Phosphorylation of CRN2 by CK2 regulates F-actin and Arp2/3 interaction and inhibits cell migration

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CRN2 (synonyms: coronin 1C, coronin 3) functions in the re-organization of the actin network and is implicated in cellular processes like protrusion formation, secretion, migration and invasion. We demonstrate that CRN2 is a binding partner and substrate of protein kinase CK2, which phosphorylates CRN2 at S463 in its C-terminal coiled coil domain. Phosphomimetic S463D CRN2 loses the wild-type CRN2 ability to inhibit actin polymerization, to bundle F-actin, and to bind to the Arp2/3 complex. As a consequence, S463D mutant CRN2 changes the morphology of the F-actin network in the front of lamellipodia. Our data imply that CK2-dependent phosphorylation of CRN2 is involved in the modulation of the local morphology of complex actin structures and thereby inhibits cell migration.

Coronins play an essential role in the structural and functional organization of actin-dependent cellular processes like protrusion formation, secretion, migration, and invasion. Phylogenetic analyses have revealed twelve subfamilies of coronin proteins, consisting of seven vertebrate paralogs and five subfamilies in non-vertebrate metazoa, fungi, and protozoa. Coronins are structured with a rather short, conserved, basic N-terminal signature motif, followed by one, or, in case of the coronin 7 ‘dimer’ subfamily, two 7-repeat WD40 domains which adopt the fold of a seven-bladed β-propeller. A unique C-terminal extension links the WD40 repeat domains with the C-terminal coiled coil domain.

The predominant form of CRN2 is isoform 1 (CRN2i1), a ubiquitously expressed 474 amino acid protein. CRN2 forms homotrimers via the coiled coil domain and has been identified as an actin filament cross-linking and bundling protein. It exists in two different pools, an actin cytoskeleton associated non-phosphorylated pool enriched at lamellipodia and a diffusely distributed phosphorylated cytosolic pool, however, the phosphorylation site and the kinase are unknown. In the murine brain, CRN2 seems to play a role in morphogenesis and in certain neuronal cell populations in the adult animal. Recently, CRN2 has also been implicated in human cancer. While normal resting astrocytes do not express CRN2, the number of CRN2-positive tumor cells is correlated with the malignant phenotype in human diffuse gliomas. Knock-down of CRN2 in human glioblastoma cell lines reduces the rate of cell proliferation, motility, and invasion. Furthermore, CRN2 is aberrantly regulated in melanoma with an increase of CRN2 expression in metastatic tumor cells. In hepatocellular carcinoma, CRN2 expression levels correlates with clinical progression. In a recent analysis of primary effusion lymphoma specimens, the CRN2 gene was found to be amplified in one-fourth of the specimens and CRN2 expression levels were elevated in three-fourths of the specimens. However, a different effect was observed in another study, where a knock-down of CRN2 in colon carcinoma cell lines appeared to induce opposite effects like enhanced cell migration and the increased number of focal adhesions.
Several reports support a role for CRN2 in signaling pathways that involve small G-proteins. A short sequence stretch that resembles the Cdc42/Rac interactive binding (CRIB) motif is present in CRN2 and could act as a potential binding site for the activated GTP-binding proteins Rac and Cdc42 involved in the regulation of the actin cytoskeleton. CRN2 has also been found to be a direct binding partner of GDP-Rab27a. GDP-Rab27a was found to increase the F-actin bundling activity of CRN2 and the protein complex was shown to be involved in the insulin secretory membrane endocytosis.

In this study, we demonstrate that CRN2 function is regulated by CK2-dependent phosphorylation. Protein kinase CK2 (synonyms: casein kinase II, CK II) was first described in a mixture with CK1 using casein as an artificial substrate. It is an evolutionarily highly conserved, ubiquitously expressed, highly pleiotropic, and constitutively active serine and threonine kinase. CK2 primarily exists as a heterotetrameric protein of either 2ββ or 2αβ subunit composition. In these CK2 complexes, the two regulatory subunits CKβ2 form a stable dimer linking together the two catalytic subunits, CK2α or CK2αβ.

We show here that a CK2-dependent phosphorylation of CRN2 at residue S463 leads to a loss of CRN2-mediated inhibition of actin polymerization as well as to a loss of its F-actin bundling activity and Arp2/3 complex interaction. Together, these changes affect the architecture of the F-actin network and result in an inhibition of cell migration. Furthermore, this work reveals that bundling of actin filaments occurs via two separate actin binding sites in CRN2 and that the CRN2 coiled coil domain forms a constitutive trimer which either can interact with F-actin or the Arp2/3 complex.

**Results**

Identification of Ser at position 463 as a specificity determining site in the mammalian and avian CRN2 subfamily. An alignment of 40 mammalian and avian proteins out of a subclassification of 60 orthologs used in previous phylogenetic analyses of coronin family homologs was first transformed into a profile hidden Markov model (pHMM) using the HMMER implementations in Unipro-Ugene (http://ugene.unipro.ru/) and visualized with LogoMat-M (http://www.sanger.ac.uk/Software/analysis/logomat-m/). This identified evolutionarily conserved amino acid patterns with the highest probabilities of information content or functional significance.

“Specificity determining positions” (SDPs) peculiar to the CRN2 subfamily were identified by SDPclust, SDPfox (http://bioinf.fbb.msu.ru/SDPfoxWeb/main.jsp), and “type II divergence” among 300 coronins representing all seven subfamily groups present in mammals and birds and were marked with an asterisk in the corresponding position of the pHMM sequence logo for CRN2 (Fig. 1A). The concentration of these SDP sites in the N- and C-terminal domains argues for a predominant role of these regions in the functional differentiation of coronin subfamilies. Other highlighted sites, which are known to be susceptible to post-translational modifications, included Tyr-301 and acetylated lysines 391 and 446 in CRN2 and various other coronin subfamilies. In contrast, the incorporation of Ser at position 463 was confined to the CRN2 subfamily. The Ser-463 phosphorylation site is uniquely conserved within the CRN2 subfamily, whereas sites of other predicted post-translational changes (P-Tyr-301, Ac-Lys-391 and Ac-Lys-446) are common to a limited number of other coronin subfamilies. Asterisks mark amino acids identified by SDPfox which show evidence of a conservation pattern able to distinguish individual coronin subfamilies and are therefore taken to confer “functional specificity”. These SDPs localized mainly to regions of the N- and C-terminal domains, in contrast to the “KGD” motif universally conserved in coronin proteins (aa485-487 in CRN2).

**Figure 1** | Phosphorylation of S463 within the CRN2 coiled coil does not induce trimer disassembly. (A) Profile hidden Markov model (pHMM) of the CRN2 subfamily. The probability distribution of amino acids within the CRN2 subfamily is reflected by letter height while the “functional significance” predicted by HMMER is given by the full column height at each site. The Ser-463 phosphorylation site is uniquely conserved within the CRN2 subfamily, whereas sites of other predicted post-translational changes (P-Tyr-301, Ac-Lys-391 and Ac-Lys-446) are common to a limited number of other coronin subfamilies. Asterisks mark amino acids identified by SDPfox which show evidence of a conservation pattern able to distinguish individual coronin subfamilies and are therefore taken to confer “functional specificity”. These SDPs localized mainly to regions of the N- and C-terminal domains, in contrast to the “KGD” motif universally conserved in coronin proteins (aa485-487 in CRN2). (B) Homology model of the trimeric CRN2 coiled coil (aa442-447, monomer chains A, B, C) based on the crystal structure of the trimeric coiled coil of hemagglutinin (HA2 chain) using residues 74 to 113 from chain B (“PDB 1e08”). Interactions of phosphorylated S463 (pS463, see C) with R461 and K464 are illustrated. (C) Size-exclusion chromatogram of synthetic pS463-CRN2 peptide (18 mg/ml) acquired on a Superose-12 column under normal (100 mM) and high salt (500 mM NaCl) conditions. The peptide eluted as single species whose molecular mass was determined using the online multi-angle light scattering (MALS) detector; theoretical mass of the trimer is 13,959 g/mol.
The location of S463 in a solvent accessible α-helical surface segment also is visualized in a three-dimensional refined model of the human CRN2 protein predicted by I-Tasser and rendered with Chimera v1.52 (Fig. S1). Finally, it is important to note that S463 is part of the sequence motif S-K-L-E coinciding with the S/T-X-X-E/D consensus target motif of the protein kinase CK2.

**Phosphorylation of S463 does not induce disassembly of the CRN2 trimer.** To address the issue of trimer stability at the experimental level, gel filtration experiments in conjunction with multi-angle light scattering detection (SEC-MALS) were carried out to determine the oligomerization state of synthetic wild-type and phospho-S463 CRN2 coiled coil peptides (aa442-472; 3.7 kDa). Under physiological buffer conditions, the S463 phosphorylated synthetic CRN2 peptide eluted as trimer (Fig. 1C). Furthermore, small-angle X-ray scattering (SAXS) was conducted in solution and also showed the presence of a trimeric particle, independent of the concentration of the sample. The molecular mass and radius of gyration derived from the SAXS experiments were in good agreement with the expected values from the atomic homology model (Tab. S1). The shape restored from the X-ray scattering data in solution was fitted with the atomic homology model of the trimeric coiled coil as rigid body and revealed an excellent agreement (Fig. S2). The goodness of fit between the experimental and theoretical scattering curves was calculated by CRYSOL as \( \chi = 5.5 \). Additional gel filtration analyses were carried out using purified recombinant wild-type, S463D phosphomimetic and S463A phospho-resistant C-terminal polypeptides (aa300-474; 19.9 kDa), full-length wild-type and S463D phosphomimetic proteins purified from insect cells (53.2 kDa), and lysates of mammalian cells over-expressing GFP-fusion proteins of wild-type, S463D and S463A CRN2. These experiments clearly demonstrate a strong CRN2 trimer that is not disassembled by S463 phosphorylation. With \( \sim 40 \text{ nM} \) the concentrations of full-length CRN2 were far lower than the lowest concentration (4.5 mg/ml \( \approx 1.2 \text{ mM} \)) used for of the synthetic peptides and the protein could only be detected by immunoblotting.

CRN2 directly interacts with protein kinase CK2. Since our in silico analysis indicated that CRN2 S463 is part of a consensus CK2 target motif, an interaction between the CK2α catalytic subunit and CRN2 was tested by pull-down assays. Experiments employing purified GST-tagged CK2α coupled to glutathione beads as bait and purified soluble CRN2 wild-type or phosphomimetic S463D mutant C-terminal polypeptides as prey showed an interaction between both CRN2 variants and CK2α (Fig. 2A). The experiment was repeated with endogenous as well as GFP-tagged full-length CRN2 proteins from HEK293 cell lysates and showed identical results (Fig. 2B). In both experiments CRN2 also interacted with a dead kinase K68A mutant of CK2α.

S463 within the CRN2 coiled coil domain is phosphorylated by CK2α. We performed in vitro kinase assays to address the question whether the interaction between CK2α and CRN2 leads to phosphorylation of CRN2. When the wild-type CRN2 C-terminal polypeptide was incubated with CK2α, a CRN2 phosphorylation signal was detected which increased with the incubation time (Fig. 3A). In contrast, the incubation of the S463D mutant resulted in very low levels of phosphate incorporation from [γ-32P]ATP. In addition, kinase assays were performed with recombinant full-length CRN2 proteins purified from insect cells. Wild-type full-length CRN2 was also phosphorylated in a time dependent manner (Fig. 3B). In case of the S463D mutant, phosphorylation signals were hardly detectable at 30 min, and did not reach the intensity of wild-type CRN2 after an extended incubation time of 120 min. These experiments demonstrate that S463 is phosphorylated by CK2α.

**Figure 2** CRN2 directly interacts with CK2α. (A) pull-down assay employing purified recombinant full-length GST-tagged CK2α coupled to glutathione beads and purified soluble CRN2 wild-type and phosphomimetic S463D C-terminal polypeptides (aa300-474). Both polypeptides bind to CK2α and CK2αdead, the latter lacks kinase activity. (B) pull-down assay employing purified recombinant full-length GST-tagged CK2α coupled to glutathione beads and lysates from HEK293 cells over-expressing endogenous CRN2 (57 kDa) as well as GFP-CRN2 (82 kDa) fusion proteins. Endogenous, wild-type GFP-CRN2, and GFP-CRN2 S463D bind to CK2α. All CRN2 polypeptides were detected with antibody K6-444, and GST-CK2α immunoblotting was done with a rabbit polyclonal GST-antibody. Asterisk, two additional CK2α bands are the result of degradation. Controls contained beads coated with GST alone. For illustration purposes individual lines from the original western blots were digitally re-arranged.

CK2 contributes to the pool of phosphorylated CRN2 in vivo. We performed de-phosphorylation experiments to verify the presence of phosphorylated CRN2 within the cell. Lysates from samples of murine tissues were incubated with alkaline phosphatase and
analyzed by two-dimensional gel electrophoresis in conjunction with CRN2 immunoblotting. A single spot of CRN2 was detected in untreated samples of skeletal muscle tissue. This spot shifted to a more alkaline pI after treatment with alkaline phosphatase. The distance between both spots was approximately 0.5 pH units and corresponded to a calculated loss of three phosphate residues. Similar results, however with the presence of multiple spots, were obtained for murine brain tissue (Fig. 4A).

In a next step, we confirmed that phosphorylation of CRN2 also occurs in a CK2-dependent manner in vivo. 293TN cells over-expressing GFP-CRN2 were grown in the presence of the CK2 inhibitor TBB, the CK2 activator 1-ethyl-4,5-dicarbamoylimidazole, or solvent control before addition of 32P-orthophosphate. We determined a 40% reduction of CRN2 phosphorylation in case of the CK2 inhibitor and an 80% increase in case of the CK2 activator strongly supporting a physiologically relevant CK2 dependent phosphorylation of CRN2 in vivo (Fig. 4B). Since it has been reported that S13 phosphorylation of the Hsp90 co-chaperone cdc37 is a marker of

![Figure 4](https://www.nature.com/scientificreports)

**Figure 4 | CK2 phosphorylates CRN2 in vivo.** (A) Presence of a phosphorylated pool of endogenous CRN2. Lysates of murine skeletal muscle (upper three panels) and brain tissue (lower two panels) were separated by 2D-gel electrophoresis. Controls where the CRN2 polypeptide, [γ-32P]ATP, or CK2α were omitted and use of a kinase-dead CK2α are indicated. Upper panel, autoradiograph of phosphorylation of CRN2 (32P). Lower panel, the corresponding Coomassie brilliant blue stained gel. For all lines containing CK2α identical volumes from the same preparation of purified enzyme were used. Note the minor phosphorylation levels of the mutant polypeptide after 90 min. (B) in vitro kinase assay employing recombinant full-length GST-CK2α (66 kDa, arrow) and GST-His-tagged full-length CRN2 purified from insect cells (90 kDa, arrowheads). Time course (1, 5, 10, 30 minutes) of phosphate incorporation from [γ-32P]ATP into wild-type CRN2. Controls where CRN2, [γ-32P]ATP, or CK2α were omitted and use of a kinase-dead CK2α are indicated. Upper panel, autoradiograph of phosphorylation of CRN2 (32P). Lower panel, the corresponding Coomassie brilliant blue stained gel. For all lines containing CK2α identical volumes from the same preparation of purified enzyme were used. CRN2 is phosphorylated in a time-dependent manner. Note, that only after longer incubation time (120 min) the phosphorylation level of S463D mutant CRN2 reaches the one of wild-type CRN2.
in vitro CK2 activity\cite{44,45}, blots were probed for pS13-cdc37, total cdc37, and CK2\(\beta\) for control. We observed a complete suppression of cdc37 phosphorylation in presence of TBB while the levels of cdc37 and CK2 proteins remained unchanged (Fig. 4B, bottom panel). The pattern of an effective TBB induced suppression of cdc37 phosphorylation and an effective 1-ethyl-4,5-dicarbamoylimidazole induced stimulation of CRN2 phosphorylation suggests less CK2-dependent phosphorylation of CRN2 under basal cellular conditions.

CRN2 and CK2 co-localize at the front of lamellipodia. Immunofluorescence analyses were carried out in lamellipodia-rich Pop10 cells to determine the subcellular distribution of CR2 relative to CRN2 and F-actin. Previous studies have shown that the subcellular localization of CRN2 does not change upon CRN2 over-expression\cite{8} and that the amount of F-actin is not influenced by the level of CRN2 expression\cite{7}. In Pop10 cells over-expressing GFP-CRN2 fusion proteins, CRN2 and CK2 were enriched and co-localized in the perinuclear region (Fig. 5, arrowheads) and at the front of lamellipodial structures (Fig. 5, arrows). Lamellipodia showed a co-localization of CRN2, CK2, and F-actin. F-actin stress fibers, which were co-stained by CRN2, did not show any overt enrichment of CK2 (Fig. 5, double-arrowheads). No differences in these patterns were detected, when the cells over-expressed phosphomimetic S463D or phospho-resistant S463A CRN2 instead of the wild-type protein.

Wild-type but not S463D phosphomimetic CRN2 inhibits actin polymerization. An influence of CRN2 on actin polymerization was determined in actin polymerization experiments employing G-actin and the Arp2/3 complex together with its activator, the VCA domain of N-WASP. Wild-type CRN2 and the S463A mutant, but not the phosphomimetic S463D CRN2 C-terminal polypeptide, effectively inhibited actin polymerization. CRN2 reduced the velocity of actin filament growth (Fig. 6A; slopes decreased) and the final amount of F-actin (Fig. 6A; plateaus decreased). This inhibitory effect of CRN2 was apparent in the presence or absence of Arp2/3 complex and VCA, although the CRN2 mediated inhibition of actin polymerization always could be antagonized to a limited extent by addition of the Arp2/3 complex (Fig. 6B).

To verify the specificity of these assays, the CRN2 polypeptides were added to pre-polymerized actin. Here, a small and identical quenching effect of the fluorescence signal of F-actin was observed with every polypeptide tested. It is noteworthy, that the inhibitory effect of CRN2 on actin polymerization was dose-dependent. Low starting concentrations of CRN2 C-terminal polypeptides caused only decreasing velocities of actin polymerization, whereas the highest CRN2 concentration (but lower than the one used in Fig. 6A) additionally reduced the final amount of F-actin (Fig. 6C). A dose-dependent effect was only detected for the wild-type and S463A mutant CRN2 polypeptides, while the phosphomimetic S463D CRN2 C-terminal polypeptide did not show such an effect (Fig. 6D).

However, high S463D CRN2 to actin ratios (see figure legend) as used in the experiments shown in Fig. 6A caused a partial inhibition of actin polymerization.

To address the possibility that the inhibitory effect of CRN2 on actin polymerization might result from sequestration of G-actin, we performed fluorescence-based G-actin binding assays. Here, a change in the fluorescence signal of G-actin upon binding of a test protein was only detected for the WH2-domain of CAP2 as control, but not for any of the CRN2 polypeptides. A potential capping effect of CRN2 that might reduce actin polymerization can be excluded due to the high molar ratio of CRN2 vs. G-actin in these assays (see Materials and Methods).

Phosphorylation of CRN2 at S463 affects its interaction with F-actin and Arp2/3 complex. Two-step F-actin co-sedimentation assays were employed to study the interactions of the CRN2 C-terminal polypeptides with F-actin. Wild-type and S463A mutant CRN2 induced the formation of F-actin bundles (Fig. 7A, 10,000xg first pellet), with less binding and co-sedimentation with actin filaments (100,000xg second pellet). S463D phosphomimetic CRN2 demonstrated opposite effects with enrichment in the 100,000xg actin filament pellet and markedly reduced F-actin bundling activity (10,000xg pellet). Addition of Arp2/3 did not change these patterns. However, the CRN2 C-terminal polypeptides competed with the Arp2/3 complex for F-actin binding. Arp2/3 immunoblots of samples derived from 100,000xg F-actin co-sedimentation assays indicated a partial release of the Arp2/3 complex into the supernatant upon the addition of either wild-type or mutant CRN2 polypeptides (Fig. 7B). The interaction of the CRN2 polypeptides as well as of full-length wild-type, S463D and S463A mutant CRN2 proteins with the Arp2/3 complex was further studied by pull-down and co-immunoprecipitation experiments. Both, wild-type and S463A mutant CRN2 bound directly to the Arp2/3 complex, while phosphomimetic S463D CRN2 showed essentially no binding (Fig. 7C,D,E).

Phosphomimetic S463D CRN2 changes the architecture of the F-actin network in the front of lamellipodia and inhibits cell migration. To demonstrate a functional role of CRN2 phosphorylation at S463, U373 human glioblastoma cells with a stable shRNA-mediated knock-down of endogenous CRN2 were transfected with GFP-tagged, shRNA-resistant wild-type, phosphomimetic S463D or phospho-resistant S463A mutant CRN2 expression constructs. Replacement of the endogenous CRN2 by the S463A mutant CRN2 led to cells with a smooth and regular co-distribution of CRN2, F-actin, and Arp2/3 complex at the lamellipodia (Fig. 8A). In contrast, cable-like enrichments of CRN2 and F-actin and a disrupted distribution of the Arp2/3 complex were detected in the front of lamellipodial extensions in case of the S463D mutant (Fig. 8B). Furthermore, the latter cells showed a thinner region of CRN2 and Arp2/3 complex (p34-Arc antibody) co-localization, which was restricted to the very tip of lamellipodia (Fig. 8A,B, right panels, distance labels). Cells that expressed GFP-tagged wild-type

Figure 5 | CRN2, CK2, and F-actin co-localize in the front of lamellipodia. Pop10 cells over-expressing GFP-tagged CRN2 were fixed and CK2\(\beta\) was immunolabeled with primary antibody 1AD9 followed by Alexa-633 labeled secondary antibody; F-actin and nuclei were visualized by TRITC-phalloidin and DAPI, respectively. CRN2 and CK2 co-localize in the peri-nuclear region (arrowheads); CRN2, CK2, and F-actin co-localize in the front of lamellipodia (arrows). Double-arrowheads, F-actin fibers decorated by CRN2.
CRN2 displayed a combination of both phenotypes; a statistical analysis is given in Fig. S3. The sole reduction of the CRN2 expression level in U373 cells did not affect the morphology of lamellipodia (see Fig. 3 in reference 8).

In order to evaluate if these lamellipodial alterations lead to changes in cell migration we monitored the formation of cellular protrusions of HEK293 cells stably expressing the GFP-tagged CRN2 variants. Compared to the wild-type and S463A mutant CRN2 situation, the expression of S463D phosphomimetic CRN2 led to a reduction in the number of cellular protrusions by a factor of two (Fig. 9A). In addition, confluent monolayers of HEK293 cells were used for in vitro wound healing assays. A reduced velocity in wound closure was detected in case of S463D phosphomimetic CRN2 (23 μm/h), compared to wild-type (29 μm/h) and S463A CRN2 (28 μm/h) expressing cells (Fig. 9B).

S463D CRN2 displays a delayed integration into the podosome core structure. Alterations in the molecular composition of podosomes, which are prominent adhesion and invasion structures that play an important role in the migration of macrophages and other cell types, further illustrate the cellular relevance of CRN2 S463 phosphorylation. Expression of GFP-CRN2 constructs in primary human macrophages demonstrated an enrichment of wild-type, S463D and S463A mutant CRN2 at podosomes, and all three CRN2 species co-localized with the F-actin core structure (Fig. S4A-C, ctrl). Various podosomal parameters were investigated in
the transfected macrophages. Determination of the number, morphology, size, subcellular distribution, and F-actin content of podosomes revealed no differences with respect to the three different CRN2 constructs. Also, a nearly complete knock-down of the endogenous CRN2 demonstrated that podosomes are assembled independently of CRN2 (Fig. S5). However, when the podosomes were disrupted by treatment with the Src family kinase inhibitor PP2 and allowed to re-form after washout of the inhibitor, the phospho-resistant S463A mutant CRN2 was in most cases excluded from the re-assembled podosomes (Fig. S4C, re-formation; Fig. S6). Further, fluorescence recovery after photobleaching (FRAP) experiments were carried out and showed that CRN2 is a fully mobile component (plateau after recovery approximately reaches pre-bleach intensity) of the podosomal structure (Fig. S7, left graphs). An analysis of the fluorescence recovery via bi-exponential equation resulted in fitted curves which indicated a significantly reduced k\text{off} value of 0.11 s\textsuperscript{−1} for S463D mutant CRN2 compared to 0.31 s\textsuperscript{−1} and 0.25 s\textsuperscript{−1} for wild-type and S463A mutant CRN2, respectively (Fig. 9C). The dissociation constants were used for the calculation of the half-life times which accordingly showed an elevated half life time (t\textsubscript{1/2}) of 6.56 s for the S463D mutant in contrast to half-life times of 2.27 s for wild-type and 2.81 s for S463A mutant CRN2 (Fig. S7, right graphs).

Figure 7 | Phosphorylation of S463 controls F-actin bundling activity and Arp2/3 interaction of CRN2. (A) two-step F-actin spin-down assay employing rabbit skeletal muscle G-actin, bovine Arp2/3 complex and purified recombinant CRN2 wild-type (W), phosphomimetic S463D (D), and S463A (A) mutant C-terminal fragments. Coomassie brilliant blue stained SDS-PAGE gels are shown. The left panel (10,000xg first pellet) demonstrates F-actin bundling activity of wild-type and S463A mutant CRN2 in comparison to reduced bundling activity of S463D mutant CRN2. Vice versa the middle panel (100,000xg second pellet) shows increased co-sedimentation of S463D mutant CRN2. Arp2/3 does not influence the results. Right panel, 100,000xg supernatant given as control. As further control, intensities of actin bands were analyzed by densitometry and the measurements demonstrated equal sums for the six triplets n+n'+n''. (B) Arp2/3 immunoblot of a 100,000xg F-actin spin-down experiment. Presence of all CRN2 polypeptides releases Arp2/3 from F-actin into the supernatant. This experiment only allows a qualitative assessment due to difficulties to completely dissolve the pellets in SDS sample buffer and transfer the proteins onto the blot membrane. (C) pull-down assay employing purified recombinant His-tagged CRN2 wild-type as well as S463A and phosphomimetic S463D mutant C-terminal polypeptides coupled to Ni-beads and soluble purified Arp2/3 complex. In comparison to wild-type and S463A mutant CRN2, the S463D mutant shows reduced direct binding to Arp2/3. Arp2/3, p34 immunoblot; CRN2pep, mAb K6-444 immunoblot. Beads, Ni-sepharose beads lacking CRN2; flow-through, Arp2/3 flow-through from these blank beads. (D) co-immunoprecipitations using GFP mAb K3-167-26 coupled to Protein G coated beads and lysates from 293TN cells expressing GFP-tagged full-length wild-type CRN2 as well as S463D and S463A mutants. Immunoblotting was performed with CRN2 mAb K6-444 and p34 pAb (Upstate #07-227). S463D mutant GFP-CRN2 shows reduced interaction with the Arp2/3 complex. Prior to preparation of the lysates cells were treated with latrunculin B to prevent unspecific co-precipitation of proteins tied together via F-actin bridges. (E) bar chart, densitometry analysis of the Arp2/3 signal intensity from three independent experiments, where GFP-CRN2 wild-type and S463D mutant were parallelly immunoprecipitated; one experiment is shown in D. Arp2/3 values are normalized to the respective GFP-CRN2 values.
Discussion

We identified CRN2 as a novel direct binding partner and substrate of CK2α. This interaction results in phosphorylation of S463 within the coiled coil domain of CRN2. Although it has been shown that CK2 phosphorylation sites in many proteins overlap with sites of caspase cleavage46, this is not the case for S463 of CRN2, since this residue is not part of the consensus motif of caspase 3. Instead, the S463 phosphorylation inhibits the actin filament crosslinking activity and the Arp2/3 binding capacity of CRN2.

Phosphorylation is a common mechanism to regulate coronin protein activity. CRN1 (synonyms: coronin 1B, coronin 2) and CRN4 (synonyms: coronin 1A, coronin 1) are substrates of protein kinase C (PKC). In the case of CRN1 PKC phosphorylates serine 2, a residue that is not present in CRN2, and thereby inhibits the interaction between Arp2/3 and CRN1. As a consequence, cell migration velocity is reduced and the PMA-induced membrane ruffling is suppressed47. For CRN4 the specific PKC phosphorylation site is unknown. However, phosphorylation of CRN4 leads to its dissociation from phagosomes and a role of CRN4 in the maturation of phagosomes has been postulated48.

Thus far, only three studies have reported a specific interaction of CK2 with actin or actin-associated proteins in mammalian cells. First, rabbit skeletal muscle G-actin directly binds to the CK2α subunit and inhibits the activity of CK2 in a dose-dependent manner in vitro49. More importantly, CK2 phosphorylates the VCA domain of WASP at serine residues 483 and 484, which in turn enhances the interaction of VCA domain with the Arp2/3 complex and thereby increases the velocity of Arp2/3 mediated actin polymerization50. Furthermore, CK2 synergizes with CKIP-1 to inhibit the actin capping protein CapZ at the barbed ends of actin filaments in actin de-polymerization assays and actin polymerization assays starting from spectrin-F-actin seeds, but neither protein has an influence on the dissociation of CapZ from F-actin in uncapping assays51. In summary, CK2 is able to promote actin polymerization and reduce the formation of crosslinked actin filaments (Fig. 10).

From our results we conclude that wild-type, S463D and S463A mutant CRN2 polypeptides do not exert any capping effect, but most likely bind to the side facing the actin filaments as described for CRN452. However, only the direct binding of wild-type and S463A mutant CRN2 polypeptides to F-actin leads to a reduced polymerization velocity. All CRN2 polypeptides were able to partially displace the Arp2/3 complex at actin filaments and, moreover, wild-type and S463A mutant, but not the phosphomimetic S463D CRN2 C-terminal polypeptides, were found to interact with free Arp2/3 complex. Thus, the wild-type and S463A mutant CRN2 polypeptides may additionally inhibit actin polymerization in an indirect manner (Fig. 10).

A competition of the Arp2/3 complex binding to F-actin has also been reported for CRN1, which results in a reduced F-actin density in the front of lamellipodia and a disturbed formation and persistence of cell protrusions53. The exact binding site of the Arp2/3 complex on CRN2 is unknown, but our pull-down and CoIP experiments employing CRN2 polypeptides and full-length protein, respectively, suggest that Arp2/3 binds to a motif in the coiled coil domain of CRN2 harboring S463. For mammalian CRN4 and yeast CRN1 (synonym: Crn1p) the Arp2/3 binding site has also been mapped to the coiled coil region3,54,55. CRN2 probably inactivates the Arp2/3 complex in a similar way as it has been described for CRN4, CRN1 and yeast CRN11, where coronin holds the Arp2/3 complex in an inactive open conformation away from the actin filaments55,56,57,58.
Figure 9 | Phosphorylation of serine residue 463 controls protrusion formation and cell migration. (A) Live cell imaging of formation and retraction of cell protrusions. Single 293TN cells expressing wild-type, S463D or S463A mutant CRN2 were monitored. Expression of the S463D mutant led to a distinct reduction (~50%) of the number of cellular protrusions. CRN2 wild-type vs. CRN2 S463D mutant: mean no. of cellular protrusions 6.0 vs. 2.8, standard deviation 2.1 vs. 2.2, 90 measurements each, Student’s t-test \( p < 10^{-11} \). (B) In vitro wound healing assays employing 293TN cells expressing full-length wild-type, S463D or S463A mutant CRN2. Cells expressing the S463D mutant show a small but statistically significant defect in wound closure velocity. CRN2 wild-type vs. CRN2 S463D mutant: mean wound closure velocity 29 μm/h vs. 23 μm/h, standard deviation 8.6 μm/h vs. 7.5 μm/h, 60 measurements for each, Student’s t-test \( p = 0.04 \). (C) GFP-fused CRN2 wild-type, S463D, or S463A was transiently expressed in primary human macrophages. FRAP experiments were performed to determine protein turn-over rates and dissociation constants in F-actin-rich podosome cores (for details see Fig. S7). Note the reduced turn-over rate of the S463D variant (lower \( k_{\text{off}} \) value of 0.11 s\(^{-1}\); standard deviation 0.017 s\(^{-1}\); Mann-Whitney test wild-type vs. S463D mutant \( p = 0.0036 \)) compared to wild-type (0.31 s\(^{-1}\); standard deviation 0.09 s\(^{-1}\)) and S463A mutant CRN2 (0.25 s\(^{-1}\); standard deviation 0.021 s\(^{-1}\)). Each bar represents mean value and standard deviation from 15 measurements from podosomes of at least 3 different cells.
Previous studies indicated that full-length CRN2 possesses binding sites for F-actin in the conserved WD40-repeat domain forming the seven-bladed $\beta$-propeller and in the conserved part of the C-terminal linker region. More specifically, a conserved arginine residue, R30 in CRN1 and R28 in CRN2, which is surface exposed and located within the seventh $\beta$-propeller blade, has turned out to be essential for F-actin binding of both coronin proteins. A belt-shaped actin binding region on the surface of the $\beta$-propeller has been identified in yeast CRN1, which is conserved in all coronin proteins. Moreover, for mammalian CRN4 a specific F-actin binding motif was shown in the C-terminal linker region (aa400-416), which is conserved in all coronin proteins. Subsequent studies have demonstrated that the residues of CRN4 that bind to actin span over the entire molecule and specifically locate to the $\beta$-propeller and the C-terminal linker region. The corroborative data from all these reports indicate that the scattered positions of actin binding sites in coronin proteins map from the N-terminal part to the C-terminal linker and form one large F-actin binding region which can make contacts to several actin molecules. In this scenario one coronin molecule apparently makes contacts to three actin molecules, namely between two actin molecules of one and an additional actin molecule of the second “substrand” of the two-start helix of an actin filament.

Apart from this widespread actin binding region another actin binding site has been detected in the coiled coil domain of CRN4 and yeast CRN1. Our data show that CRN2 also contains an additional actin binding site in the coiled coil domain. The C-terminal CRN2 polypeptides used in our in vitro analyses were proven to fold properly and comprise a part of this large belt-shaped actin binding region as well as the coiled coil domain. Phosphorylation of S463 within the coiled coil inactivated this second actin binding site so that the actin filament crosslinking activity of CRN2 was reduced but the F-actin binding activity retained. Thus, the second actin binding site within the coiled coil domain of CRN2 is a CK2 dependent regulatory element for actin filament crosslinking.

Our data strongly suggest that the coiled coil domain of CRN2 has three major functions, i) formation of a constitutively trimeric CRN2 quaternary structure, ii) binding to actin filaments, and iii) interaction with the Arp2/3 complex. However, only trimerization and F-actin binding, or alternatively trimerization and Arp2/3 interaction of (non-phosphorylated) CRN2, can occur at the same time. A simultaneous binding to F-actin and Arp2/3 apparently is not possible as CRN2 was found to displace the Arp2/3 complex from actin filaments. This agrees with results of the FRAP experiments in this study, which show a 3.5-fold increased half-life time of the phosphomimetic CRN2 S463D mutant in podosome F-actin cores. As these cores are rich in Arp2/3 complex the S463D mutant CRN2 lacking the ability to interact with the Arp2/3 complex exhibited a prolonged interaction with F-actin.

Based on our data we assume that in vivo the cellular fraction of non-phosphorylated CRN2, previously described as cytoskeleton associated pool, inhibits actin polymerization, bundles actin filaments, binds to and inactivates Arp2/3 complexes, and accordingly leads to the formation of ‘stabilized’ F-actin structures (Fig. 10). Phosphorylation of CRN2 by CK2 inhibits these CRN2 functions. This is supported by our observations where the presence of S463D phosphomimetic full-length CRN2 correlated with an accumulation of F-actin structures and an irregular re-distribution of the Arp2/3 complex in the front of lamellipodia, a reduced velocity of cell migration, and a decreased number of cell protrusions.

**Methods**

**Molecular modeling.** The sequence of CRN2 aa315-474 was subjected to multimetric coiled coil prediction by the program MultiCoil, which predicted, in addition to experimental evidence, a trimeric coiled coil for the peptide sequence CRN2 aa438-467 with a probability of 62%. While the three-dimensional crystal structure of the coiled coil domain of CRN4 has been determined, the amino acid sequence alignment of the CRN4 and CRN2 coiled coil domains showed that CRN4 lacks a hexapeptide in this region. We have therefore re-constructed the trimeric coiled coil of hemagglutinin (HA2 chain) using residues 74 to 113 from chain B in PDB entry 1eo8, and used this as a template for comparative modeling. Twenty independent models for CRN2 aa435-474 were generated with MODELLER and the one with the best structure that fit the experimental evidence and the calculated secondary structure was selected for this work.
lowest energy was selected. The overall geometry was scrutinized using PROCHECK.

Molecular cloning and protein expression. A human CRN2 cDNA fragment coding for a C-terminal polypeptide cloned into the pQE30 (Qiagen) vector (aa304-474) and a full-length CRN2 cDNA cloned into pGFP-C1 (Invitrogen) vector (aa1-474) were used as templates. Using the QuickChange Site-Directed Mutagenesis kit (Stratagene) in combination with primer pairs I) CRN2mutS463Dfer 5'-GCCATCTGCTGTCTACTTTGAATACGGTAC-3' and CRN2mutS463Drev 5'-GGCTACGGCTTTTATGTTTACTCATAGGCTC-3', and ii) CRN2mutS463Afer 5'-CATTACAGATTGGATTGCCAAGACGACAGATGC-3' and CRN2mutS463Arev 5'-GCCATCTGCTGTCTACTTTGAATACGGTAC-3', CRN2s were expressed in E. coli BL21 and subsequently used for the purification of CRN2 WT, S463D, and S463A. The CRN2s' expression constructs were used for transfections of cells in which a stable expression of the CRN2 specific shRNA oligo 5'-CCATCTGCTGTCTACTTTGAATACGGTAC-3' is described in 8.

Generation of U373 cells stably expressing the CRN2 specific shRNA oligo 5'-CCATCTGCTGTCTACTTTGAATACGGTAC-3' was confirmed by mass spectrometry.

Expression and purification of CRN2 WT, S463D, S463A, and CRN2 WT, S463D, and S463A. The CRN2s' expression constructs were used for transfections of cells in which a stable expression of the CRN2 specific shRNA oligo 5'-CCATCTGCTGTCTACTTTGAATACGGTAC-3' was described in 8.

Full-length human GST-His6-tagged CRN2 wild-type and S463D mutant proteins were purified from S9 insect cell cultures according to the manufacturer's protocol (bac-to-bac expression system, Invitrogen). In brief, the DH10Bac E. coli strain was transformed with a pDEST10 donor vector containing a GST-His6-tagged CRN2 subclone and subsequent expression from either His6- or GST-tagged recombinant baculovirus. Insect larvae was used to transfect S9 cells and resulting baculoviruses were amplified several times in order to finally infect S9 cells cultivated in spinner flasks (Technne) using SF-900 III SFM medium (Invitrogen).

pGEX-2T based vectors for expression of GST-tagged chicken CK2 and CK2 kinase domain were kindly provided by Drs. Odlo during purification of the phosphorylated CRN2 coiled coil peptide (435–474) was acquired on a SAXSess instrument (Anton Paar, Austria) with a sealed tube microsource (Cu-Kα). Data collection was performed by the program SAFIR from the PCSB collection; theoretical scattering curves and their fit to experimental data were obtained with CRYSOL.

**GST pull-down analyses.** Cell extracts were prepared by lysing HEK293 cells with buffer A (10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 0.5% NP40) and E. coli cells with buffer B (200 mM NaCl, 5% glycine, 100 μg/ml lysozyme, 0.5% NP40, 10 mM DTT, 0.5 mM PMSF, 2 mM Benzanidin, 10 μg/ml aprogin and leupeptin) on ice before homogenization. Cleared soluble extracts were obtained by centrifugation at 100,000×g for 5 min at 4°C. In parallel, GST-C2k fusion proteins purified from bacteria were mixed with 100 nM of equilibrated glutathione sepharose beads (GE Healthcare) and incubated for 2 h at 4°C. After washes with buffer C (4.3 mM NaH2PO4, 1.47 mM KH2PO4, 1.37 mM NaCl, 2.7 mM KCl), aliquots of the beads were incubated together with the recombinant expression vector containing His6-tagged CRN2 C-terminal polypeptides. Further incubation for 2 h at 4°C was carried out to pull-down respective proteins. Finally, the washed beads were washed three times with wash buffer D (4.3 mM NaH2PO4, 1.47 mM KH2PO4, 1.37 mM NaCl, 2.7 mM KCl, 5% glycerol) and proteins were eluted with SDS sample buffer and analyzed by immunoblotting. Control experiments were performed with GST-coated beads or soluble GST protein alone.

**In vitro kinase assays.** In vitro CK2 kinase assays were performed with phosphorylation buffer (50 mM MOPS pH 7.0, 150 mM NaCl, 10 mM MgCl2, ATP and γ-32P-ATP (10 μCi/ml, Amersham)) according to His6-tagged CRN2 C-terminal polypeptides (~4 μg) as well as full-length GST-tagged CRN2 (~0.2 μg) proteins were added to 50 μl of 2x phosphorylation buffer without γ-32P-ATP. The reaction was initiated by addition of CK2 (~2.0 μg) with the final addition of 5 μl ATP mix (6 μl 2 mM ATP, 2.4 μl 10 μCi/ml γ-32P-ATP, 51.6 nM H2O2). After adjusting the reaction volume to 100 μl, the reaction mixtures were incubated for 1, 5, 10, 30, 60, 90, and 120 min at 30°C. The reactions were terminated by addition of 20 μl 0.1 M EDTA, 30 μl 5× SDS sample buffer, and boiling for 10 min at 95°C. All samples were analyzed by SDS-PAGE followed by Coomassie brilliant blue staining and autoradiography.

**In vitro de-phosphorylation assays and 2D gel electrophoresis.** Alkaline phosphatase is able to hydrolyze phosphate esters of primary and secondary alcohols, amines, and phenols, including serine, threonine and tyrosine residues in proteins. For in vitro de-phosphorylation 20 μg of murine skeletal muscle tissue was powdered on dry ice, de-phosphorylation dissolved in de-phosphorylation buffer (100 mM Tris/HCl pH 7.9, 100 mM NaCl, 10 mM MgCl2, 1 mM DTT, 1: 200 protease inhibitor (Sigmar)), and centrifuged for 10 min at 16,000×g. 5 μl (5 U) alkaline phosphatase (Roche) were added to the supernatant and incubated for 30 min at 30°C. Positively controls additionally contained p-nitrophenylphosphate (pNPP), negative controls lacked the phosphatase. Samples were subjected to two-dimensional gel electrophoresis in conjunction with immunoblotting according to 9. CRN2 protein spots were visualized with enhanced chemiluminescence followed by exposure to x-ray films (Kodak).
with wash buffer 2 (50 mM Tris/HCl pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% Na-deoxycholate, 0.1% SDS), and finally once with buffer 3 (20 mM Tris/HCl pH 7.5). The lysis buffer contained phosphatase (Sigma) and protease (Roche) inhibitor cocktails in double concentration. Immunoprecipitated GFP-CRN2 was eluted with 70 μl elution buffer (50 mM Tris/HCl pH 6.8, 50 mM DTT, 1% SDS, 1 mM EDTA, 10% glycerol, 0.005% bromphenol blue), subjected to SDS-PAGE, Coomassie brilliant blue staining, and autoradiography. A duplicate set of three plates was treated in parallel in order to harvest cells for immunoblotting. The duplicates were not incubated with “P-orthophosphate but with medium containing phosphate at the appropriate steps of the experiment.

**Actin polymerization assays.** Actin polymerization assays were performed using the Actin polymerization Biochem kit (RK030, Cytoskeleton) in which the rate of prenylated G-actin conversion into F-actin was monitored. Pyrene fluorescence signals were monitored in black flat bottom 96 well plates (Nunc) using an Infinite M1000 device (Tecan) equipped with Tecan i-control (version 1.6.19.2) with the following settings: excitation 350 nm with 20 nm bandwidth, emission 407 nm with 20 nm bandwidth, gain 80, 20 μs integration time, 200 ms settle time, 20198 μm Z-position, 30 s measurement interval, polymerization start by dispersion of 13 μl 10x polymerization buffer (500 mM KCl, 20 mM MgCl₂, 10 mM ATP), final volume per well 125 μl. Pyrene labeled rhodamine skeletal muscle G-actin, bovine brain Arp2/3 complex (RP01, Cytoskeleton), recombinant human GST-tagged VCA-domain of WASP (VCG03, Cytoskeleton), and His6-tagged CRN2 C-terminal polypeptides were prepared in G-buffer (5 mM Tris/HCl pH 8.0, 0.2 mM CaCl₂). Final concentrations were: G-actin 1.5 μM (Fig. 6A,B,E) or 2 μM (Fig. 6C,D), Arp2/3 complex (1.75 μM) (Fig. 6A,B,E). The reactions were equilibrated to 25 °C, fluorescence top reading, excitation 350 nm with 20 nm bandwidth, emission 407 nm with 20 nm bandwidth, gain 80, 20 ms integration time, 200 μs settle time, 20198 μm Z-position, volume per well 125 μl. Pyrene labeled rabbit skeletal muscle G-actin, His6-tagged CRN2 C-terminal polypeptides, His6-tagged GFP-CRN2, and supernatants were resolved by SDS-PAGE and analyzed by immunoblotting. In order to verify the amounts of the CRN2 polypeptides added to the reactions, samples from all wells were taken at the end of each experiment, separated by SDS-PAGE, and proteins were stained by Coomassie brilliant blue. Immunofluorescence analyses, immunoblotting, and antibodies. Direct and indirect immunofluorescence analyses as well as immunoblotting were performed as described in 87. Immunofluorescence images were captured on a Leica TCS SP5/AOBS/tandem scanning system equipped with the Leica LAS-AS software (version 2.2.1 build 4842) and further processed using CorelDraw Graphics Suite X4. Visualization of immunoblots was done by enhanced chemiluminescence in conjunction with the imaging system Fluorchem SP (Alpha Innotech) or exposure to x-ray films (Kodak). Antibodies used in this study specifically recognized CRN2 (mAb K6-444-4‘), Arp2/3 complex (p34-Arc subunit, rabbit polyclonal Ab, Upstate), GST (rabbit polyclonal antibody 506), CK2x (mAb IAD9, KinaseDetect), and cdc37 phosphorylated at serine 13 (28, note that the rabbit polyclonal Ab ab61797 (Abcam) also recognizes non-phosphorylated cdc37); F-actin was labelled with 200 ng/ml TRITC-phallolin (Sigma).

**Fluorescence recovery after photobleaching (FRAP) experiments.** Transfected primary human macrophages grown in glass-bottom culture dishes were placed in a heating insert P covered with a small incubator S 2 for warm air incubation and CO₂- atmosphere of the tissue culture chamber. Bovine cells were kept at 37 °C in a humidified atmosphere with 5% CO₂ and observed using an oil immersion HCX PL APO 63x/1.4-0.6 lambda blue objective lens. Subcellular localization of GFP-tagged proteins was monitored using the 488 nm laser line of an argon ion laser. Photobleaching experiments were performed as follows: before the bleaching event 10–15 frames were acquired, then, by illumination of a selected region of interest, 100% laser light illumination was applied. In several fields of view the total number of cells showing a clear co-localization of GFP-CRN2 constructs with mRFP-Lifeact at podosomes. Graphs were generated using Excel 2004 for Mac (Microsoft) and Prism 5.0c for Mac (GraphPad).

1. Morgan, R. O. & Fernandez, M. P. in The Coronin Family of Proteins Vol. 84 Subcellular Biochemistry (eds C. S. Clemen, L. Eichinger, & V. Rybak) Landes Bioscience & Springer, 2008. http://www.landesbioscience.com/cuiri/chapter/3620
2. Rybak, V. & Clemen, C. S. in Actin and actin binding proteins as multifunctional regulators of the cytoskeleton and membrane trafficking. Bioessays 27, 625–632 (2005).
3. McArdle, B. & Hofmann, A. in The Coronin Family of Proteins Vol. 84 Subcellular Biochemistry (eds C. S. Clemen, L. Eichinger, & V. Rybak) Landes Bioscience & Springer, 2008. http://www.landesbioscience.com/cuiri/chapter/3821
4. Spierling, A., Stumpf, M., Novak, A., Ochs, T., and Erler, J. Actin interaction, and membrane association of the ubiquitous mammalian coronin 3 isoforms in muscle. J Mol Biol 393, 287–299 (2009).
5. Rosentret, A. et al. Coronin 3 involvement in F-actin-dependent processes at the cell cortex. Exp Cell Res 315, 889–895 (2009).
6. Haase, A. et al. Coronin 3 and its role in murine brain morphogenesis. Eur J Neurosci 21, 1155–1168 (2005).
7. Thal, D. et al. Expression of coronin-3 (coronin-1C) in diffuse gliomas is related to malignancy. J Pathol 214, 415–424 (2008).
17. Allende, J. E. & Allende, C. C. Protein kinases. 4. Protein kinase CK2: an enzyme

22. Salvi, M., Sarno, S., Cesaro, L., Nakamura, H. & Pinna, L. A. Extraordinary

21. Pinna, L. A. The raison d’etre of constitutively active protein kinases: the lesson of

20. Pinna, L. A. A historical view of protein kinase CK2.

19. Guerra, B. & Issinger, O. G. Protein kinase CK2 in human diseases.

18. Canton, D. A. & Litchfield, D. W. The shape of things to come: an emerging role

et al

15. Kimura, T.

14. Kimura, T., Taniguchi, S. & Niki, I. Actin assembly controlled by GDP-Rab27a is

13. Xavier, C.-P., Eichinger, L., Fernandez, M. P., Morgan, R. O. & Clemen, C. S. in

12. Samarin, S. N., Koch, S., Ivanov, A. I., Parkos, C. A. & Nusrat, A. Coronin 1C

The Coronin Family of Proteins

11. Luan, S. L.

et al

29. Niefind, K., Guerra, B., Ermakowa, I. & Issinger, O. G. Crystal structure of human

31. Mazin, P. V.

30. Chester, N., Yu, I. J. & Marshak, D. R. Identification and characterization of

39. Svergun, D., Barberato, C. & Koch, M. CRYSOL - a program to evaluate X-ray

et al

38. Pettersen, E. F.

36. Fleury, D., Daniels, R. S., Skehel, J. J., Knossow, M. & Bizebard, T. Structural

35. Choudhary, C.

et al

34. Rush, J.

et al

32. Reikhardt, B. A., Kulikova, O. G., Borisova, G. Y., Aleksandrova, I. Y. & Sapronov,

29. Svergun, D. I. & Egelhofer, A. Protein kinase CK2: a dynamic player in cell biology.

28. Honig, B. & Kim, P. S. A general method for protein structure comparison.

27. Svergun, D. I. & Zhang, D. Biophysical Methods of Structural Biology.

26. Filhol, O. & Cochet, C. Protein kinase CK2 in health and disease: Cellular

25. Harada, J. K. et al. Regulation of protein phosphatase 2A by direct interaction with

24. Quadsorff, M. et al. A concerted action of HNF4alpha and HNF4alpha links hepatitis B virus replication to hepatocyte differentiation. Cell Microbiol 10, 1478–1490 (2008).

23. Van den Abbeele, P. et al. Structure and function of the human SH3 domain.

22. Kudlicki, W. et al. Integrative genomics to identify candidate susceptibility genes for rheumatoid arthritis.

21. Brahante, D. S., Choudhary, C., Germain, J. D. & Blomberg, J. A. Phosphorylation

20. Brautigam, C. A., Khan, R., McRorie, C. M., Pande, V. S. & Teichmann, S. A. How

19. Cobbold, S. R. & Shepherd, J. J. Diversity in the alphavirus envelope protein.

18. Deng, D. Y., Cui, J. H., Zhang, W. Z., Zhang, J. et al. Small interfering siRNA targeting the AKAP-LIM domain of the human PRKCD isoform.

17. Song, D. F., Li, J., Wang, H., Jia, B. et al. The plasma membrane protein

16. Lassleben, M. et al. ERG protein is a novel transcriptional activator of the human

15. Reikhardt, B. A., Kulikova, O. G., Borisova, G. Y., Aleksandrova, I. Y. & Sapronov,

14. Richter, B. A., Kulikova, O. G., Borisova, G. Y., Aleksandrova, I. Y. & Sapronov,

13. Xiao, L. et al. A novel protein phosphatase 2A anchoring complex.

12. D’Addio, A. et al. A novel protein phosphatase 2A anchoring complex.

11. Xu, Y. et al. A novel protein phosphatase 2A anchoring complex.

10. Cristofari, G. et al. A novel protein phosphatase 2A anchoring complex.

9. Cheetham, M. M. et al. A novel protein phosphatase 2A anchoring complex.

8. Rouiller, A. et al. A novel protein phosphatase 2A anchoring complex.

7. Schlesinger, P. J. et al. A novel protein phosphatase 2A anchoring complex.

6. Liu, Y. et al. A novel protein phosphatase 2A anchoring complex.

5. Riedel, H. et al. A novel protein phosphatase 2A anchoring complex.

4. Weiszmann, R. et al. A novel protein phosphatase 2A anchoring complex.

3. Wang, J. et al. A novel protein phosphatase 2A anchoring complex.

2. Wang, Y. et al. A novel protein phosphatase 2A anchoring complex.

1. Wang, X. et al. A novel protein phosphatase 2A anchoring complex.
73. Kopp, P. et al. The kinesin KIF1C and microtubule plus ends regulate podosome dynamics in macrophages. *Mol Biol Cell* 17, 2811–2823 (2006).

74. Konarev, P. V., Volkov, V. V., Sokolova, A. V., Koch, M. H. J. & Svergun, D. I. PRIMUS: a Windows PC-based system for small-angle scattering data analysis. *Journal of Applied Crystallography* 36, 1277–1282 (2003).

75. Semenyuk, A. & Svergun, D. GNOM: a program package for small-angle scattering data processing. *Journal of Applied Crystallography* 24, 537–540 (1991).

76. Volkov, V. & Svergun, D. Uniqueness of ab initio shape determination in small-angle scattering. *Journal of Applied Crystallography* 36, 860–864 (2003).

77. Hofmann, A. & Wlodawer, A. PCSB--a program collection for structural biology and biophysical chemistry. *Bioinformatics* 18, 209–210 (2002).

78. Skjerpen, C. S., Nilsen, T., Wesche, J. & Olsnes, S. Binding of FGF-1 variants to protein kinase CK2 correlates with mitogenicity. *EMBO J* 21, 4058–4069 (2002).

79. Clemen, C. S. et al. Hsp27-2D-gel electrophoresis is a diagnostic tool to differentiate primary desminopathies from myofibrillar myopathies. *FEBS Lett* 579, 3777–3782 (2005).

80. Duncan, J. S. et al. An unbiased evaluation of CK2 inhibitors by chemoproteomics: characterization of inhibitor effects on CK2 and identification of novel inhibitor targets. *Mol Cell Proteomics* 7, 1077–1088 (2008).

81. Pagano, M. A. et al. Optimization of protein kinase CK2 inhibitors derived from 4,5,6,7-tetramethylbenzimidazole. *J Med Chem* 47, 6239–6247 (2004).

82. Ruzzene, M., Penzo, D. & Pinna, L. A. Protein kinase CK2 inhibitor 4,5,6,7-tetramethylbenzotriazole (TBB) induces apoptosis and caspase-dependent degradation of hematopoietic lineage cell-specific protein 1 (HS1) in Jurkat cells. *Biochem J* 364, 41–47 (2002).

83. Jung, E., Fucini, P., Stewart, M., Noegel, A. A. & Schleicher, M. Linking microfilaments to intracellular membranes: the actin-binding and vesicle-associated protein comitin exhibits a mannose-specific lectin activity. *EMBO J* 15, 1238–1246 (1996).

84. Morita, T., Mayanagi, T., Yosio, T. & Sobue, K. Changes in the balance between caldesmon regulated by p21-activated kinases and the Arp2/3 complex govern podosome formation. *J Biol Chem* 282, 8454–8463 (2007).

85. Clemen, C. S. et al. Strumpellin is a novel valosin-containing protein binding partner linking hereditary spastic paraplegia to protein aggregation diseases. *Brain* (2010).

86. Xiong, H. et al. Dictyostelium Sun-1 connects the centrosome to chromatin and ensures genome stability. *Traffic* 9, 708–724 (2008).

87. Himmel, M. et al. Control of high affinity interactions in the talin C terminus: how talin domains coordinate protein dynamics in cell adhesions. *J Biol Chem* 284, 13832–13842 (2009).

88. Chew, C. S. et al. Lasp-1 binds to non-muscle F-actin in vitro and is localized within multiple sites of dynamic actin assembly in vivo. *J Cell Sci* 115, 4787–4799 (2002).

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**Author contributions**

CPX, BHR, MB, MS, MH, ROM, MPF, CW, AO, AH, SL, and CSC designed, performed, and analyzed experimental work. YM and RAG provided new methods and essential chemicals. LE, AAN and CSC designed experiments and evaluated the overall data. CSC wrote the main manuscript text and prepared the figures. All authors reviewed the manuscript.

**Additional information**

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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