Neuronal remodeling and apoptosis require VCP-dependent degradation of the apoptosis inhibitor DIAP1

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SUMMARY
The regulated degeneration of axons or dendrites (pruning) and neuronal apoptosis are widely used during development to determine the specificity of neuronal connections. Pruning and apoptosis often share similar mechanisms; for example, developmental dendrite pruning of Drosophila class IV dendritic arborization (da) neurons is induced by local caspase activation triggered by ubiquitin-mediated degradation of the caspase inhibitor DIAP1. Here, we examined the function of Valosin-containing protein (VCP), a ubiquitin-selective AAA chaperone involved in endoplasmic reticulum-associated degradation, autophagy and neurodegenerative disease, in Drosophila da neurons. Strong VCP inhibition is cell lethal, but milder inhibition interferes with dendrite pruning and developmental apoptosis. These defects are associated with impaired caspase activation and high DIAP1 levels. In cultured cells, VCP binds to DIAP1 in a ubiquitin- and BIR domain-dependent manner and facilitates its degradation. Our results establish a new link between ubiquitin, dendrite pruning and the apoptosis machinery.

KEY WORDS: Pruning, Apoptosis, DIAP1 (Thread), VCP (TER94), Drosophila

INTRODUCTION
Developmentally programmed neuronal cell death and developmental pruning of axons or dendrites are important processes that shape the connections in the nervous system. Pruning can occur by several mechanisms, including retraction and local degeneration of the affected processes. Local degeneration of axons or dendrites often involves caspase activation and thus shares features with apoptosis (Kuo et al., 2006; Nikolaev et al., 2009; Williams et al., 2006). In the Drosophila peripheral nervous system, class III and class IV dendritic arborization (da) neurons have stereotyped long and branched dendrites at larval stages (Grueber et al., 2002). During the pupal stage, a subset of class IV neurons prune and then regrow their dendrites (Kuo et al., 2005; Williams and Truman, 2005), whereas class III neurons undergo developmental apoptosis (Williams and Truman, 2005). Both pruning and developmental apoptosis in these neurons require caspase activation through ubiquitin-mediated degradation of the caspase inhibitor Drosophila inhibitor of apoptosis protein 1 [DIAP1; Thread (Th) – FlyBase] (Kuo et al., 2006; Williams et al., 2006; Williams and Truman, 2005).

VCP (TER94 – FlyBase; CDC-48/CDC48 in C. elegans and yeast; also known as p97 in vertebrates) is an abundant AAA ATPase that is required for the degradation of a subset of substrates of the ubiquitin-proteasome system (Jentsch and Rumpf, 2007; Schuberth and Buchberger, 2008). Its best-understood function is the degradation of misfolded proteins of the endoplasmic reticulum (ER) in the ER-associated degradation (ERAD) pathway (Ye et al., 2001), but it has also been linked to a number of other processes including nucleus reformation after mitosis (Ramadan et al., 2007), myofibril assembly (Janisch et al., 2007) and autophagosome maturation (Ju et al., 2009; Tresse et al., 2010). Interestingly, certain autosomal dominant mutations of VCP cause a degenerative disease called inclusion body myopathy with Paget’s disease of bone and frontotemporal dementia (IBMPFD), which mostly affects muscles and brain (Watts et al., 2004), thus linking VCP to brain function. It has been proposed that VCP is required for the degradation of ubiquitylated substrate proteins when they need to be extracted from the ER membrane or a tight protein complex. However, VCP is also sometimes required for the degradation of monomeric soluble proteins, such as GFP-based model substrates.

A potential explanation for this observation is that these substrates are particularly strongly folded and VCP might be needed to unfold them prior to degradation (Beskow et al., 2009). However, the exact mechanism of action of VCP in many processes remains unclear owing to a lack of knowledge concerning its substrates.

We investigated neural developmental functions of VCP in Drosophila class III and class IV da neurons. We find that VCP inhibition in larval neurons induces proteotoxic stress, which, depending on the strength of the inhibition, can be cell lethal. Remarkably, intermediate levels of VCP inhibition are not lethal, but instead suppress dendrite pruning, apoptosis and caspase activation at the pupal stage. We show that these phenotypes are caused by impaired caspase activation and DIAP1 degradation. Thus, VCP-dependent DIAP1 degradation is required for proper neuronal remodeling and apoptosis.

MATERIALS AND METHODS
Fly stocks
Dendritic arborization neurons were labeled using the following GAL4 lines: ppk-GAL4 (Grueber et al., 2007), ppk-GeneSwitch [gift from Rebecca Yang (Duke University Medical Center, NC, USA) and Jay Parrish (University of Washington, WA, USA)] Gal80ts12 [gift from H.-H. Lee and Y. Xiang (National Taiwan University Medical School)], GALA10628, UAS-GMA (Dutta et al., 2002; Medina et al., 2006) and ppk-CD4::tdTomato [gift from P. Soba, (University of California, San Francisco, CA, USA)]. VCP constructs (wild type, QQ dominant-negative, R152H) were cloned into pUAST (Brand and Perrimon, 1993) and injected

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DEVELOPMENT
into w1118 flies according to standard procedures. The JNK reporter line was puc1253;193 (Bloomington) and UAS-Xbp1-GFP (Ryoo et al., 2007) was used to assess ER stress. UAS-tau::GFP was a gift of W. Song (University of California, San Francisco, CA, USA); other lines included UAS-mCD66GFP (Lee and Luo, 1999), UAS-dVCP-IR (inverted repeat, VDRC 24354) and UAS-dcr2 (Dietzl et al., 2007), UAS-p35 (Hay et al., 1994) (Bloomington) and UAS-mCD8::PARP::Venus (Williams et al., 2006). Mutant alleles included dvcp26-2 and dvcp2-1 (Goldstein et al., 2001; Ruden et al., 2000) and th4 (Hay et al., 1995) (all from Bloomington).

**Live imaging**

Live imaging of da neurons in appropriately staged larvae or pupae was carried out on a Leica SP5 confocal microscopy. Images were taken from neurons in abdominal segments A2-A6. In all images shown, anterior is left and dorsal is up. Fisher’s exact test (GraphPad) was used for statistical comparisons.

**Immunocytochemistry**

Larvae or appropriately staged pupae (4 hours APF) were dissected as described (Kuo et al., 2006). Antibodies used were rat anti-VCP (1:500) (Leon and McKearin, 1999), mouse anti-ubiquitin (1:50; P4D1, Sigma), rabbit anti-p-galactosidase (1:5000; Cortex Biochem), rabbit anti-activated caspase (1:100; Trevigen), rabbit anti-cleaved PARP (1:20; Abcam 2317), rabbit anti-DIAP1 (1:1000) (Ryoo et al., 2002), rat anti-mCD8 (1:200; Invitrogen) and rabbit anti-GFP (1:3000; Jan lab). Donkey Cy2- or Rhodamine Red X-conjugated secondary antibodies (Jackson Laboratory) were used at 1:200.

**Transfections, immunoprecipitation and Western blotting**

FLAG-tagged forms of Drosophila VCP and HA-tagged wild-type and mutant forms of DIAP1 were in pUAST. For expression in S2 cells, plasmids were co-transfected with Actin-GAL4 using the Effectene Kit (Qiagen) according to the manufacturer’s instructions. After 48 hours, cells were harvested and lysed in RIPA buffer containing 1 mM PMSF and complete protease inhibitors (Roche). Where indicated, the proteasome inhibitor MG132 (10 μM) was added 3 hours before lysis. For immunoprecipitations, cells were lysed in lysis buffer (100 mM NaCl, 50 mM Tris-HCl pH 7.5, 5 mM MgCl2, 10% glycerol, 1% Triton X-100, 1 mM PMSF, 1× complete protease inhibitors). Extracts were precleared with IgG Sepharose 6 Fast Flow beads (Pharmacia) and immunoprecipitated with mouse anti-HA beads (Sigma) for 2 hours, washed with lysis buffer and eluted with SDS loading buffer. Primary antibodies for Western blotting were mouse anti-DIAP1 (Yoo et al., 2002), mouse anti-FLAG (M2, Sigma), rat anti-HA (3F10, Roche), mouse anti-tubulin (DM1a, Sigma) and mouse anti-GAPDH (clone 71.1, Sigma).

**RESULTS**

**Drosophila VCP is required for proper neuronal morphology and viability**

In order to characterize the function of VCP in neurons, we used the GAL4/UAS system (Brand and Perrimon, 1993) to express either wild-type VCP, an ATPase-deficient VCP transgene (E375Q, E575Q, referred to as VCP QQ) that acts as a dominant-negative (Ye et al., 2001), or an RNAi construct directed against VCP (Dietzl et al., 2007). We expressed VCP RNAi or VCP QQ in class III neurons using the GAL4 driver Gal419-12 (Goldstein et al., 2001) or used two copies of the UAS-VCP QQ transgene. Whereas the heterozygous mutations did not visibly affect the ddaC neuron (Fig. 1I), expression of VCP RNAi in vcp12/12+ animals frequently led to a drastic reduction of the dendritic field and also induced lethality, as neurons were sometimes missing or displayed blebbing and fragmenting dendrites (drastic dendrite reduction or cell death occurred in 37% of all neurons; Fig. 1I). Similar results were seen when the copy number of VCP QQ was increased (strong defects occurred in 64% of all neurons; Fig. 1K). We also performed mosaic analysis with a repressible cell marker (MARC3) to test whether cells homozygous for the element insertion vcp10/15502 showed similar defects. At the third instar stage, the morphology of vcp10/15502 mutant neurons was almost wild-type, which was likely to be due to maternal contribution and protein perdurance, although these cells died shortly after the onset of the pupal phase (data not shown).

**VCP inhibition induces several cellular stress pathways**

To address the mechanism underlying cell death upon strong VCP inhibition, we next asked whether class IV neurons expressing the dominant-negative VCP QQ showed signs of proteotoxic stress. First, we asked whether we could detect signs of impaired proteasomal degradation. When we stained class IV neurons with an antibody against ubiquitin, we found that class IV neurons expressing VCP QQ showed strongly increased ubiquitin levels, indicating that ubiquitylated substrate proteins had accumulated (Fig. 2A,B). We also asked whether stress signaling pathways, such as the pro-apoptotic Jun N-terminal kinase [JNK; also known as stress-activated protein kinase (SAPK) or Basket] pathway or the unfolded protein response (UPR), were activated. To address JNK activation, we used a lacZ-containing enhancer-trap insertion in the JNK target gene puckered (puc-lacZ), which acts as a reporter for JNK activity. puc-lacZ activity was low in control class IV neurons compared with two neighboring class I neurons (ddaD and ddaE), but it was strongly increased in class IV neurons expressing VCP QQ (Fig. 2C,D). In order to assess UPR activation, we expressed an Xbp1-GFP reporter (Ryoo et al., 2007) in class IV neurons. UPR activation induces correct splicing of this construct such that GFP fluorescence becomes detectable upon ER stress. Whereas no GFP could be detected in control neurons, neurons expressing VCP QQ frequently showed GFP signals in the nucleus. Thus, consistent with previous reports (Kobayashi et al., 2002; Ye et al., 2001), VCP inhibition interferes with proteasomal protein degradation and induces JNK signaling and the UPR. Both these signaling pathways can induce apoptosis; for example, JNK signaling can induce the expression of pro-apoptotic genes such as reaper and Hid (Wrinkled – FlyBase) (McEwen and Peifer, 2005). We conclude that, depending on the strength of VCP inhibition, these stresses can eventually lead to cell death.
VCP is required for neuronal remodeling and apoptosis

During metamorphosis, the larval dendrites of class IV neurons are pruned (i.e. severed and degraded) in a ubiquitin- and caspase-dependent process, and new, adult-type dendrites grow out later (Kuo et al., 2005; Kuo et al., 2006; Williams et al., 2006). As expression of VCP RNAi or one copy of the dominant-negative VCP QQ did not affect cell viability, we were able to examine the effects of VCP inhibition at this stage. At 18 hours after puparium formation (APF), control class IV neurons and neurons overexpressing wild-type VCP had lost all their larval dendrites [0% not severed (n=16) and 2% not severed (n=52), respectively] (Fig. 3A,C). By contrast, a high proportion of class IV neurons expressing VCP RNAi or VCP QQ retained long, attached dendrites [63% not severed (n=65) and 54% not severed (n=54), respectively] (Fig. 3B,D). In order to exclude the possibility that these defects were the consequence of the earlier dendritic morphology defects described above, we sought to inhibit VCP after dendrite morphogenesis was completed using the RU486-inducible GasSwitch technique (Osterwalder et al., 2001). To this end, we used a ppk-GAL4::tdTomato transgene to visualize neuronal morphology and a ppk-GasSwitch (ppk-GS) transgene to induce the expression of VCP QQ. In the absence of drug, the morphology of class IV neurons appeared wild type at the third instar larval stage (data not shown) and most neurons had pruned their dendrites at 18 hours APF [defects occurred in 2/28 neurons (7%); Fig. 3E]. Addition of 100 μM RU486 24 hours before the onset of metamorphosis did not visibly change the morphology of the larval neurons (data not shown); however, a high proportion of class IV neurons in these drug-treated animals retained their dendrites at 18 hours APF [defects occurred in 24/34 neurons (70%); Fig. 3F], indicating that the phenotypes observed during metamorphosis can be disassociated from the defects at the larval stage.

We next examined the effect of VCP inhibition on class III neurons, which normally undergo developmental apoptosis soon after puparium formation such that they are completely removed at 18 hours APF (Williams and Truman, 2005). Control class III neurons...
neurons or class III neurons overexpressing wild-type VCP had indeed undergone apoptosis and disappeared at this stage (0/48 surviving; Fig. 3G,J), whereas expression of the baculovirus caspase inhibitor p35 (Hay et al., 1994) caused most class III neurons to survive [49/56 (87.5%); Fig. 3H]. Interestingly, expression of VCP RNAi or VCP QQ inhibited apoptosis in a large proportion of class III neurons, allowing them to persist until 18 hours APF (Fig. 3I,K). In these experiments, the ddaF neuron was also examined and showed swellings and started to fragment, and caspase activity could often be detected in fragmented dendrites with the caspase reporter (Fig. 4A, A’). When we expressed VCP QQ, at 5 hours APF class IV dendrites developed substantially fewer swellings, did not fragment and did not contain detectable caspase activity (Fig. 4B, B’). However, in class IV neurons expressing VCP QQ, we sometimes detected low levels of reporter activation around the nucleus, possibly indicating caspase activation by elevated stress levels (Fig. 4B, B’). Next, we assessed caspase activity in class III neurons at 4 hours APF. All control class III neurons examined showed high levels of active caspases in the soma, indicating that they were undergoing apoptosis \( n=11 \); Fig. 4C, C’). By contrast, expression of VCP QQ in class III neurons strongly suppressed this activity, such that these neurons did not display caspase activity \( n=12 \); Fig. 4D, D’).

DIAP1 is a crucial regulator of apoptosis in flies (Wang et al., 1999). High DIAP1 levels inhibit caspase activation, and DIAP1 degradation induces apoptosis (Ryoo et al., 2002; Yin and Thummel, 2004; Yoo et al., 2002) and dendrite pruning (Kuo et al., 2006; Lee et al., 2009). Although the limited resolution of the immunofluorescence experiments, especially at the pupal phase, prevented us from assessing DIAP1 levels in class IV neuron dendrites, DIAP1 levels could be easily examined in class III neurons. Consistent with the results of the caspase immunostaining, control class III neurons had very low levels of DIAP1 at 4 hours APF, as compared with adjacent cells (Fig. 5A, A’). By contrast, class III neurons expressing VCP QQ displayed much higher DIAP1 levels, which were comparable to those of adjacent cells (Fig. 5B, B’ and about four times higher than in control neurons (Fig. 5C).

VCP inhibition causes defects in caspase activation and degradation of the caspase inhibitor DIAP1

Both dendrite pruning and developmental apoptosis require caspase activity (Kuo et al., 2006; Williams et al., 2006; Williams and Truman, 2005). Caspase activity can be detected in severing class IV neuron dendrites and dying class III neurons during the early pupal stage at ~4–6 hours APF. We asked whether caspase activity and the levels of apoptotic regulators were changed in these neurons when VCP was inhibited. To visualize caspase activity, we either used a caspase reporter construct based on mammalian poly(ADP-ribose) polymerase 1 (PARP1), a known caspase substrate (Williams et al., 2006), or an antibody that recognizes cleaved mammalian and Drosophila caspases (Yu et al., 2002). At 5 hours APF, control class IV neuron dendrites

![Image](image_url)
Next, we assessed a potential link between VCP and DIAP1 using a genetic approach. If impaired caspase activation through blocked DIAP1 degradation is the reason for the pruning defects upon VCP inhibition, then these defects should be reversed by lowering DIAP1 levels genetically. To this end, we expressed VCP QQ in class IV neurons either in wild type or in a heterozygous diap1 mutant background (th4/+ ) that should decrease the amount of DIAP1 by half. As expected, heterozygous th4 did not affect pruning (2.6% pruning defects, n=38) (Fig. 5D), but a high proportion of neurons expressing VCP QQ in the wild-type background still had attached dendrites at 15 hours APF (59%, n=19; P<0.0005) (Fig. 5E). By contrast, the majority of class IV neurons expressing VCP QQ in the th4/+ background had pruned their dendrites (12.5% still attached at 15 hours APF, n=24) (Fig. 5F), indicating that lowering DIAP1 levels suppressed the pruning defects induced by VCP inhibition. Notably, lowering DIAP1 levels also sensitizes cells to stress-induced apoptosis, and a high proportion of the VCP QQ-expressing neurons in the th4/+ background underwent apoptosis during early metamorphosis (~60% at 15 hours APF). Taken together, inhibition of VCP in class III and class IV neurons inhibits caspase activation, probably through an effect on DIAP1.

### VCP is required for DIAP1 degradation in cultured Drosophila cells

Our histochemical and genetic data suggest that VCP might be involved in the degradation of DIAP1. In order to test this hypothesis further, we performed biochemical experiments. When we expressed VCP QQ in S2 cells, the levels of endogenous DIAP1 were increased (Fig. 6A). In the following experiments, we used an HA-tagged form of DIAP1 for ease of detection. When we co-expressed HA-tagged DIAP1 with VCP QQ, we observed additional HA-reactive high molecular weight species in western blots, indicative of the accumulation of poly-ubiquitylated DIAP1 (data not shown). To verify this, we co-expressed tagged DIAP1 with wild-type VCP or VCP QQ and immunoprecipitated DIAP1 under stringent buffer conditions. Anti-ubiquitin blots of...
the precipitates showed that poly-ubiquitylated DIAP1 did indeed accumulate upon VCP inhibition, indicating a defect in the degradation of ubiquitylated DIAP1 (Fig. 6B). In order to test whether DIAP1 and VCP interact with each other or can be found in the same protein complexes, we performed co-immunoprecipitation experiments with tagged VCP and DIAP1. In these experiments, a weak interaction between the two factors could indeed be detected (Fig. 6C). Furthermore, the DIAP1-VCP interaction was strongly enhanced with the dominant-negative VCP QQ (Fig. 6C), which acts as a substrate trap (Ye et al., 2001).

**Molecular determinants of the DIAP1-VCP interaction**

VCP is usually involved in ubiquitin-mediated degradation when a ubiquitylated substrate protein is part of a tight protein complex, or when it is tightly folded. To explore why VCP might be required for DIAP1 degradation, we tested whether DIAP1 forms oligomers or whether VCP QQ would change a protein interaction with a binding partner. We found that DIAP1 dimerizes (or oligomerizes) in a manner dependent on its RING domain, but only to a relatively low extent (see Fig. S3A in the supplementary material). Furthermore, the presence of VCP QQ did not alter the interaction of DIAP1 with the caspase Dronc (Nedd2-like caspase – FlyBase) (see Fig. S3B in the supplementary material), indicating that VCP might not be required to disrupt DIAP1-containing protein complexes.

Next, we asked whether specific DIAP1 domains are required for the interaction with VCP. DIAP1 has three major domains (Fig. 7A): two baculovirus inhibitor of apoptosis repeat (BIR) domains (BIR1 at residues 42-112 and BIR2 at residues 224-295, respectively), which are...
required for interactions with caspases and the RHG proteins Reaper, Hid and Grim; and a C-terminal RING finger domain that is required for the ubiquitylation of bound caspases and for the auto-ubiquitylation of DIAP1 under apoptosis-inducing conditions. We focused on the roles of the BIR1 and RING domains because both are located relatively close to the respective N- and C-termini of DIAP1 and might thus represent obstacles for proteasome-dependent unfolding. In addition, auto-ubiquitylation catalyzed by the RING domain might contribute to VCP binding. BIR and RING domains contain Zn\(^{2+}\) ions that are chelated by cysteine residues. We disrupted the BIR1 domain by replacing one of the coordinating cysteines with serine (C107S). This mutation lowered the levels of DIAP1, owing to increased proteasomal degradation (Fig. 7B). Interestingly, expression of VCP QQ did not increase the levels of DIAP1 C107S, indicating that the degradation of DIAP1 C107S was independent of VCP (Fig. 7B).

Next, we asked whether replacing one of the coordinating cysteines in the RING domain (C406S) would alter DIAP1 ubiquitylation. As shown above, ubiquitylated DIAP1 can be detected in S2 cells and ubiquitylated DIAP1 accumulates upon VCP inhibition. The DIAP1 C406S mutation strongly decreased the amount of ubiquitylated DIAP1, indicating that auto-ubiquitylation is responsible for a large portion of the DIAP1-ubiquitin conjugates (Fig. 7C). Residual ubiquitylation of DIAP1 C406S is consistent with the existence of several ubiquitin ligases for DIAP1 (Herman-Bachinsky et al., 2007). When we assessed the ability of these two DIAP1 mutants to interact with VCP QQ, both showed a strongly reduced interaction (Fig. 7D). These results suggest that a functional BIR1 domain and ubiquitylation are necessary for DIAP1 to be recognized as a VCP substrate.

**DISCUSSION**

In this study, we have analyzed the role of VCP during the development of peripheral da neurons in *Drosophila*. We found that VCP inhibition affects neural development and cell viability in a biphasic manner. Mild VCP inhibition causes defects in caspase activation and therefore affects apoptosis and pruning. This effect is caused by interference with the ubiquitin-dependent degradation of the caspase inhibitor DIAP1, as our data suggest that DIAP1 is a VCP substrate. By contrast, strong VCP inhibition causes severe morphological defects and cell death, probably owing to increased proteotoxic stress. A likely explanation for these seemingly contradicting phenotypes is that VCP inhibition activates pro-apoptotic signaling cascades, such as JNK signaling or the UPR. Upon strong VCP inhibition, these signals eventually override the anti-apoptotic effects through slowed DIAP1 degradation (Fig. 7E). Importantly, caspase activation can occur in the presence of DIAP1; for example, the Hid protein can displace caspases from DIAP1 and thereby activate them (Wang et al., 1999). Similar biphasic phenotypes have been reported for the ubiquitin-activating enzyme E1 (UbA1 – FlyBase): hypomorphic E1 alleles support cell viability and inhibit apoptosis via stabilization of DIAP1, whereas strong loss-of-function alleles have effects on mitosis and cell viability (Lee et al., 2008). It is interesting to speculate whether our results could be relevant for the pathogenesis of VCP-related neurodegeneration. In fact, we observed mild pruning defects in class IV neurons upon expression of a VCP disease variant (VCP R152H) (see Fig. S4 in the supplementary material). Although these defects were relatively subtle, similar defects in neuronal remodeling in humans could contribute to dementia. In addition, our results suggest that VCP mutation might induce dementia not only through stress-induced cell death but also through inhibition of other VCP-dependent neuronal processes.

Importantly, our work has identified VCP as a new regulator of DIAP1 degradation. An interesting question is how VCP might contribute to DIAP1 degradation. Our biochemical analysis suggests that an intact BIR1 domain is a major determinant for VCP binding. As our results suggest that VCP is not required to break up an interaction between DIAP1 and a binding partner, we speculate that it might be required to unfold ubiquitylated DIAP1, and specifically the BIR1 domain, prior to proteasomal degradation (Fig. 7F), as has been proposed for a GFP-based model substrate (Beskow et al., 2009). A stable BIR1 domain, in turn, might be favorable because caspase cleavage in the DIAP1 N-terminus (after amino acid 20) exposes an N-end rule degradation signal (Ditzel et al., 2003).
In other VCP-dependent pathways, such as ERAD, VCP often requires adaptor proteins to perform its functions. We also tested RNAi lines directed against the VCP interactors Ufd1-like, Npl4 (CG4673), p47 and Ufd2 (CG9934) for their effects on dendrite pruning but did not observe defects with any of these lines, indicating that VCP might not need adaptors to act on DIA1; alternatively, other, as yet unknown adaptors might be involved. Taken together, we have identified VCP as a new regulator of neuronal remodeling and developmental apoptosis, and we have identified DIA1 as the relevant substrate. Our data therefore provide a new link between the ubiquitin system and apoptosis.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

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