A single structurally conserved SUMOylation site in CRMP2 controls NaV1.7 function

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ABSTRACT
The neuronal collapsin response mediator protein 2 (CRMP2) undergoes several posttranslational modifications that codify its functions. Most recently, CRMP2 SUMOylation (addition of small ubiquitin like modifier (SUMO)) was identified as a key regulatory step within a modification program that codes for CRMP2 interaction with, and trafficking of, voltage-gated sodium channel NaV1.7. In this paper, we illustrate the utility of combining sequence alignment within protein families with structural analysis to identify, from several putative SUMOylation sites, those that are most likely to be biologically relevant. Co-opting this principle to CRMP2, we demonstrate that, of 3 sites predicted to be SUMOylated in CRMP2, only the lysine 374 site is a SUMOylation client. A reduction in NaV1.7 currents was the corollary of the loss of CRMP2 SUMOylation at this site. A 1.78-A-resolution crystal structure of mouse CRMP2 was solved using X-ray crystallography, revealing lysine 374 as buried within the CRMP2 tetramer interface but exposed in the monomer. Since CRMP2 SUMOylation is dependent on phosphorylation, we postulate that this state forces CRMP2 toward a monomer, exposing the SUMO site and consequently, resulting in constitutive regulation of NaV1.7.

KEYWORDS
CRMP2; NaV1.7; post-translational modifications; SUMOylation; trafficking; X-ray crystallography

Introduction
Small ubiquitin-like modifier 1 (SUMO1) protein was first identified in 1996 as a covalent modifier of nuclear proteins. In the years that immediately followed, the definition of SUMOylation expanded to include a family of 3 ~11 kilodalton (kDa) proteins (SUMO 1–3) able to target ε-amino groups of lysine residues for posttranslational modification. It is now known that SUMO proteins are transferred via covalent interaction through the E1-SUMO-activating, E2-SUMO-conjugating, and E3-SUMO-ligating enzyme cascade allowing for recognition and binding to SUMO-targeted lysine residues. Despite evidence that SUMOylation is a transient and reversible modification typically bound to only ~1% of SUMO-targeted lysine residues, the consequences of protein SUMOylation are numerous. The rapid turnover of SUMOylation has led to the hypothesis that the effects of protein SUMOylation persist long after the modification has been lost. Put another way, only ~1% of substrate might be SUMOylated but a much greater fraction of protein may have undergone recent SUMOylation and this may be sufficient to actively exert longer-term effects of the modification on the protein’s function. The effects of protein SUMOylation include alteration of conformation, regulation of stability via competition with ubiquitin modifications, and alteration of interactome via enhancement or restriction of interaction with protein binding partners (see for review).

Predicting clients for protein SUMOylation originally relied on protein sequence comparison to the canonical SUMOylation consensus sequence. SUMOylation most commonly occurs at a lysine within a canonical SUMO-
consensus motif defined as $\psi$KxD/E, where $\psi$ is a large hydrophobic amino acid (~60% of all SUMOylation$^*$), or an inverted canonical motif (D/ExK$\psi$). As SUMOylation literature expands and more non-canonical SUMOylation motifs are identified, pattern-based predictions are becoming more accurate. However, tertiary structure undoubtedly has a strong influence on whether a lysine can be efficiently SUMOylated and although some algorithms do incorporate empirical data on local secondary structure, the 3-dimensional environment of the predicted site presents a challenge to SUMO-site prediction that has yet to be met. Nevertheless, several freely available algorithms (PTMProber,$^9$ JASSA,$^10$ GPS-SUMO,$^{11}$ SUMOhydro,$^{12}$ SUMOpplot (http://www.abgent.com/sumoplot)) are useful for comparing protein sequences to positively identify SUMOylation targets. These algorithms compare hydrophobicity of amino acids that flank lysine residues and rely on machine learning techniques to improve prediction of SUMOylation based on positively identified sites of modification. Analyzing the sequence of the mouse collapsin response mediator protein 2 (CRMP2; Uniprot ID: O08553) with the above SUMO site prediction algorithms consistently predicts K20, K269, K374, and K390 as likely sites of SUMOylation. As a protein interacting partner of tubulin, actin, and vimentin, CRMP2 participates in processes that regulate cytoskeletal dynamics and the balance between neurite collapse and outgrowth.$^{13-15}$ Proximity of CRMP2 to cytoskeletal proteins and the cell membrane led to the discovery of additional functions in cellular trafficking.$^{16-21}$ Our laboratory has further expanded this role by demonstrating that CRMP2 mediates trafficking of N-type voltage-gated calcium (CaV2.2) channels.$^{22,23}$ An interaction between calcium channels and CRMP2 is regulated by Cdk5 phosphorylation.$^{24}$ Indeed, CRMP2 functions are determined by an interplay of multiple posttranslational modifications including glycosylation, oxidation, proteolysis, phosphorylation, and SUMOylation (for review see$^{25}$). Most recently, we identified SUMOylation as a posttranslational modification that targets CRMP2.$^{26,27}$ We determined that CRMP2 SUMOylation regulates proteostasis – trafficking and surface expression – of the voltage-gated sodium channel NaV1.7 in sensory neurons and that CRMP2 SUMOylation status is controlled by CRMP2 phosphorylation by Cdk5 and Fyn kinases.$^{26}$ This arrangement of interacting posttranslational modifications determines CRMP2 interactions with endocytosis related proteins Numb and Eps15, and the NaV1.7 targeting E3 ubiquitin ligase Nedd4–2 to regulate sodium current density and properties of sensory neuron excitability.

In this paper detailing the modification and signaling program for CRMP2s control of NaV1.7 in sensory neurons,$^{26}$ we analyze predicted CRMP2 SUMOylation sites and illustrate how the combination of protein sequence conservation and tertiary structural information can be used to more accurately predict residues likely to be biologically relevant clients for SUMOylation. To this end, we report the X-ray crystal structure of mouse CRMP2 to 1.78 Å resolution, revealing an additional 6 amino acids at the C-terminus, compared to previous X-ray structures of CRMP2. Analyzing the environment of the top predicted SUMOylation sites in this structure reveals features relevant to SUMOylation that could not be ascertained from either the primary sequence or secondary structure predictions.

**Results and discussion**

*Predicted CRMP2 SUMOylation motifs are phylogenetically conserved*

An analysis of the CRMP2 primary amino acid sequence with several SUMOylation algorithms identified at least 5 sequences that conformed to the SUMO-consensus motif $\psi$KxD/E ($\psi$, a hydrophobic amino acid, such as A, I, L, M, P, F, V or W; x, any amino acid residue) (Fig. 1A). Notably, these sequences were conserved between most of the other 4 members of the CRMP family (Fig. 1B). In addition to comparison of lysine residue conservation between CRMP family proteins, we also performed protein BLAST analysis of predicted CRMP2 SUMOylation sequences. We used 14 amino acid sequences surrounding the predicted lysine target of SUMOylation and identified 47 animal species that have perfect sequence conservation of either lysine 20, 374, or 390. Phylogenetic relationships of animals with CRMP2 sequence homology are represented in Fig. 1C. Lysine residue 374 was conserved in 100% of these species compared with 68% and 64% for lysine residues 390 and 20, respectively. For putative targets of SUMOylation, higher rates of sequence conservation between
related proteins and between species may be predictive of endogenous SUMOylation.

**Primacy of the K374 SUMOylation site in CRMP2 in control of NaV1.7 function**

Of these sites, K374 was particularly interesting due to (i) 100% conservation between CRMPs 1–5, (ii) conformity to the canonical SUMO-consensus motif ψ/KxD/E, and (iii) the presence of downstream aspartic and glutamic acid residues that typify this motif as the more stringent negative charge dependent SUMO motif (NDSM).4 We previously reported that loss of CRMP2 SUMOylation, via expression of a CRMP2-K374A mutant construct, decreased NaV1.7 trafficking (by up to ~85%) and current density (by
Consistent with this reduction, we also demonstrated that expression of this SUMO-null CRMP2 mutant lead to a more than 80% reduction in biochemically detectable CRMP2 SUMOylation levels, suggesting this site is the predominant target of SUMOylation within CRMP2. However, as the SUMOylation algorithms predicted not only K374 but also K20 and K390 as high-probability sites for SUMOylation in CRMP2 (Fig. 1A), we also made mutants harboring lysine to alanine (K→A) at these sites (K20A and K390A) to test the possibility that these sites may also contribute to CRMP2’s SUMOylation status and, consequently, to diminution of NaV1.7 function.

Catecholamine A Differentiated (CAD) cells were used in these studies as they endogenously express CRMP2 protein and mRNAs for voltage-gated sodium channel (VGSC) subtypes NaV1.1, NaV1.3, NaV1.7, and NaV1.9. NaV1.7 accounts for about ~95% of the total VGSC gene transcripts in CAD cells. The current-voltage (IV) protocol (Fig. 2A, top) was used to survey properties of current-voltage relationship like peak current and activation (Fig. 2A, bottom) and fast inactivation (Fig. 2A, bottom) observed in CAD cells. Blue data points represent half-maximal values of activation and inactivation curves fitted using Boltzmann equation: $y = \frac{1}{1 + \exp(V_{1/2} - V)/k}$, in which $V_{1/2}$, $V$, and $k$ represent midpoint voltage of kinetics, test potential, and slope factor, respectively. Voltage pulses and current responses that are equivalent to $V_{1/2}$ and peak current values are highlighted in blue in (A) and (B). (E) Representative peak current traces and summary data (F) before and 5 minutes after application of 125 nM Huwentoxin-IV. Some error bars are hidden by symbols. Asterisk denotes statistical significance compared with pre-drug (Student’s t-test). Data are means with SEM $n = 5–9$ cells per condition for each property shown.

**Figure 2.** Properties of CAD cell VGSC currents. (A) Protocols used to generate current-voltage (IV) relationship and activation (top) and fast inactivation (bottom) and (B) families of current traces generated by these protocols. (C) Summary of I-V relationship. Activation of currents begins near $-40$ mV, current peaks between 0 mV to $+10$ mV ($+10$ mV in this experiment), and reversal potential for sodium is near $+70$ mV. (D) Typical fast inactivation (left curve, squares) and activation (right curve, circles) observed in CAD cells. Blue data points represent half-maximal values of activation and inactivation curves fitted using Boltzmann equation: $y = \frac{1}{1 + \exp(V_{1/2} - V)/k}$, in which $V_{1/2}$, $V$, and $k$ represent midpoint voltage of kinetics, test potential, and slope factor, respectively. Voltage pulses and current responses that are equivalent to $V_{1/2}$ and peak current values are highlighted in blue in (A) and (B). (E) Representative peak current traces and summary data (F) before and 5 minutes after application of 125 nM Huwentoxin-IV. Some error bars are hidden by symbols. Asterisk denotes statistical significance compared with pre-drug (Student’s t-test). Data are means with SEM $n = 5–9$ cells per condition for each property shown.
maximal voltages of fast inactivation and activation. Notably, NaV1.7 is the dominant sodium channel in CAD cells, representing about 80% of functional sodium channels determined by NaV1.7-selective block with 125nM Huwentoxin-IV (Fig. 2E, F).

The sodium currents of CAD cells (predominantly NaV1.7) were significantly reduced when CRMP2-K374A was expressed but were not altered by expression of CRMP2-K20A, or CRMP2-K390A (Fig. 3A). None of the mutations had any effect on activation or fast-inactivation of NaV1.7 (Fig. 3B). Total levels of CRMP2 were not affected by any of the lysine mutations (Fig. 3C, D). Mutating K374, but not K20 or K390, to alanine resulted in a dramatic loss of CRMP2 SUMOylation (Fig. 3E, F), in agreement with a reduction in NaV1.7 currents.

We paired this sodium current and biochemical analyses of predicted CRMP2 SUMOylation targets with a post-hoc analysis of protein sequence homology within CRMP proteins (CRMPs 1–5). The analysis compared only the presence of a lysine at the predicted residue and revealed 60% conservation of lysine at position 390, 80% conservation of lysine at position 20, and a 100% conservation of lysine at position 374 (Fig. 1B). This result allows for a hypothesis that biologic relevance of protein SUMOylation is
Table 1. Comparative current densities and Boltzmann–fits of voltage–dependence of channel activation and fast-inactivation for the respective transfection conditions in CAD cells.

| Condition     | Peak Current | n | Activation |           | Fast Inactivation |           |
|---------------|--------------|---|------------|-----------|-------------------|-----------|
| CRMP2         | 100.0 ± 9.7  | 5 | V_{1/2} (mV) | 3.0 ± 1.3 | k (mV/e-fold) n | V_{1/2} (mV) k n |
| CRMP2-K374A   | 42.9 ± 10.0  | 5 | 10.8 ± 1.0 | 5          | −64.4 ± 1.9      | 5          |
| CRMP2-K20A    | 109.3 ± 16.5 | 5 | 10.5 ± 0.9 | 4          | 5.6 ± 0.7        | 3          |
| CRMP2-K390A   | 93.4 ± 5.0   | 5 | 9.8 ± 0.9  | 3          | 5.7 ± 0.9        | 3          |

Note: Values of peak current density (picoamp/picofarad) were normalized to the CRMP2 condition. Values for V_{1/2}, the voltage of half–maximal activation and fast inactivation (in millivolts, mV), and slope k, were derived from Boltzmann distribution fits to the individual recordings and averaged. The mean and standard error of the mean (± SEM) is reported. n values indicates the number of cells per condition. All transfection conditions contained dsRed for identification of construct expression. Asterisks represent statistically significant differences as compared with control (i.e., CRMP2 alone condition) within tested groups (p < 0.05, ANOVA with Tukey’s post–hoc test or Student’s t–test).

correlated with more stringent conservation of the motif within related proteins. Within our study, the prediction of CRMP2 SUMOylation sites was aided by protein sequence analysis. When applicable, this criterion could be an additional resource for prediction of SUMOylation sites within other proteins.

**Accessibility and local environments of predicted SUMOylation sites in the CRMP2 structure**

Additional insight about the availability of predicted SUMOylation motifs can be inferred from 3-dimensional structures, when available. Many predicted SUMOylation motifs are not accessible to the SUMOylation machinery proteins due to positioning within internal or highly structured regions of the protein. This is especially relevant when the SUMOylation motif occurs at an interface within an oligomeric assembly. CRMP2 has been shown to exhibit multiple oligomeric states in a metal dependent manner as well as form heterotetramers with other CRMPs.

In addition, the structure of human CRMP2 is known. We report here the X-ray crystal structure of mouse CRMP2 determined to 1.78 Å resolution (Table 2).

The structure contains a dimer in the asymmetric unit with the primary biologically relevant tetramer being formed by crystal symmetry. This is not unusual for CRMPs, which crystallize in a variety of space groups. Our structure is highly similar to published CRMP2 structures with RMSDs of less than 0.30 Å among the 4 structures (Fig. 4A).

The 3 top-predicted SUMOylation sites are all present and well-ordered. Site K20 is located in the solvent accessible, flexible N-terminus and is within 10 Å of the Fyn kinase phosphorylation site Y32, which may have an impact on whether K20 gets SUMOylated in vivo. Sites K374 and K390 are located in the interface between subunits and could be accessible in the monomeric state (Fig. 4B). Although no monomeric structure is available, we can predict the change in solvent accessibility by calculating the solvent accessible surface area for the oligomer as well as each subunit in isolation (Fig. 4C). K20 is highly accessible to solvent in either case and K374 is partially buried in the oligomer but more exposed in the isolated subunit. In contrast, K390 is largely solvent inaccessible in the tetramer, though slightly more accessible in the monomer. More importantly, comparing the accessibility of the 4 residue SUMOylation motif reveals that 2 key residues of the K20 motif (I19 and G22) and 3 residues of the K390 motif (AG389, MV391, and F392) are, in fact, not solvent accessible in either the tetramer or monomer. On the other hand, the 3 residues of the K374 motif (G373, M3375, and D376) are highly accessible in the monomeric state. Examining the local

Table 2. Crystallographic data collection and refinement statistics.

| Data Collection |        |        |
|-----------------|--------|--------|
| Wavelength (Å)  | 1.13   |        |
| Resolution range (Å) | 39.22 – 1.78 | |
| Space group     | C 1 2 1 |        |
| Unit cell       | 133.4, 107.2, 81.7, 90, 120.7, 90 | |
| Total reflections| 552,156 | |
| Unique reflections| 94,721 | |
| Multiplicity    | 5.8 (5.8) | |
| Completeness (%)| 100 (99.8) | |
| Mean I/σ(I)     | 23.1 (3.4) | |
| Wilson B-factor | 15.3   |        |
| R-merge         | 0.059 (0.54) | |

| Refinement |        |        |
|------------|--------|--------|
| R-work (%) | 13.5   |        |
| R-free (%) | 17.3   |        |
| RMSD bonds (Å) | 0.018  | |
| RMSD angles (°) | 1.82 | |
| Ramachandran favored (%) | 97.2 | |
| Clashscore  | 2.4    |        |
Figure 4. Structural analysis of predicted and validated CRMP2 SUMOylation motifs in the crystal structure of mCRMP2 at 1.78 Å. (A) Structural alignment of asymmetric unit contents of 3 published CRMP2 structures against our structure. Ribbon representation overlaid on smoothed surface with key residues shown as spheres. Letters in parentheses indicate subunits; the tetramer is formed by interaction between the A:B dimer and the C:D dimer. (B) Subunit A of our structure, rotated approximately 90° from panel A to show the interface between subunits A and B. Ribbon colored by B-factor overlaid on smoothed surface. Low B-factors (dark blue) consistent with regions of contact with subunit B and high B-factors (orange) indicative of flexibility. (C) Accessible surface area (1.4 Å probe) for each amino acid in the top SUMOylation motifs averaged over all subunits in 4 structures, calculated for asymmetric unit contents (with interfaces intact, top) and as isolated monomers (with interfaces exposed, bottom). (D) Close up view of each 4 residue SUMOylation motif (carbon atoms white) with the surrounding protein shown in green surface representation. Top row shows dimer, bottom row shows monomer, total accessible surface area of SUMOylation motif in lower right corner of each panel. Solvent accessible motif residues are labeled.
structural environment of each site corroborates these values (Fig. 4D). Although K20 is present at the surface, it sits in a shallow pocket with only 2 residues of the motif accessible to solvent. Furthermore, the surrounding residues likely present a barrier to interaction with the SUMOylation machinery; at the very least some conformational changes would be required for efficient SUMOylation of this residue. The K390 motif sits in a deep pocket in both the tetramer and monomer with only the lysine accessible to solvent, making this site unlikely to be accessible to the SUMOylation machinery without drastic conformational changes. In contrast, the K394 site, though buried in the tetrameric interface, is nearly 4-fold more exposed in the monomeric state and furthermore is presented on the surface without any local barriers to protein-protein interactions required for SUMOylation (Fig. 4D, middle panel).

Thus, from our analysis as well as that of a recent publication,34 SUMOylation of K374 will require exposure of the interface. This could be triggered by interaction with the SUMOylation machinery, but since the K374 motif is partially obscured in the interface this would require an additional recognition site outside of the interface. However, as mentioned above, CRMP2 forms heterotetramers with other CRMPs.30,31 This implies a certain level of equilibrium between the tetrameric and monomeric states as we have observed with analytical ultracentrifugation.36 Additionally, with this new structure, we confirm for the first time that CRMP2 forms a domain-swap handshake across the AC and BD interfaces as previously seen in CRMP4,37 CRMP5,29 and for one subunit in CRMP138 as well as other DHPs (for example, see39) (Fig. 5A). We observe clear electron density for the backbone and several side chains out to conserved residue arginine 496 (Fig. 5B), confirming that the conserved residues R496 and E221 form a salt bridge as previously observed for other CRMPs.29,37,38 The CRMP2 sequence extends another 76 residues beyond this point and contains multiple phosphorylation sites (see review25) but has been predicted to be disordered.34 We speculate that the role of this conserved salt bridge is to anchor and position the C-terminus and it’s multiple regulation sites at the surface of the protein to facilitate recognition and modification. It is likely that there are other points of contact as well, but structural evidence has been elusive. Further, in our recent paper,26 we have shown that SUMOylation at site 374 is dependent on phosphorylation at S522 but not vice-versa. Thus we suggest that phosphorylation at site S522 weakens the handshake, driving the equilibrium toward the monomeric state and exposing the K374 SUMOylation motif, ultimately culminating in constitutive regulation of Nav1.7.

**A crosstalk among posttranslational modifications – the case of CRMP2**

Protein structure analysis may aid in prediction of the interplay between SUMOylation and phosphorylation. Our recent publication indicated that CRMP2 phosphorylation by Fyn kinase at tyrosine 32 prevented CRMP2 SUMOylation of lysine 374 and that CRMP2...
phosphorylation by cyclin depend kinase 5 (Cdk5) at serine 522 enabled CRMP2 SUMOylation of lysine 374. Previous work has indicated that phosphorylation and SUMOylation can interact within the same protein to alter prevalence of the other modification. Examples of this include focal adhesion kinase where SUMOylation promotes autophosphorylation, heat-shock factors 1 and 4b, myocyte enhancer factor 2, GluK2 kainate receptor, or estrogen receptor b that require phosphorylation for secondary SUMOylation, DNA binding protein SATB1 where phosphorylation prevents SUMOylation, and microtubule stabilizing Tau protein where either phosphorylation or SUMOylation promotes the other modification. In the case of CRMP2, Fyn phosphorylation reduces SUMOylation, consequently reducing CRMP2s interaction with NaV1.7. It is possible that this relationship results from Fyn phosphorylation of tyrosine 32 blocking access to lysine 374 by SUMOylation machinery and proximity within the CRMP2 crystal structure supports this hypothesis. Other relationships between phosphorylation and SUMOylation have been described as proximity-dependent but only in terms of primary protein structure, i.e. within about 10 amino acids.

**Conclusions**

In summary, our data show that multiple levels of CRMP2 sequence analysis aided our prediction of CRMP2 SUMOylation and enhanced our understanding of CRMP2 structure-function relationships. This description of our analysis puts forward evidence that protein sequence conservation and positive identification of protein SUMOylation sites can be correlative. However, additional studies of SUMOylated proteins and sequence conservation are needed to determine if these properties have predictive value.

**Methods**

**Plasmids, antibodies and other materials**

Biochemical analysis of protein content by Western blot analysis used the following antibodies: βIII-Tubulin (Promega Cat# G7121 RRID:AB_430874), CRMP2 (Sigma-Aldrich Cat# C2993 RRID:AB_1078573).

Mutations to plasmid dsRed2-N1-CRMP2 used in this study were introduced by QuickChange II XL mutagenesis kit (Cat# 200521, Agilent, Santa Clara, CA) in the pdsRed2-CRMP2 plasmid. Plasmids were purified from DH5α E. coli using the NucleoBond® Xtra Maxi kit (Cat# 740414, Machery-Nagel, Germany). The coding sequence of mouse CRMP2 (residues 1–500) was amplified by PCR using primers 5’-ACCATCACAGCCATATGTCTTATCAGGGGAAGAAAATAT-3’ (forward) and 5’-GAAGTTCCAGTCGACTTAGCTCATAAGGCACAGGG-3’ (reverse) (Eurofins Genomics (Louisville, KY, USA)), and subcloned into the pET15_NESG vector (purchased from DNAasu, Phoenix, AZ, USA) between Ndel and SalI restriction sites, resulting in a construct with an N-terminal His tag. Amplified sequences and introduced mutations were verified by DNA sequencing.

**Protein expression and purification**

Recombinant mouse CRMP2 (residues 1–500) without the C-terminal tail was expressed and purified as described previously. The pure CRMP2 protein was concentrated to 30 mg/mL, flash frozen in liquid nitrogen and stored at −80°C.

**Crystallization**

CRMP2 1–500 was crystallized using the hanging drop method at room temperature, by mixing 2 μL of the well solution (0.1 M Bis-Tris pH 6, 25% PEG3,350) and 2 μL of protein (16 mg/mL diluted in 50mM HEPES pH7.5, 300 mM Na Cl, 0.5mM TCEP, 10% glycerol, 200mM CaCl2) and equilibrating against 0.5 mL of the well solution. Crystals were soaked in a cryoprotectant solution (100 mM HEPES, pH 7.4, 45% PEG 3,350) before data collection.

**Data collection and processing**

X-ray data were collected at Stanford Synchrotron Radiation Lightsource (SSRL) beamline BL7–1. Data were processed using X-ray Detector Software to 1.78 Å and the structure was solved by molecular replacement using the published human CRMP2 structure (2GSE.pdb). The asymmetric unit contains a dimer. The appropriate amino acid substitutions were made and the structure was refined using Refmac49 in the CCP4 suite with automatic weighting with manual rebuilding in Coot. The final model contains residues 15 – 496 (subunit A) and residues 15 – 49 and 54 – 496 (subunit B). Density for residues 50–53 was poorly resolved in subunit B, likely due to
disorder caused by crystal packing. Simulated annealing omit maps were generated with PHENIX. Coordinates and structure factors for CRMP2 are deposited in the Protein Data Bank (accession code 5UQC).

**Culturing and transfection of catecholamine A differentiated (CAD) cells**

Mouse neuron derived CAD (ECACC Cat# 08100805, RRID:CVCL_0199) cells were grown in standard cell culture conditions (DMEM/F12 media supplemented with 10% fetal bovine serum (Hyclone)), 37°C in 5% CO2, as described previously. For patch clamp experiments, CAD cells were transfected with 1 µg/µl of polyethyleneimine (PEI, Sigma) and 2 µg of various CRMP2 constructs. Under these conditions efficiencies of ~50% were observed. For biochemistry experiments, CAD cells were transfected with Lipofectamine 2000 (Invitrogen) with ~80–95% efficiency. All experiments were performed 48 h after transfection.

**Patch clamp electrophysiology**

Whole cell voltage clamp recordings were performed at room temperature using an EPC 10 Amplifier-HEKA as described previously. The internal solution for voltage clamp cell recordings contained (in mM): 110 CsCl, 5 MgSO4, 10 EGTA, 4 ATP Na2-ATP, and 25 HEPES (pH 7.3, 290–300 mOsm/L) and external solution contained (in mM): 100 NaCl, 10 tetraethylammonium chloride, 1 CaCl2, 1 CdCl2, 1 MgCl2, 10 D-glucose, 4 4-aminopyridine, 0.1 NiCl2, 10 HEPES (pH 7.3, 310–315 mosM/L).

**Voltage clamp protocols**

CAD cells were subjected to current-density (I-V) and fast-inactivation voltage protocols. In the I-V protocol, cells were held at a −80 mV holding potential before depolarization by 20 ms voltage steps from −70 mV to +60 mV in 5 mV increments. This allowed for collection of current density data to analyze activation of sodium channels as a function of current versus voltage and also peak current density which was typically observed near ~0 to −10 mV and normalized to cell capacitance (pF). Values of current in nA and cell size in pF are available in figure legends. In the fast-inactivation protocol, cells were held at a −80 mV holding potential before hyperpolarizing and depolarizing pulses for 500 ms between −120 mV to −10 mV in 5 mV increments. This step conditioned various percentages of channels into fast-inactivated states so that a 0 mV test pulse for 20 ms could reveal relative fast inactivation normalized to maximum current. The current density of CAD cells undergoes a slight drift between passages, but averages around 60 pA/pF peak currents. Due to drift, controls were repeated within each experiment to normalize data. Importantly, all relationships between conditions were conserved regardless of drift.

**Immunoprecipitation (IP) of endogenously SUMOylated proteins from cell lysates**

CAD cells were lysed into the immunoprecipitation buffer as described previously. Buffer composition was 20 mM Tris-HCl pH = 7.4, 50 mM NaCl, 2 mM MgCl2, 10 mM N-Ethylmaleimide (NEM), 1% (vol/vol) NP-40, 0.5% (mass/vol) sodium deoxycholate, 0.1% (mass/vol) sodium dodecyl sulfate (SDS) with Protease inhibitors (Cat# B14002, Bio- tool, Houston, TX), phosphatase inhibitors (Cat# B15002, Biotool, Houston, TX) and BitNuclease (Cat# B16002, Biotool, Houston, TX). Total protein concentration was determined by BCA protein assay (Cat# PI23225, Thermo Fisher Scientific, Waltham, MA). For immunoprecipitation of endogenously SUMOylated proteins, SDS was added to the lysates at 0.5% (mass/vol) final concentration, before boiling for 5 min at 95°C. Then, 500 µg of total proteins were incubated with 5 µg of SUMO1 antibody overnight at 4°C under gentle agitation. Protein G magnetic beads (Cat# 10009D, Thermo Fisher Scientific, Waltham, MA), pre-equilibrated with the immunoprecipitation buffer, were then added to the lysates and incubated for 1 h at 4°C to capture immuno-complexes. Beads were washed 4 times with immunoprecipitation buffer to remove non-specific binding of proteins, before re-suspension in Laemmli buffer and boiling at 95°C for 5 min before immunoblotting.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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