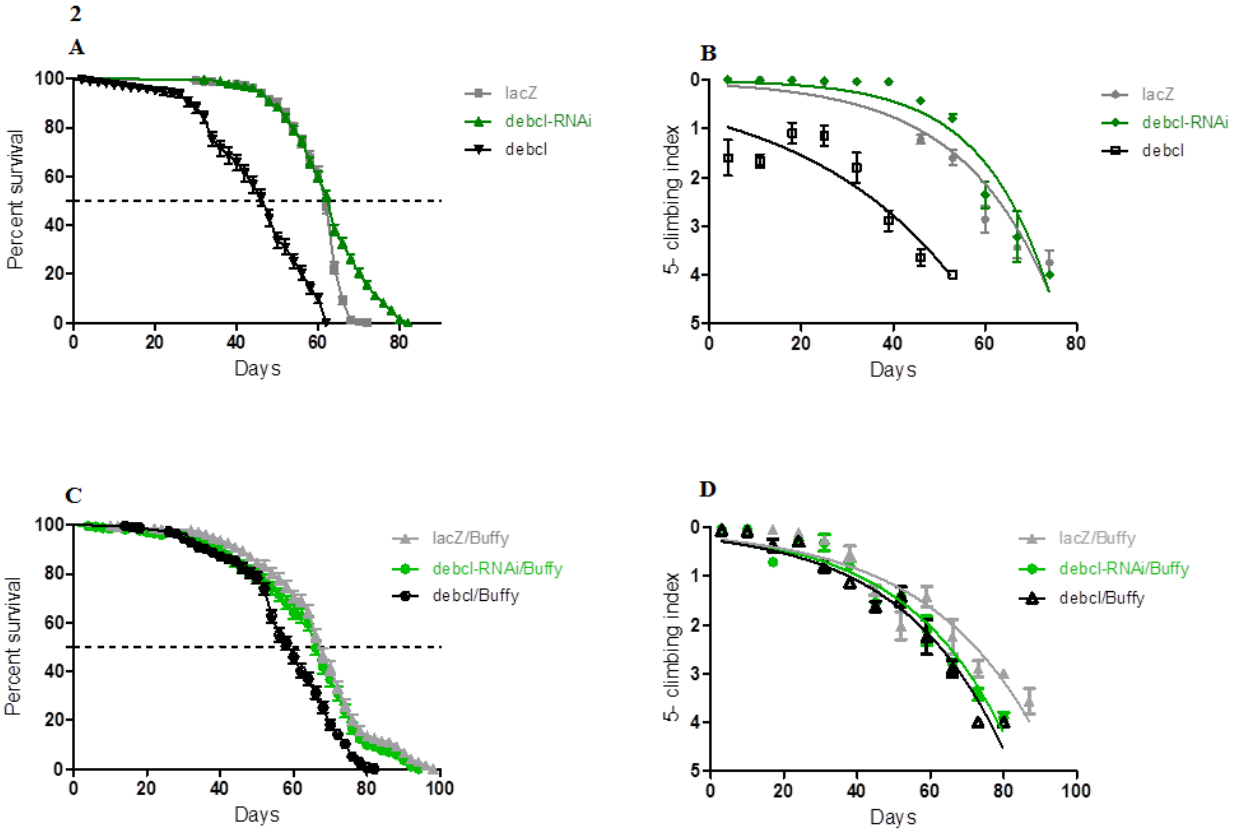

A
Percent survival
Days
lacZ
debcl-RNAi
debcl
B
5- climbing index
Days
lacZ
debcl-RNAi
debcl
C
Percent survival
Days
lacZ/Buffy
debcl-RNAi/Buffy
debcl/Buffy
D
5- climbing index
Days
lacZ/Buffy
debcl-RNAi/Buffy
debcl/Buffy

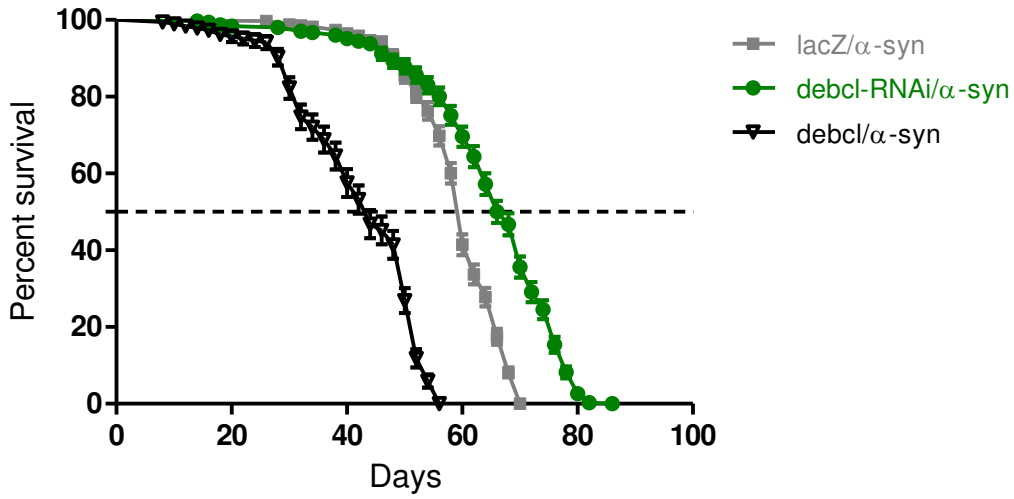
Figure 3(on next page)

Figure 3 – Overexpression of *debcl* enhances the *α-synuclein*-induced phenotypes

A) Directed overexpression of *debcl* in the DA neurons severely decreases longevity whereas its inhibition shows an improvement in lifespan. Genotypes are *UAS-α-synuclein; Ddc-Gal4/UAS-lacZ; UAS-α-synuclein; Ddc-Gal4/ UAS-debcl-RNAi;* and *UAS-α-synuclein; Ddc-Gal4/ UAS-debcl.* Longevity is shown as percent survival ($P < 0.01$, determined by log-rank and $n \geq 200$). B) The co-expression of *debcl* in the *α-synuclein* model of PD enhanced the age-dependent loss in climbing ability. The directed inhibition of *debcl* in the DA neurons improved the climbing ability over time compared to the control. The genotypes are *UAS-α-synuclein; Ddc-Gal4/UAS-lacZ, UAS-α-synuclein; Ddc-Gal4/ UAS-debcl-RNAi,* and *UAS-α-synuclein; Ddc-Gal4/ UAS-debcl.* Analysis of the climbing curves and significance was determined by comparing the 95% confidence intervals. Error bars indicate the SEM and $n=50$.

3

A.



B.

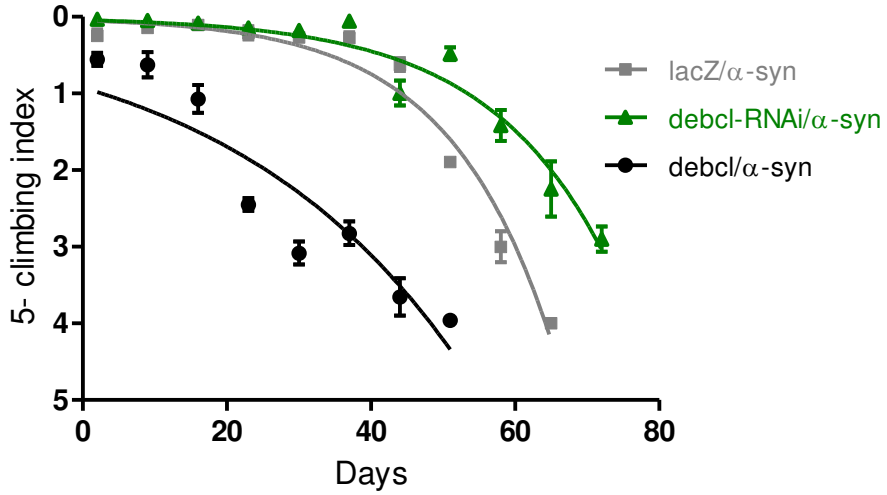


Figure 4(on next page)

Figure 4 – *Buffy* partially rescues the *debcl*-induced developmental eye defects

Scanning electron micrographs when *debcl* is overexpressed or inhibited in the eye with the eye-specific *GMR-Gal4* transgene; (I) *GMR-Gal4/ UAS-lacZ;* (II) *GMR-Gal4/ UAS-debcl-RNAi;* (III) *GMR-Gal4/ UAS-debcl,* when co-expressed with *α-synuclein*; (IV) *UAS-α-synuclein; GMR-Gal4 / UAS-lacZ;* V) *UAS-α-synuclein; GMR-Gal4 / UAS-debcl-RNAi* VI) *UAS- α-synuclein; GMR-Gal4/ UAS-debcl;* and when co-expressed with *Buffy* VII) *UAS-Buffy; GMR-Gal4/ UAS-lacZ* VIII) *UAS-Buffy; GMR-Gal4/ UAS-debcl-RNAi* and IX) *UAS-Buffy; GMR-Gal4/ UAS-debcl*.