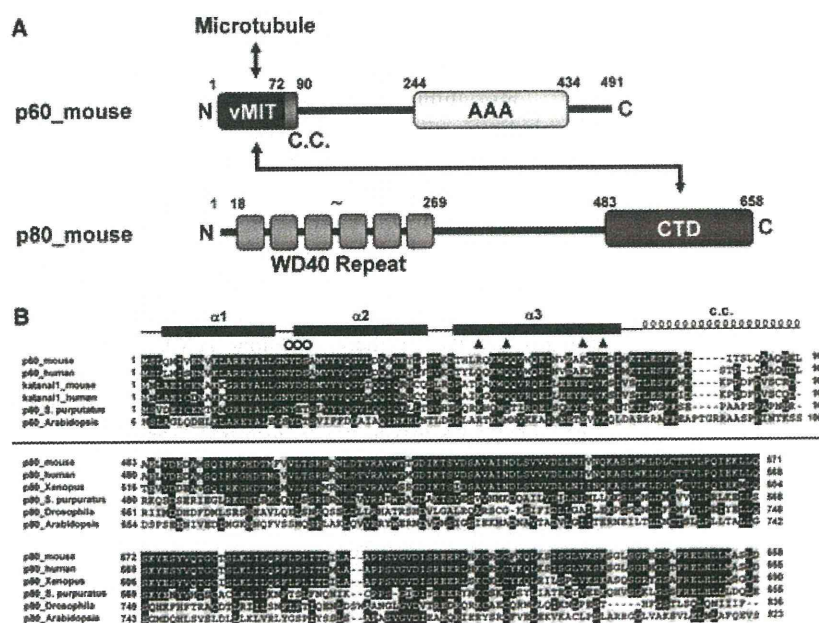


catalytic factor responsible for breaking MTs in an ATP-dependent manner and contains a single ATPase associated with various cellular activities (AAA) domain. Adaptor-p80 is a regulatory factor containing six WD40 repeats, and is not essential for severing activity (Fig. 1) [6,7]. The MT-severing function of katanin is specific for certain cell types and cell cycles. For example, katanin promotes mitosis in proliferating cells, where it severs MTs at the mitotic spindle poles and increases the number of minus ends, resulting in the accumulation of  $\gamma$ -tubulin at the mitotic centrosomes [8]. However, katanin exhibits severing of the metaphase spindles at the spindle poles [9].

Recently, MT severing by katanin in neuronal cells has received increased attention, because of its contribution to neurite outgrowth and its implication in neurodegenerative diseases. In nascent neurons, it seems critical that the MTs are chopped by katanin at the cell body prior to being translocated into axons; subsequently, the chopped MT fragments disassemble into tubulin dimers that then reassemble into long MTs [10]. By contrast, excessive MT severing by exogenous overexpression of p60-katanin results in a shortening

of the total process length of MTs in the hippocampal neuron [11]. In a model of neurodegenerative disease, MT breakdown by katanin triggers the loss of neurite spikes, but not the apoptosis of neurons, while the microtubule-associated protein, tau may protect MTs against attack by katanin [12]. Thus, the mechanism of p60-katanin regulation by adaptor-p80 or other factors such as tau in various cells and tissues, and at different developmental stages, is an important and unresolved issue.

We have previously shown that the substrate recognition mechanism is evolutionarily conserved between p60-katanin and its related AAA ATPase Vps4 [13,14]. Vps4 is responsible for the dissociation of membrane skeleton ESCRT-III fibrils. Note that p60-katanin and Vps4 share a common domain organization typical of type I AAA-ATPases, which consists of an N-terminal substrate-binding region, followed by a single AAA domain at the C-terminus. Despite the low (~20%) amino acid identity, we have shown that the 3D structure of the N-terminal domain of p60-katanin is strikingly similar to that of the microtubule interacting and trafficking (MIT) domain of Vps4 and other MIT



**Fig. 1.** Domain architectures and multiple sequence alignment of p60-katanin and adaptor-p80. (A) Domain architectures of mouse p60-katanin and adaptor-p80. The regions of interaction between each domain are indicated by an arrow. vMIT, variant MIT domain; cc, coiled-coil; AAA, AAA domain; CTD, C-terminal domain. (B) Multiple sequence alignment of p60-vMIT and p80-CTD, which interact with each other. The secondary structure elements of p60-vMIT are shown at the top. (▲) Residues involved in tubulin binding. (○) Residues involved in Ca<sup>2+</sup> binding (see Fig. 6). The protein names and UniProtKB accession numbers are as follows: p60 mouse ([Q9WV86](#)), p60 human ([Q75449](#)), katanal1 mouse ([Q8K0T4](#)), katanal1 human ([Q9BW62](#)), p60 *Stroglyocentrotus purpuratus* ([Q61577](#)), p60 *Arabidopsis* ([Q9SEX2](#)), p80 mouse ([Q8BG40](#)), p80 human ([Q9BVA0](#)), p80 *Xenopus* ([Q4V7Y7](#)), p80 *Stroglyocentrotus purpuratus* ([O61585](#)), p80 *Drosophila* ([Q9NHFO](#)) and p80 *Arabidopsis* ([Q8HOT9](#)). The sequence alignment was generated using CLUSTAL x [45].

domains. Therefore, we named this domain 'variant MIT' (vMIT). Helices 2 and 3 of p60-vMIT make up the interface with MTs, which is similar that Vps4-MIT makes with its substrate ESCRT-III [14]. We further predicted that the binding site for p60-katanin in MTs is located at helix 12 of the  $\alpha$ -tubulin subunit. Interestingly, this helix was recently proposed as the binding site for tau [15]. On the basis of our structural model of the p60-MT complex, competition between tau and katanin may occur at this 12th helix of  $\alpha$ -tubulin. This unexpected coincidence is, however, consistent with the hypothesis proposed by Qiang *et al.* [12], in which the physiological role of tau is to protect the MT bundle in axons against the MT-severing activity of p60-katanin. Sudo & Baas [16] recently proposed this competition between tau (MT-stabilizing) and katanin (MT-severing) as a new therapeutic target for dementia and other neurodegenerative diseases.

In this study, we focused in detail on the role of the vMIT domain in regulating the ATPase activity of p60-katanin. It is known that either MT (substrate) alone or MT + p80 (adaptor) enhances the ATPase activity of p60-katanin [6,17,18], although the underlying molecular mechanism remains unclear. We therefore hypothesized that substrate/adaptor-dependent ATPase activation originates from p60-vMIT via a change in the location of this domain relative to the AAA domain. During the course of biochemical studies, we found that Ca<sup>2+</sup> ions inhibit the MT-severing activity of p60-katanin. Furthermore, we found that Ca<sup>2+</sup> directly affects the vMIT domain and then cancels the elevated ATPase activity of p60-katanin that occurs in the presence of either MT or the C-terminal domain of adaptor-p80 (p80-CTD). Given that Ca<sup>2+</sup> is a key intracellular signal that indirectly regulates MT stability, as well as rearranging the cytoskeleton *in vivo*, we discuss the physiological implications of this finding.

## Results

### p80-CTD interacts with helix 1 of p60-vMIT

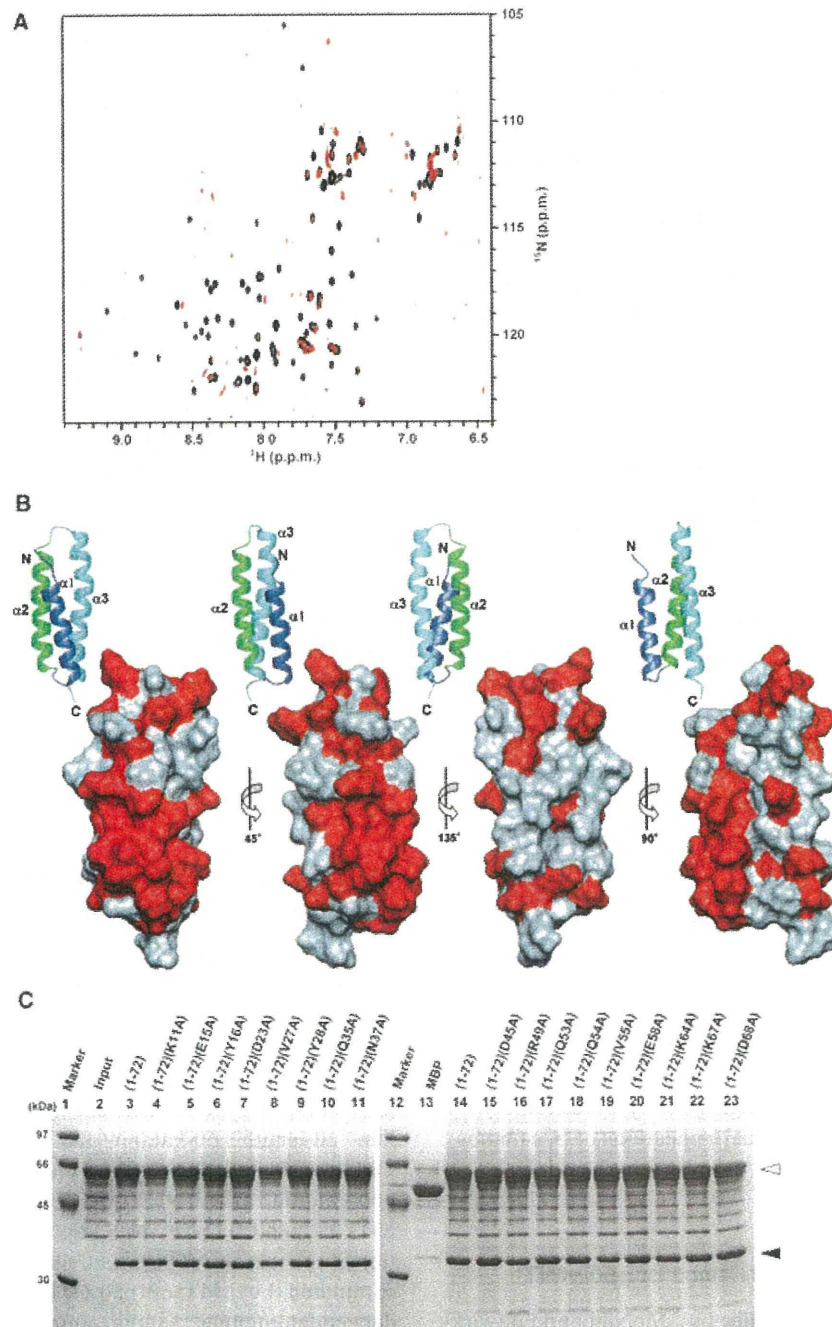
Previous biochemical studies by McNally and co-workers [7,19] showed that the N-terminal half of p60-katanin interacts with the C-terminal half of adaptor-p80, and in a previous study we showed from the 3D structure of p60-vMIT that the MT interface of p60-vMIT is located on the surface of helix 2/3. Therefore, we first examined the amino acid sequence of adaptor-p80 to identify the boundary of its C-terminal p60-binding domain. Figure 1 summarizes the domain organization of p60-katanin and adaptor-p80, and presents multiple sequence alignments of the two domains (p60-vMIT

and p80-CTD) that interact with each other. Both domains are genetically conserved among many higher eukaryotes. Finally, we chose residues 480–655 of human p80 and 483–658 of mouse p80 for expression and purification because this region is markedly conserved among vertebrates (Fig. 1B).

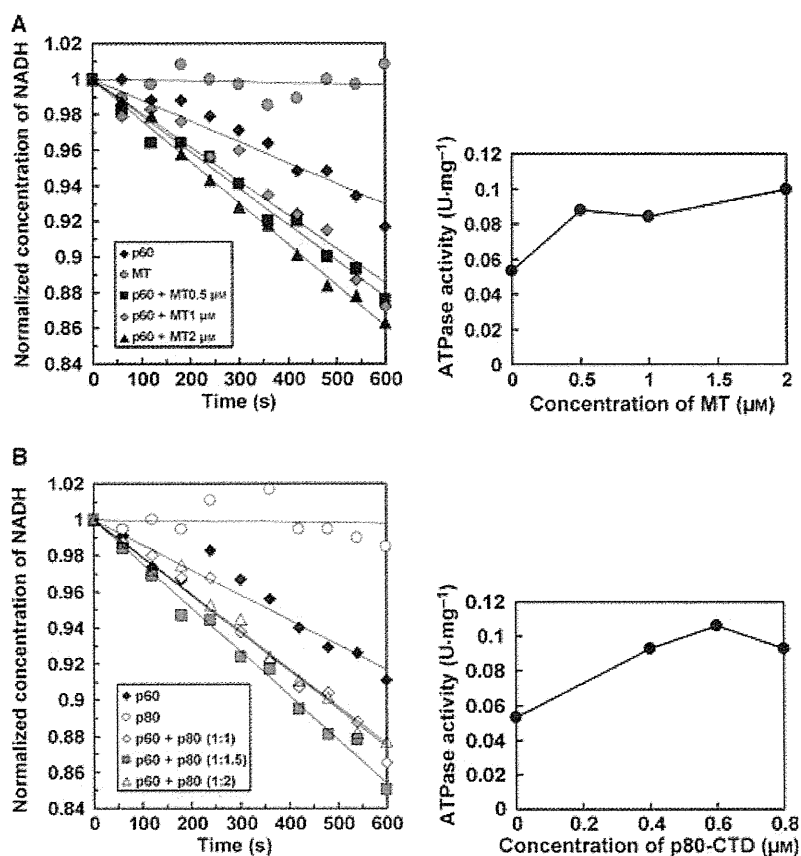
Next, we determined the interface between p60-vMIT and p80-CTD using <sup>1</sup>H-<sup>15</sup>N HSQC experiments. Binding of p80-CTD induced drastic line broadening of NH signals from <sup>15</sup>N-labeled p60-vMIT (Figs 2A and S1A). Mapping of the broadened signals upon binding to p80-CTD suggested that a wide area covering the surface of helices 1/2 and 1/3 of p60-vMIT might form the binding site for p80-CTD (Figs 2B and S1B; Table S1). We further examined the key residues involved in p80 binding by using Ala-substituted mutants of p60-vMIT in an *in vitro* pull-down assay (Fig. 2C). Wild-type p60-vMIT bound to p80-CTD in a ratio of almost 1 to 1, as judged from the density of the bands in SDS/PAGE (Fig. 2C, lanes 3 and 14). All Ala mutants also showed similar binding to p80-CTD. Therefore, we assumed that a polar single mutation does not completely inhibit the interaction between p60-vMIT and p80-CTD, probably because the interface is multivalent and covers a wide area, including helix 1.

### Binding of either MT or p80-CTD to p60-vMIT stimulates the ATPase activity of p60-katanin

The ATPase activity of sea urchin and plant p60-katanin is stimulated by MTs alone, or MTs and adaptor-p80 [6,17,18]. In addition, the MT-severing activity of p60-katanin is stimulated by p80/con80 (412–655), according to a 4'-6-diamidino-2-phenylindole assay [6,7,18,20]. We performed experiments using mammalian full-length p60-katanin and p80-CTD, the domain that we isolated above. We investigated the ability of p60-katanin to hydrolyze ATP in the presence of either MTs or p80-CTD *in vitro* (Fig. 3). Under both conditions, the basal ATPase activity of p60-katanin was stimulated by MTs or p80-CTD in a concentration-dependent manner. In the presence of MTs, stimulation of the ATPase activity of p60-katanin reached almost a maximum at 0.5  $\mu$ M MTs, corresponding to a p60/MT molar ratio of 0.8. We also found that the ATPase activity of p60-katanin is stimulated by the addition of p80-CTD alone. Stimulation of the ATPase activity of p60-katanin reached almost a maximum at 0.4  $\mu$ M p80-CTD, corresponding to a p60/p80-CTD molar ratio of 1.0. Similar stimulation of p60-katanin was observed in the presence of both MTs and p80-CTD (Fig. S2A). These results show that binding



**Fig. 2.** Analyses of the interaction between p60-vMIT and p80-CTD. (A)  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of p60-vMIT in the absence (black) and presence (red) of p80-CTD. Because a  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum of p60-vMIT in the presence of p80-CTD was drastically broadening, it was represented at a high threshold level corresponding to the noise. (B) Residues whose NH signals of p60-vMIT were broadening in the presence of p80-CTD are mapped on the surface in red. The surface orientations are shown by the ribbon diagram of p60-vMIT (PDB: [2rpa](#)). The residues in helix 1 had mostly been eliminated from the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra, because those regions are involved in binding to p80-CTD. (C) Pull-down assays of MBP-tagged p80-CTD with GST-tagged p60-vMIT (wild-type and Ala mutants) *in vitro*. MBP-tagged p80-CTD was used as the input. Molecular sizes are shown in lanes 1 and 12. MBP was used as a negative control (lane 13). Recombinant proteins used for pull-down are indicated at the top of the gel. Filled and open arrowheads show MBP-tagged p80-CTD and GST-tagged p60-vMITs, respectively. The SDS/PAGE gel was stained with Coomassie blue.



**Fig. 3.** The ATPase activity of full-length p60-katanin is stimulated in the presence of MT or p80-CTD. (A) ATPase activity of p60-katanin (0.4  $\mu\text{M}$ ) was monitored at 340 nm in the absence (black diamond) or presence of 0.5  $\mu\text{M}$  (black square), 1  $\mu\text{M}$  (gray diamond) and 2  $\mu\text{M}$  (black triangle) of taxol-stabilized MTs (left). The gray circle indicates taxol-stabilized MTs (1  $\mu\text{M}$ ) without p60-katanin. ATPase activity was further expressed as  $\mu\text{moles}$  of ATP hydrolyzed per min per mg of p60-katanin ( $\text{units}\cdot\text{mg}^{-1}$ ) in 0, 0.5, 1 and 2  $\mu\text{M}$  of taxol-stabilized MTs (right). (B) ATPase activity of p60-katanin (0.4  $\mu\text{M}$ ) was monitored at 340 nm in the absence (black diamond) or presence of 0.4  $\mu\text{M}$  (open diamond), 0.6  $\mu\text{M}$  (gray square), and 0.8  $\mu\text{M}$  (open triangle) of p80-CTD (left). Open circle indicates p80-CTD (0.4  $\mu\text{M}$ ) without p60-katanin. The ATPase activity was further expressed as  $\mu\text{moles}$  of ATP hydrolyzed per min per mg of p60-katanin ( $\text{units}\cdot\text{mg}^{-1}$ ) in 0, 0.4, 0.6 and 0.8  $\mu\text{M}$  of p80-CTD (right).

of either MTs or p80-CTD to the N-terminal domain of p60-katanin stimulates the basal ATPase activity of p60-katanin. In other words, p60-vMIT serves as a sensory regulator for the ATPase activity of p60-AAA.

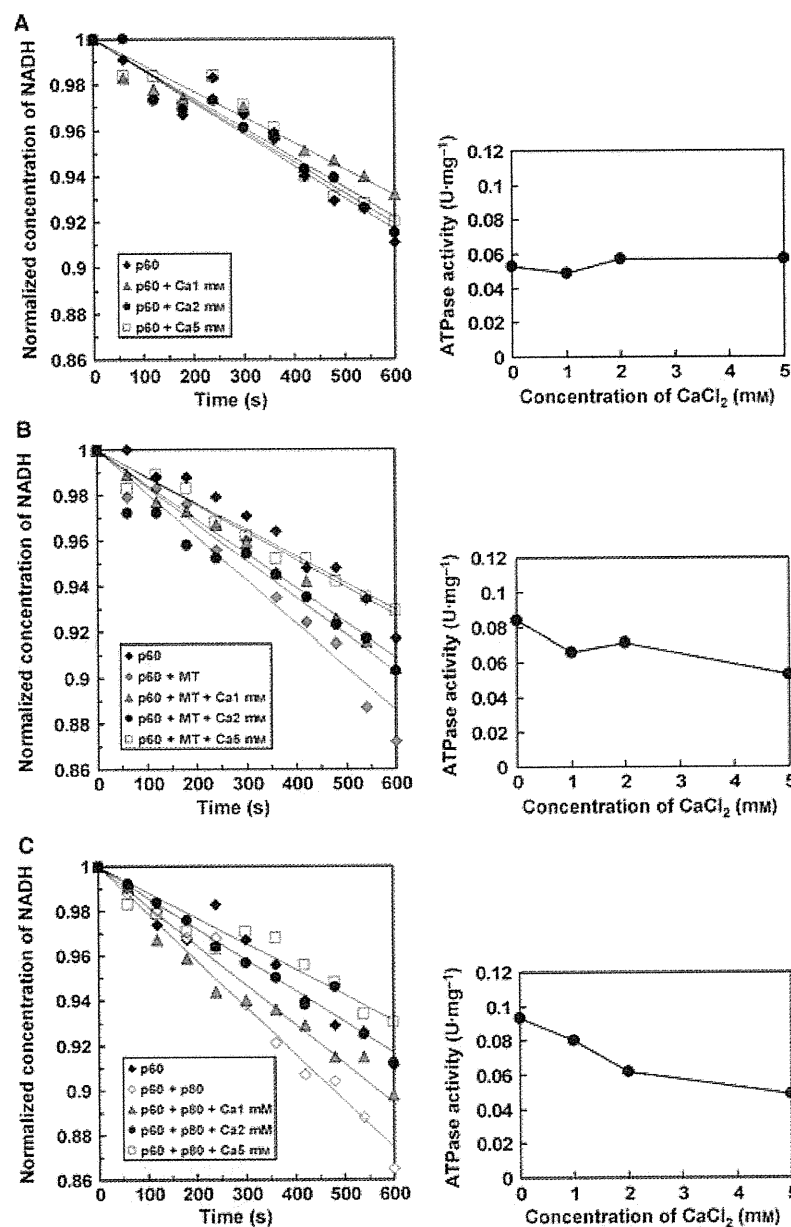
### $\text{Ca}^{2+}$ negatively regulates the ATPase activity enhanced by MT or p80-CTD

Next, we examined the effect of  $\text{Ca}^{2+}$  on the ATPase activity of p60-katanin. Interestingly, we found that  $\text{Ca}^{2+}$  reduced the increase in ATPase activity induced by MTs or p80-CTD to the basal level (Fig. 4). Basal ATPase activity of p60-katanin in the absence of MTs or p80-CTD was not affected by  $\text{Ca}^{2+}$  (Fig. 4A), even at a higher concentration such as 10 mM (data not shown). In the presence of MTs, however, the ATPase

activity of p60-katanin decreased as the  $\text{Ca}^{2+}$  concentration increased, finally decreasing to the basal level at 5 mM  $\text{Ca}^{2+}$  (Fig. 4B). A similar tendency was observed in the presence of p80-CTD, where the ATPase activity decreased to the basal level at 2 mM  $\text{Ca}^{2+}$  (Fig. 4C). These results suggest that  $\text{Ca}^{2+}$  regulates a putative On/Off mechanism for the ATPase activity of p60-katanin, which may be associated with either the vMIT or AAA domain.

### $\text{Ca}^{2+}$ inhibits the MT-severing activity of p60-katanin, but does not inhibit its binding to MTs

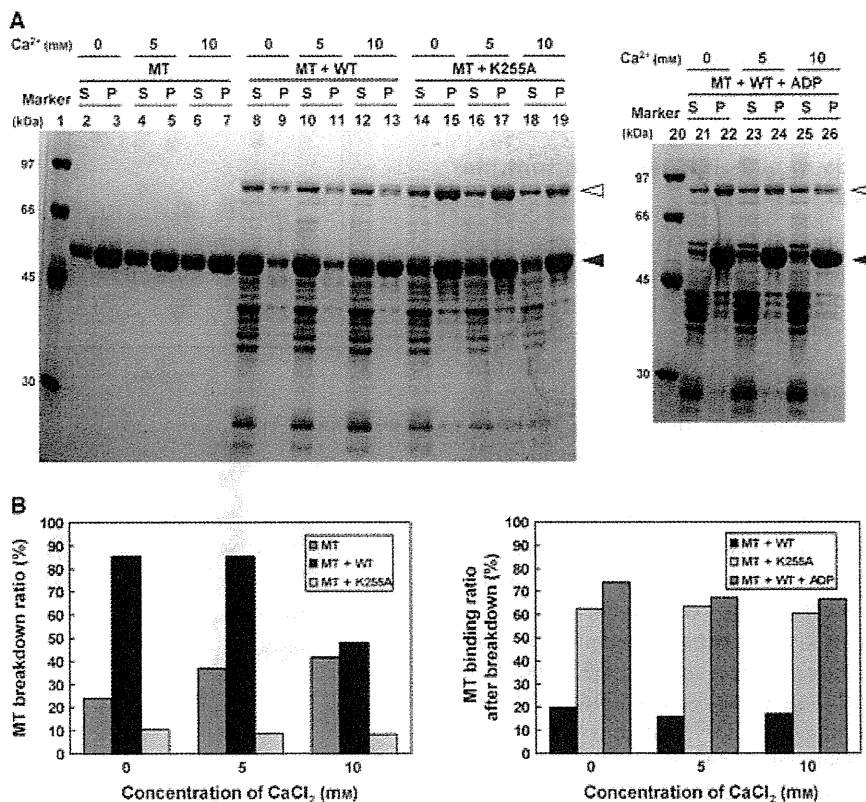
The dynamics of the polymerization–depolymerization of MTs is called ‘dynamic instability’ [21]. Growing



**Fig. 4.** Substrate/adaptor-stimulated ATPase activity of full-length p60-katanin is suppressed in the presence of  $\text{Ca}^{2+}$ . (A) ATPase activity of p60-katanin ( $0.4 \mu\text{M}$ ) (black diamond) was monitored at 340 nm with increasing  $\text{Ca}^{2+}$  concentrations: 1 mM (gray triangle), 2 mM (black circle) and 5 mM (open square) (left). ATPase activity was further expressed as  $\mu\text{moles}$  of ATP hydrolyzed per min per mg of p60-katanin ( $\text{units}\cdot\text{mg}^{-1}$ ) in 0, 1, 2 and 5 mM of  $\text{Ca}^{2+}$  (right). (B, C) ATPase activity of p60-katanin ( $0.4 \mu\text{M}$ ) + taxol-stabilized MTs ( $1 \mu\text{M}$ ) (gray diamond) (B) or p60-katanin ( $0.4 \mu\text{M}$ ) + p80-CTD ( $0.4 \mu\text{M}$ ) (open diamond) (C) was monitored at 340 nm with increasing  $\text{Ca}^{2+}$  concentrations: 1 mM (gray triangle), 2 mM (black circle) and 5 mM (open square) (left). ATPase activity in presence of MTs or p80-CTD was further expressed as  $\mu\text{moles}$  of ATP hydrolyzed per min per mg of p60-katanin ( $\text{units}\cdot\text{mg}^{-1}$ ) in 0, 1, 2 and 5 mM of  $\text{Ca}^{2+}$  (right).

MTs suddenly start depolymerizing rapidly, known as a 'catastrophe'. However, shortening MTs often are 'rescued' and start polymerizing again [22]. *In vitro*, higher concentrations of  $\text{Ca}^{2+}$  in the mM range induce

destabilization of MTs [23,24]; therefore,  $\text{Ca}^{2+}$  has been used for biochemical experiments to inhibit polymerization or induce the depolymerization of MTs. Figure 5A shows the effects of  $\text{Ca}^{2+}$  on the interaction



**Fig. 5.** The effects of Ca<sup>2+</sup> on full-length p60-MT interactions. (A) *In vitro* MT cosedimentation assay performed with increasing Ca<sup>2+</sup> concentrations (0–10 mM) using GST-tagged p60-katanin (wild-type and K255A mutant) in the presence of ATP (left) or using GST-tagged p60-katanin (wild-type) in the presence of ADP without Mg<sup>2+</sup> (right). Taxol-stabilized MTs and associated p60-katanin were separated from depolymerized tubulin and unbound p60-katanin by sedimentation in a glycerol cushion buffer. P and S represent the pellet fraction and the supernatant fraction, respectively. Molecular sizes are shown in lane 1. Open and filled arrowheads indicate GST-tagged p60-katanin and tubulin, respectively. The SDS/PAGE gel was stained with Coomassie Brilliant blue. (B) Quantitative analysis of degraded tubulin fragments from taxol-stabilized MTs (left) and p60-katanin fragments binding to MT after degradation (right). Signals corresponding to the MT/tubulin and p60-katanin fragments in (A) were integrated and normalized by their molecular weights, using IMAGEJ.

between full-length p60-katanin and MTs in a cosedimentation assay. We quantified a normalized amount of the degraded tubulin fragments from taxol-stabilized MTs (Fig. 5B, left) and p60-katanin cosedimented with the remaining MT filaments (Fig. 5B, right). In the absence of p60-katanin, marked depolymerization of MT was observed in a Ca<sup>2+</sup>-dependent manner, probably caused by a catastrophe effect [24] (Figs 5A, lanes 2–7 and 5B, left). In the presence of wild-type p60-katanin, most of the MTs were severed by p60-katanin (Figs 5A, lanes 8–11 and 5B, left). However, MT severing by p60-katanin was mostly inhibited by the presence of 10 mM Ca<sup>2+</sup> (Figs 5A, lanes 12 and 13 and 5B, left). We observed a partial inhibition in the presence of 5 mM Ca<sup>2+</sup> in multiple independent experiments (data not shown), thus we concluded that 5 mM

of Ca<sup>2+</sup> is an approximate threshold concentration for the inhibition of MT severing.

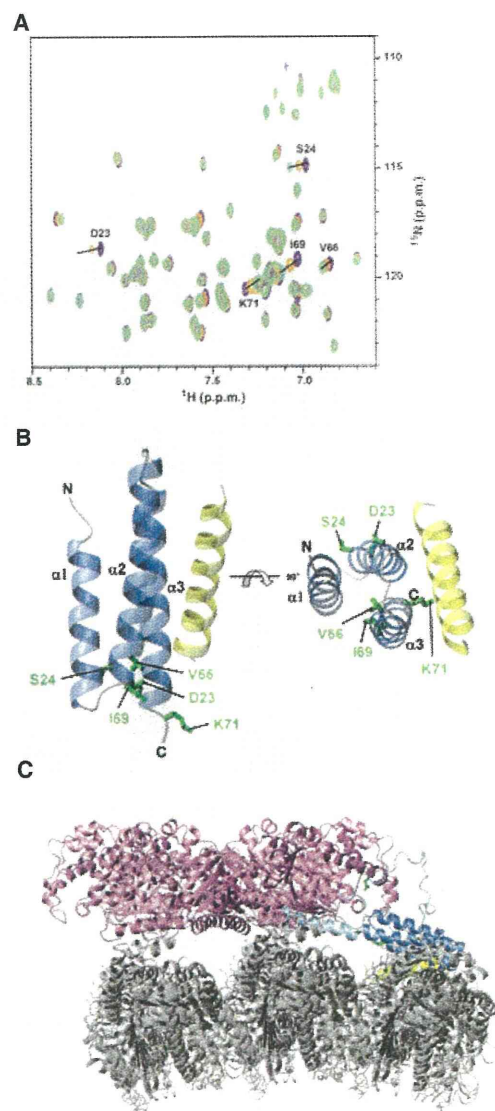
We further examined the MT-binding activity of p60-katanin using a K255A mutant that lacks ATPase activity (data not shown). K255A bound to MTs regardless of the Ca<sup>2+</sup> concentration (Figs 5A, lanes 14–19 and 5B, right). Furthermore, K255A might stabilize MTs, because the Ca<sup>2+</sup>-dependent catastrophe of MTs was suppressed (Figs 5A, left, lanes 14–19 and 5B, left). Recently, McNally & McNally [19] showed that ATPase-deficient katanin promoted the assembly of meiotic spindles. Our observation of K255A is partially consistent with their result. In addition, we examined the MT-binding activity of p60-katanin with ADP in the absence of Mg<sup>2+</sup>. p60-katanin with ADP bound to MTs without MT severing regardless of the

Ca<sup>2+</sup> concentration (Fig. 5A, right). Taken together, we concluded that Ca<sup>2+</sup> inhibits the MT-severing activity of p60-katanin without affecting its MT-binding activity.

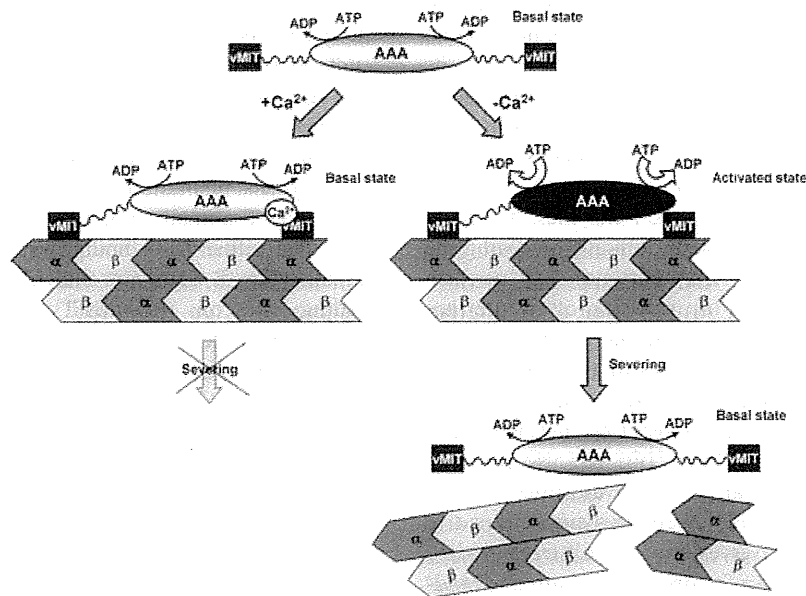
### Ca<sup>2+</sup> directly binds to p60-vMIT different from the interface with MT

With the aim of obtaining information on the possible involvement of metal ions in the action mechanism of p60-vMIT, we examined its interaction with Ca<sup>2+</sup>, and with Ce<sup>3+</sup> as an ideal paramagnetic probe for Ca<sup>2+</sup> (Figs 6A, S3 and S4A) [25,26]. NMR titration analyses of p60-vMIT at various Ca<sup>2+</sup> concentrations are shown in Fig. S3. In the case of Ca<sup>2+</sup>, residues showing a major chemical shift perturbation were V66, I69 and K71. Because these residues are adjacent to the C-terminus of p60-katanin vMIT domain (residues 1–72), this binding site is assumed to be an artifact of the use of the engineered domain-only construct. When using paramagnetic Ce<sup>3+</sup> to enhance the chemical shift changes upon metal binding, residues showing a large change, D23 and S24, were additionally found (Figs 6A and S4A). We succeeded in determining this Ce<sup>3+</sup> binding site by analyzing pseudocontact shifts due to Ce<sup>3+</sup> (Fig. S4B). The metal was chelated close to D23 and S24. From these results, we assumed that D23 and S24, which are evolutionarily conserved among many higher eukaryotes, form the binding site for Ca<sup>2+</sup>. This Ca<sup>2+</sup>-binding site differs from the putative interface between p60-vMIT and tubulin/MT, and so is consistent with our observation that Ca<sup>2+</sup> did not inhibit MT binding.

From the structural model of the p60–MT complex, we hypothesized that the Ca<sup>2+</sup>-binding site is close to the putative interface between the vMIT domain and hexameric AAA ATPase domains (Figs 6B,C and S5), enabling us to propose a model for the regulatory role of the vMIT domain in MT severing (Fig. 7). p60-katanin may form a putative ring-shaped hexamer, as indicated by Hartman & Vale [17]. We assumed that hexameric p60-katanin possesses flexible linkers between the vMIT and AAA domains, and may move over MTs using them freely. In the absence of Ca<sup>2+</sup>, the ATPase activity of p60-katanin is elevated upon binding to an MT through contact between the vMIT and AAA domains. The enhanced activity of p60-katanin may lead to MT severing. After the reaction, p60-katanin is released from the MT and goes back to the basal state. In the presence of Ca<sup>2+</sup>, however, p60-katanin does not adopt the activated state regardless of MT binding.



**Fig. 6.** Ca<sup>2+</sup>-binding sites of p60-katanin. (A) <sup>1</sup>H-<sup>15</sup>N HSQC spectra of p60-vMIT in the absence (violet) and presence of 1 mM (orange) and 2 mM (pale green) Ce<sup>3+</sup> as a paramagnetic probe for Ca<sup>2+</sup>. Residues with the greatest chemical shift perturbations are shown. (B) Side and top views of the ribbon diagram of p60-vMIT (PDB: [2rpa](#)). Side chains of residues binding Ca<sup>2+</sup> are shown in green. Tubulin helix 12, a putative interface of p60-vMIT, is colored yellow. (C) Proposed model for tubulin binding with full-length p60-katanin. Model complex between tubulin oligomer (gray) and hexameric full-length p60-katanin, composed of p60-vMIT (blue), a coiled-coil (light blue), a flexible linker (light blue) and AAA ATPase (violet) domains is shown. One of the six p60-vMITs on the hexameric AAA ATPase is drawn. One helix 12 on tubulin, the putative interface of p60-katanin, is colored yellow. Residues bound to Ca<sup>2+</sup> on p60-vMIT and residues on hexameric AAA ATPase domains close to the Ca<sup>2+</sup>-binding sites on p60-vMIT are shown in green.



**Fig. 7.** Model of the mechanism of MT-severing by p60-katanin in the absence and presence of  $\text{Ca}^{2+}$ . Hexameric p60-katanin represents the enzyme in a basal state of ATP-dependent ATPase activity. Four of the six p60-vMIT domains, followed by flexible linkers on the side view of the hexameric AAA ring are not drawn for clarity. In the absence of  $\text{Ca}^{2+}$ , p60-katanin is in an activated state, whereby its ATPase activity is elevated by binding MT through an interaction between the vMIT and AAA domains, resulting in MT-severing. By contrast, when p60-katanin binds  $\text{Ca}^{2+}$ , p60-katanin remains in a basal state regardless of MT-binding and does not sever MT.

## Discussion

### The function of p80-CTD to capture and release p60-vMIT

Our NMR and mutation studies suggest that p80-CTD binds to a wide surface of p60-MIT, especially helix 1/2 and helix 1/3, which differs from the MT-binding surface, helix 2/3 (Figs 2B and S1B) [14]. If so, p80-CTD and MT could simultaneously bind to p60-vMIT, forming a ternary complex. However, our ATPase assay showed only a limited additive effect of p80-CTD and MT on ATPase activity (Fig. S2A). In addition, p80-CTD was excluded from the p60/MT complex (Fig. S6, lanes 9–12). Thus, in our experiments, we did not observe a ternary complex of p80-CTD, p60-vMIT and MT. Steric hindrance caused by p80-CTD binding to the surfaces of helices 1/2 and 1/3 may occur. This unique activity of p80-CTD may explain how adaptor-p80 recruits p60-katanin to MTs. That is, using the N-terminal WD40 domain, adaptor-p80 might localize on the  $\gamma$ -tubulin ring complex at the minus end of an MT [6], whereas p80-CTD captures p60-vMIT. p60-katanin would then relocate to the MT, whereas p80-CTD releases p60-vMIT. Note that, the *in vivo* activity of adaptor-p80 against p60-katanin also remains controversial, because adaptor-p80 localizes p60-katanin to the  $\gamma$ -tubulin ring complex

and stimulates MT severing [8], whereas overexpression of adaptor-p80 suppresses the MT-severing activity of p60-katanin in certain types of cell [11].

### Existence of an MT-/p80-activated state of p60-katanin

The phenomenon that MT (substrate) as well as p80 (adaptor) binding to p60-katanin increases the ATPase activity of p60-katanin has been reported previously [6,17,18], although the molecular mechanism remains unclear. One explanation might be an equilibrium shift between an inactive dimeric state and an active hexameric state that forms upon binding to the substrate and/or adaptor. For example, the katanin-related type I AAA enzyme Vps4 has been shown to act in a dimer-hexamer equilibrium in a nucleotide-dependent manner, although substrate-dependent ATPase activation of Vps4 has not been reported to date [27,28]. Because multivalent binding between active katanin and MT is expected, MT-binding may promote hexamer formation in p60-katanin. We can rule out this possibility, however, because activation of ATP hydrolysis was observed even upon p60-katanin binding to p80-CTD, which was monomeric in solution (data not shown). This suggests the existence of a unique MT-/p80-activated state of p60-katanin. This activated



state of p60-katanin is more likely attributed to an allosteric effect depending on the intramolecular spatial rearrangement between the N-terminal vMIT and the central AAA domains, rather than an equilibrium shift.

The regulatory role of p60-vMIT on its own AAA domain has not been predicted. Nevertheless, it is not surprising if the analogy to other AAA-ATPases, including type II rather than type I enzymes, is considered. In general, the N-terminal domains preceding the AAA domain of type II AAA enzymes may perform three molecular functions: (a) provision of an interface for substrates for ATP hydrolyzing energy; (b) provision of an interface for adaptor proteins that mainly contribute to subcellular localization; and (c) modulation of ATPase activity. We previously reported that p60-vMIT serves as an interface for MTs [14]. We also reported that the N-terminal domain of both valosin-containing protein (VCP)/p97 and *N*-ethylmaleimide-sensitive fusion protein (NSF) serves as the substrate-binding site, as well as the surface for organelle membrane binding [29]. However, it has been reported that binding to substrates or membranes regulates the ATPase activity of these proteins [30,31]. In VCP/p97, the residues at the contact site between the N-domain and the first AAA domain (D1-domain) were identified in conjunction with Paget's disease of the bone and front temporal dementia (e.g. R155, A232, T262, and N387) [30,31]. A nucleotide-dependent conformational change in the N-domain relative to the D1-domain was observed by X-ray crystallography [30,31]. A mutation on A232 (A232E), one of the key residues, is associated with increased disease severity [32]. Biochemically, an A232E mutant exhibits elevated ATPase activity [33]. In NSF, a similar mechanism in which the contact site between the N-domain and D1 is associated with enhancement of ATPase activity has also been identified [34]. Note that, although both the sequences and the structures of the N-terminal domains of katanin and the VCP/NSF family are rather different, the AAA domains are highly conserved. Finally, we illustrate the MT-activated state of p60-katanin in Fig. 6C, assuming that the edge of p60-vMIT comes close enough to these interfacial residues on the AAA domain with a minimum structural change upon MT binding. In this model, residues E397 and G237 of p60-katanin are highlighted, and correspond to the interfacial residue N387 and the key residue A232 in VCP, respectively.

#### Ca<sup>2+</sup> alters the spatial domain rearrangement of p60-katanin in its MT-/p80-activated state

In this study, we showed that Ca<sup>2+</sup> inhibits the MT-severing activity of katanin, as well as reducing its

MT-elevated ATPase activity to basal levels. Although the mechanism of this modulation seems complicated, studying the effect of Ca<sup>2+</sup> on p60-katanin may provide clues to the working model of MT severing, which is driven by ATP hydrolyzing energy and catalyzed by katanin, as shown in Fig. 6C. First, we showed that Ca<sup>2+</sup> affects p60-katanin activity by directly binding to the vMIT domain (Fig. 6). By contrast, Ca<sup>2+</sup> does not affect either MT binding or p80 binding by the domain (Fig. 5, data not shown). Because the Ca<sup>2+</sup>-binding site on p60-vMIT is different from its MT-binding site, these observations are consistent. Next, we found that Ca<sup>2+</sup> does not affect the basal ATPase activity level of p60-katanin in the absence of MT and/or adaptor-p80 (Fig. 4A). By contrast, Ca<sup>2+</sup> has an effect only on the MT- and/or p80-elevated ATPase activity, but not the basal activity (Figs 4B,C and S2B). Because a flexible linker (~114 amino acids) connects the N-terminal vMIT domain and the AAA domain, this Ca<sup>2+</sup> effect is difficult to explain unless these two domains move spatially close to each other. As discussed above, we assume the existence of the MT-/p80-activated state of p60-katanin in such a domain rearrangement. It is likely that Ca<sup>2+</sup> affects this MT-/p80-activated state, while the relative orientation of the two domains, which may be a trigger to elevate ATP hydrolysis, is altered by Ca<sup>2+</sup>. This idea is further assessed by visualizing the spatial locations of the vMIT domain, Ca<sup>2+</sup> ion, and the putative interfacial residues on the AAA domain (Figs 6C and S5).

#### Biological implications of the regulatory role of Ca<sup>2+</sup> in MT-severing by katanin

In this study, Ca<sup>2+</sup> negatively regulated the MT-severing function of katanin. In other words, Ca<sup>2+</sup> contributed to the stabilization of MTs by protecting MTs from attack by katanin. The interaction of Ca<sup>2+</sup> with MTs has been extensively studied because the inhibitory effect of Ca<sup>2+</sup> on the *in vitro* assembly of MTs was reported by Weisenberg [23]. Ca<sup>2+</sup> has been shown to destabilize polymerization of MTs directly or indirectly. Serrano *et al.* [35] determined the Ca<sup>2+</sup>-binding sites on both  $\alpha$ - and  $\beta$ -tubulin molecules, which are major components of MTs, and demonstrated that tubulin deprived of these sites could still polymerize in the presence of high concentrations of Ca<sup>2+</sup>. Ca<sup>2+</sup> is also known to affect dynamic instability in the assembly of MTs: it enhances the rate of catastrophic degradation of MT [24]. Lefèvre *et al.* [15] showed that the Ca<sup>2+</sup>-binding sites of tubulin overlap with the binding sites for microtubule-associated proteins, and thus

Ca<sup>2+</sup> competed with microtubule-associated proteins, resulting in the disassembly of MTs.

The indirect effect of Ca<sup>2+</sup> on MT destabilization via one of the microtubule-associated proteins, tau, has received increased attention [36]. Calpain, a calcium-dependent nonlysosomal cysteine protease, plays critical roles in the pathway. Tau is highly relevant to regulating MT polymerization, or something of that nature. Hyperphosphorylation of tau, however, abolishes its ability to bind tubulin and promote MT assembly. When MTs release hyperphosphorylated tau, the protein aggregates into paired helical filaments, which are the neuropathological hallmarks of Alzheimer's disease. There are at least three protein kinases responsible for the hyperphosphorylation; cyclin-dependent-kinase 5, glycogen synthase kinase 3 $\beta$  and dual-specificity tyrosine-phosphorylation-regulated kinase 1A [37–39]. The activity of all the three kinases is regulated by calpain, as follows. In the presence of high concentrations of Ca<sup>2+</sup>, glycogen synthase kinase 3 $\beta$  is truncated by calpain, generating two fragments of ~40 and 30 kDa. Glycogen synthase kinase 3 $\beta$  truncation augments its kinase activity [40]. Cyclin-dependent-kinase 5 is also activated by proteolytic cleavage of its specific activator p35 by calpain [41]. Recently, dual-specificity tyrosine-phosphorylation-regulated kinase 1A was shown to be proteolyzed and activated by calpain in Alzheimer disease brain [42]. In addition, calpain directly degrades tau, which also causes its loss of MT-stabilizing activity [43,44].

To date, Ca<sup>2+</sup> has not been thought to stabilize MTs, but rather to promote their disassembly, as mentioned above. Our new findings on katanin, however, show that Ca<sup>2+</sup> can slow the disassembly of MTs, suggesting that the assembly and disassembly of MT is managed by a more complicated system than expected. Excess Ca<sup>2+</sup> is generally harmful to MTs, both directly and indirectly. To survive an accidental increase in Ca<sup>2+</sup>, cells may have developed various fail-safe mechanisms, one of which might be the Ca<sup>2+</sup> inhibition of katanin, which would function to avoid the rapid breakdown of MTs for the survival of neuronal cells upon a transient Ca<sup>2+</sup> flux.

In conclusion, we have shown that p60-katanin, a type I AAA ATPase, possesses a novel mechanism for regulating its own ATPase activity, probably via a 3D domain rearrangement between the N-terminal vMIT domain and the AAA domain. Ca<sup>2+</sup> binds p60-vMIT at the edge of this domain, close to the contact site between vMIT and AAA. This putative arrangement may well explain why Ca<sup>2+</sup> inhibits MT severing without inhibiting either ATPase activity or MT-binding.

## Experimental procedures

### Production of p80-CTD

We generated a multiple sequence alignment of the C-terminal sequences of adaptor-p80 excluding the N-terminal WD40 repeats (residues 1–269) using CLUSTAL X [45], from which we decided to clone sequences 480–614, 480–630 and 480–655 of human and mouse p80. Expression vectors for the recombinant glutathione *S*-transferase (GST)-tagged form of the human and mouse p80 fragments were constructed using PRESAT vector methodology [46], as derived from the pGEX-4T3 vector (GE Healthcare, Buckinghamshire, UK). Finally, p80 (480–655), termed p80-CTD, was used for further analysis because this construct produced a more soluble protein than the others. Human p80-CTD, prepared by expression in *Escherichia coli* BL21 (DE3), followed by affinity purification on glutathione-Sepharose (GE Healthcare, Buckinghamshire, UK) and thrombin digestion, was used for ATPase assays. The expression vector for the recombinant MBP-tagged p80-CTD of mouse was also constructed by a standard protocol using PCR, and ligated into the *EcoRI*–*HindII* sites of pMAL-c2X (NEB). The fusion protein was prepared by expression in *E. coli* BL21 (DE3), followed by affinity purification on amylose-sepharose (New England Biolabs, Ipswich, MA, USA), and used for NMR experiments and binding experiments with p60-vMIT.

### Production of p60-katanin

The expression vector for the recombinant GST-tagged full-length p60-katanin of mouse was constructed by a standard protocol using PCR, and ligated into the *BamHI*–*Sall* sites of pGEX-6P3 (GE Healthcare, Buckinghamshire, UK) [14]. An Ala-substituted mutant (K255A) was engineered with the QuikChange site-directed mutagenesis kit (Stratagene, Santa Clara, CA, USA). Two complementary oligonucleotides with mutated sequences for each mutant were used as primers (Table S2). The fusion proteins were produced in *E. coli* JM109. Expression was induced with 0.1 mM isopropyl thio- $\beta$ -D-galactoside, and Luria–Bertani cultures were grown overnight at 20 °C. For cosedimentation assays, GST-tagged proteins were purified with glutathione-Sepharose and eluted in elution buffer (50 mM Tris/HCl, pH 7.4, 100 mM NaCl, 1 mM dithiothreitol, 50 mM reduced glutathione, and 5% glycerol). For ATPase assays, proteins were eluted in elution buffer (20 mM Tris/HCl, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol, 1 mM EGTA, 2 mM MgCl<sub>2</sub>, 0.25 mM ATP, 0.02% Triton X-100 and 5% glycerol) after PreScission Protease digestion on the column.

Production of the recombinant GST-tagged p60-vMIT domains of mouse, including wild-type and Ala-substituted mutants, was carried out as described previously (Table S2) [14]. The fusion proteins were expressed in *E. coli* BL21

(DE3), affinity-purified on glutathione–Sepharose and dialyzed. These fusion proteins were used for MBP-tagged p80-CTD binding assays. For NMR spectroscopy, a 1-L culture was incubated with [<sup>15</sup>N]-ammonium chloride as the sole nitrogen source by following a standard fermentation protocol at 25 °C. Purification of <sup>15</sup>N-labeled p60-vMIT was achieved by glutathione–Sepharose affinity chromatography followed by thrombin digestion, benzamidine–Sepharose chromatography and gel filtration using a Superdex 75 column (GE Healthcare, Buckinghamshire, UK). The complex of <sup>15</sup>N-labeled p60-vMIT with p80-CTD was purified from a mixture of <sup>15</sup>N-labeled GST-tagged p60-vMIT and non-labeled MBP-tagged p80-CTD prepared following FactorXa and thrombin digestion, respectively.

### Continuous ATPase assays of p60-katanin

ATPase activity was measured using an ATP regenerating system. The reaction mixture contained 50 mM Tris/HCl, pH 7.5, 50 mM KCl, 2 mM MgCl<sub>2</sub>, 2 mM phosphoenolpyruvate, 1 mM ATP, 50 µg·mL<sup>-1</sup> pyruvate kinase, 50 µg·mL<sup>-1</sup> lactate dehydrogenase and 0.2 mM NADH. The reactions were performed in the presence of 0.4 µM p60-katanin, and ATPase activity was measured by monitoring the decrease in NADH absorption at 340 nm at room temperature using a UV–Vis spectrophotometer (UV mini-1240; Shimadzu, Tokyo, Japan). The shifts in absorption at 340 nm caused by the addition of taxol-stabilizing MTs and p80-CTD were measured. The absorbance data were representative of two or three independent experiments and were normalized relative to the level of the time-point zero. ATP hydrolysis activity was then calculated and represented in the unit of micromoles of ATP hydrolyzed per min per mg of p60-katanin (units·mg<sup>-1</sup>).

### In vitro binding experiments

MBP-p80 bound to amylose resin was incubated with lysate of GST-tagged p60-vMIT domains in binding buffer (20 mM Tris/HCl, pH 7.4, 200 mM NaCl and 1 mM EGTA) for 2 h at 4 °C. The beads were washed four times in wash buffer (20 mM Tris/HCl, pH 7.4, 200 mM NaCl and 1 mM EGTA). The associated proteins were analyzed by SDS/PAGE.

### Cosedimentation assays for MT-binding and MT-severing activity of p60-katanin

Taxol-stabilized MTs (1 µM) and GST-tagged p60-katanin (3.5 µM) were incubated for 30 min at 25 °C in binding buffer (80 mM Pipes, pH 7.0, 2 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM ATP and 20 µM taxol). Reaction mixtures (50 µL) were spun through a glycerol cushion buffer (50% v/v glycerol in binding buffer; 100 µL) for 30 min at 100 000 g. Supernatants and pellets were analyzed by SDS/PAGE. The band intensity of proteins was quantified with

IMAGEJ (National Institutes of Health, Bethesda, MD, USA) (<http://rsbweb.nih.gov/ij/>).

### NMR experiments

<sup>1</sup>H–<sup>15</sup>N HSQC spectra of <sup>15</sup>N-labeled p60-vMIT in the presence or the absence of p80-CTD were acquired at 25 °C on a Bruker AVANCE III 600 MHz spectrometer equipped with a cryogenic probe. These samples were dissolved in 20 mM sodium phosphate, pH 7.5, and 150 mM NaCl, and the protein concentrations were below 50 µM because of the low solubility of p80-CTD. <sup>1</sup>H–<sup>15</sup>N HSQC spectra for Ce<sup>3+</sup> titration experiments were recorded using 0.1 mM <sup>15</sup>N-labeled p60-vMIT at 25 °C on a Bruker AVANCE 500 MHz spectrometer equipped with a cryogenic probe. <sup>1</sup>H–<sup>15</sup>N HSQC spectra for Ca<sup>2+</sup> titration experiments were similarly performed (Fig. S3). The samples were dissolved in 20 mM Hepes, pH 7.5, and 25 mM NaCl. All data were processed using NMRPIPE [47] and SPARKY [48] software. The position of the coordinating Ce<sup>3+</sup> ion was calculated by using the program FANTASIAN [49] coupled with an inhouse grid-search program with 144 pseudocontact shift value of NH signals. For calculation the coordinates of hVps4b-MIT (PDB: [1wr0](#)) and p60-vMIT (PDB: [2rpa](#)) were used. All of the figures were prepared by MOLMOL [50].

### Molecular modeling

A hexameric ring model of the AAA domains of p60-katanin was generated on the basis of the hexameric ring structure of p97 D1 (PDB: [1s3s](#)) using MODELLER (version 9v6) (<http://salilab.org/modeller/>), as described previously [14]. A molecular model of the complex of p60-vMIT with a tubulin oligomer (PDB: [3du7](#)) was constructed on the basis of the complex between Vps4a-MIT and CHMP1a (PDB: [2jq9](#)) [51] by replacing each component corresponding to p60-vMIT and helix 12 of  $\alpha$ -tubulin, respectively [14]. Finally, the complex model of full-length p60-katanin and part of an MT was built by connecting these two models by a flexible linker corresponding to residues 91–204, and by placing the hexameric AAA domain close to the vMIT domain. The model was adjusted and visualized using MOLMOL [50].

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## Supporting information

The following supplementary material is available:

**Fig. S1.** Analyses of the interaction between p60-vMIT and p80-CTD.

**Fig. S2.** ATPase activity of full-length p60-katanin in the presence of MT, p80-CTD, and increasing concentrations of Ca<sup>2+</sup>.

**Fig. S3.** <sup>1</sup>H-<sup>15</sup>N HSQC spectra of p60-vMIT in the absence (black) and presence of 5 mM (magenta), 10 mM (cyan), 15 mM (yellow), 25 mM (green) Ca<sup>2+</sup>.

**Fig. S4.** Ce<sup>3+</sup> titration experiments of p60-vMIT.

**Fig. S5.** Close-up view of the Ca<sup>2+</sup> binding region of p60-katanin in Fig. 6C.

**Fig. S6.** MT co-sedimentation assay in the presence of p60-katanin and p80-CTD *in vitro*.

**Table S1.** The broadened signals of <sup>15</sup>N-labeled p60-vMIT in the presence of p80-CTD.

**Table S2.** Oligonucleotides used as primers for Ala substitution.

This supplementary material can be found in the online version of this article.

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3 **Effect of Ca<sup>2+</sup> on the microtubule-severing enzyme p60-katanin: insight into the**  
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5 **substrate-dependent activation mechanism.**  
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**Supplementary Table 1.**The broadened signals of <sup>15</sup>N-labeled p60-vMIT in the presence of p80-CTD.

loop 1	helix 1	loop 1-2	helix 2	loop 2-3	helix 3
S2	M5	G20	D23	S42	T46
L3	V7		S24	K44	H47
	E8		Y28		L48
	N9		G31		R49
	V10		V32		Q53
	K11		L33		E58
	L12		M36		Q65
	A13		Y39		V66
	R14		Y41		K67
	E15				D68
	Y16				
	L18				
	L19				

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**Supplementary Table 2.**

Oligonucleotides used as primers for Ala substitution.

Primer	Sequence
K255A_F	CACCTGGCACTGGAG <b>CG</b> ACCCTTCTAGCTAAAG
K255A_R	CTTTAGCTAGAAGGGT <b>CGCT</b> CCAGTGCCAGGTG
K11A_F	CAAATGATTGTTGAGAATGTAG <b>CC</b> TTGGCTCGTGAATATGCACTG
K11A_R	CAGTGCATATTCACGAGCCAAG <b>GGCT</b> TACATTCTCAACAATCATTTG
E15A_F	GAGAATGTAAAATTGGCTCGT <b>GCCT</b> ATGCACTGCTGGGAAACTATGAC
E15A_R	GTCATAGTTTCCAGCAGTGCATAG <b>GGC</b> ACGAGCCAATTTTACATTCTC
Y16A_F	GAGAATGTAAAATTGGCTCGTGAAG <b>CGC</b> CACTGCTGGGAAACTATGAC
Y16A_R	GTCATAGTTTCCAGCAGTGC <b>GGCT</b> TCACGAGCCAATTTTACATTCTC
D23A_F	GCACTGCTGGGAAACTAT <b>GCCT</b> CTGCAATGGTCTACTATCAG
D23A_R	CTGATAGTAGACCATTGCAGAG <b>GC</b> CATAGTTTCCAGCAGTGC
V27A_F	GGAAACTATGACTCTGCAAT <b>GGCCT</b> ACTATCAGGGAGTTCTTGAC
V27A_R	GTCAAGAACTCCCTGATAGTAG <b>GGC</b> CATTGCAGAGTCATAGTTTCC
Y28A_F	GGAAACTATGACTCTGCAATGGT <b>CGCCT</b> ATCAGGGAGTTCTTGAC
Y28A_R	GTCAAGAACTCCCTGATAG <b>GGC</b> GACCATTGCAGAGTCATAGTTTCC
Q35A_F	CAGGGAGTTCTTGAC <b>GC</b> CATGAACAAGTACCTGTA
Q35A_R	GACTGAGTACAGGTA <b>CTT</b> GTTTGGCGTCAAGAACTCCCTG
N37A_F	CAGGGAGTTCTTGAC <b>CA</b> AAATGG <b>CCA</b> AGTACCTGTA
N37A_R	GACTGAGTACAGGTA <b>CTT</b> GGCCATTTGGTCAAGAACTCCCTG
D45A_F	CTGTA <b>CT</b> CAGTCAAAG <b>CC</b> ACACACCTCCGTCAGAAATGG
D45A_R	CCATTTCTGACGGAGGTGTGT <b>GGC</b> TTTGACTGAGTACAG
R49A_F	GTCAAAGATACACAC <b>CT</b> CG <b>CC</b> CAGAAATGGCAACAG
R49A_R	CTGTTGCCATTTCTG <b>GGC</b> GAGGTGTGTATCTTTGAC
Q53A_F	CTCCGTCAGAAATGG <b>GGCC</b> CAGGTTTGGCAGGAAATAAATGTG
Q53A_R	CACATTTATTTCTG <b>CC</b> AAAC <b>CT</b> GG <b>GGCC</b> CATTTCTGACGGAG
Q54A_F	CTCCGTCAGAAATGG <b>CA</b> AG <b>CC</b> GTTTGGCAGGAAATAAATGTG
Q54A_R	CACATTTATTTCTG <b>CC</b> AAAC <b>GGCT</b> TGCCATTTCTGACGGAG
V55A_F	CTCCGTCAGAAATGG <b>CA</b> ACAG <b>GCCT</b> GGCAGGAAATAAATGTG
V55A_R	CACATTTATTTCTG <b>CC</b> AG <b>GCCT</b> GTTGCCATTTCTGACGGAG
E58A_F	CAGAAATGGCAACAGGTTTGGCAG <b>GC</b> CATAAATGTGGAAGCTAAG
E58A_R	CTTAGCTTCCACATTTAT <b>GGCCT</b> GCCAAACCTGTTGCCATTTCTG



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Primer	Sequence
K64A_F	GTTTGGCAGGAAATAAATGTGGAAGCT <b>GCCCA</b> AGTTAAGGATATCATG
K64A_R	CATGATATCCTTAACCTT <b>GGC</b> CAGCTTCCACATTTATTTCTGCCAAAC
K67A_F	GTGGAAGCTAAGCAAGTT <b>GCC</b> GATATCATGAAAACATAATAGAGC
K67A_R	GCTCTATTATGTTTTCATGATAT <b>CGGCA</b> ACTTGCTTAGCTTCCAC
D68A_F	GTGGAAGCTAAGCAAGTTAAG <b>GCC</b> ATCATGAAAACATAATAGAGC
D68A_R	GCTCTATTATGTTTTCATGAT <b>GGCCT</b> TAACCTTGCTTAGCTTCCAC

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3 **Supplementary Fig. 1.**

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5 Analyses of the interaction between p60-vMIT and p80-CTD. (A)  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra  
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7 of p60-vMIT in the absence (black) and presence (red) of p80-CTD. Residues whose NH  
8  
9 signals of p60-vMIT were broadening in the presence of p80-CTD are shown in red.

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11 Since a  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum of p60-vMIT in the presence of p80-CTD was drastically  
12  
13 broadening, it was represented at a high threshold level corresponding to the noise. (B)

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15 Residues whose NH signals of p60-vMIT were broadening in the presence of p80-CTD  
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17 are mapped on the surface in red. Tubulin binding site of p60-vMIT is shown in yellow.

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19 The residues where the broadened signals and tubulin binding site overlap are shown in  
20  
21 green. The surface orientations are shown by the ribbon diagram of p60-vMIT (PDB:  
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23 **2rpa**).

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29 **Supplementary Fig. 2.**

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31 ATPase activity of full-length p60-katanin in the presence of MT, p80-CTD, and increasing  
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33 concentrations of  $\text{Ca}^{2+}$ . (A) ATPase activity of p60-katanin (0.4  $\mu\text{M}$ ) was monitored at 340  
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35 nm in the absence (black diamond) and presence of 1  $\mu\text{M}$  taxol-stabilized MTs (gray  
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37 diamond), 0.4  $\mu\text{M}$  p80-CTD (open diamond), or both (asterisk). (B) The ATPase activity of  
38  
39 p60-katanin (0.4  $\mu\text{M}$ ) + taxol-stabilized MTs (1  $\mu\text{M}$ ) + p80-CTD (0.4  $\mu\text{M}$ ) was monitored at  
40  
41 340 nm in the presence of increasing  $\text{Ca}^{2+}$  concentrations: 1 mM (gray triangle), 2 mM (black  
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43 circle), and 5 mM (open square) (left). ATPase activity in presence of MTs and p80-CTD was  
44  
45 further expressed as  $\mu\text{moles}$  of ATP hydrolyzed per min per mg of p60-katanin (units/mg) in  
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47 0, 1, 2, and 5 mM of  $\text{Ca}^{2+}$  (right).

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53 **Supplementary Fig. 3.**

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55  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of p60-vMIT in the absence (black) and presence of 5 mM (magenta),  
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3 10 mM (cyan), 15 mM (yellow), 25 mM (green)  $\text{Ca}^{2+}$ . Residues with the greatest chemical  
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5 shift perturbations are shown.  
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10 **Supplementary Fig. 4.**

11  $\text{Ce}^{3+}$  titration experiments of p60-vMIT. (A)  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of p60-vMIT in the  
12 absence (violet) and presence of 1 mM (orange), 2 mM (pale green), 5 mM (red), 10 mM  
13 (blue)  $\text{Ce}^{3+}$  as a paramagnetic probe for  $\text{Ca}^{2+}$ . Residues with the greatest chemical shift  
14 perturbations are shown. (B) A view of best 5 positions of  $\text{Ce}^{3+}$  ion (orange) in p60-vMIT  
15 (PDB: 2rpa). Side chains of residues binding  $\text{Ce}^{3+}$  are shown in green. The position of the  
16 coordinating  $\text{Ce}^{3+}$  ion was calculated by using the program FANTASIAN  
17 (<http://www.cerm.unifi.it/software/software-fantasia>) coupled with an inhouse grid-search  
18 program with 144 pseudocontact shift value of NH signals.  
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32 **Supplementary Fig. 5.**

33 Close-up view of the  $\text{Ca}^{2+}$  binding region of p60-katanin in Fig. 6C.  
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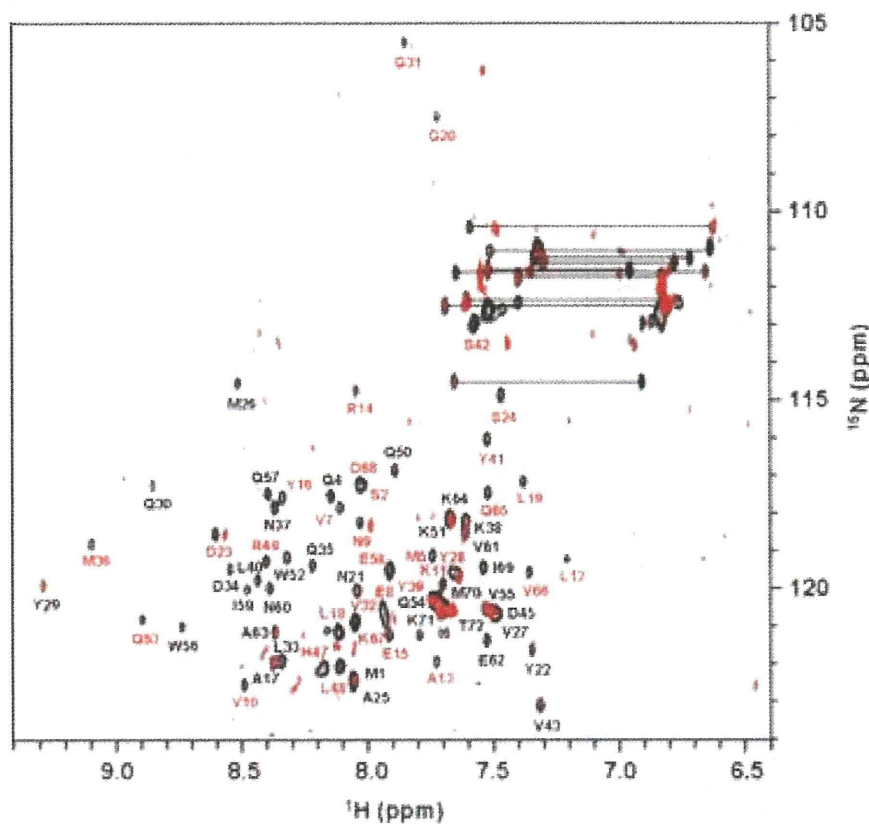
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39 **Supplementary Fig. 6.**

40 MT co-sedimentation assay in the presence of p60-katanin and p80-CTD *in vitro*. The assay  
41 used a GST-tagged K255A mutant p60 and MBP-tagged p80-CTD. MTs and associated  
42 proteins were separated from unbound proteins by sedimentation in a glycerol cushion buffer.  
43 The proteins used for co-sedimentation are indicated at the top of the gel. P and S represent  
44 the pellet fraction and the supernatant fraction, respectively. Molecular sizes are shown in  
45 lanes 1 and 8. Open, gray and filled arrowheads show GST-tagged p60, MBP-tagged  
46 p80-CTD and MT, respectively. The SDS-PAGE gel was stained with Coomassie blue.  
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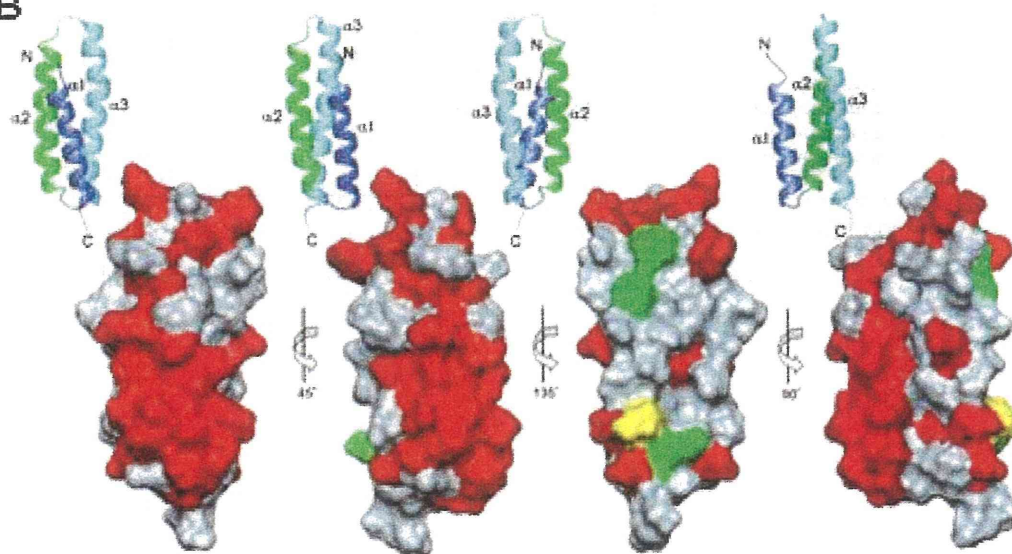
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**A**



**B**



Supplementary Figure 1