Supporting Information

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SI Methods

Expression and Purification of AKAP79 Complexes. Human cAMP-dependent protein kinase (PKA) RIIα(1–45), mouse CaM and a bicistron containing human Ca^{2+}/calmodulin (CaM)-dependent protein phosphatase (PP2B) A-B(5–170), adapted from (1), were each cloned into pGEX6P1 for expression in *Escherichia coli* BL21 cells by induction with 0.5 mM IPTG after the bacteria had reached $OD_{600\,nm}$ of 0.5. Cells were harvested after overnight growth at 18 °C. A-kinase anchoring protein 79 (AKAP79) was expressed in *Sf21* insect cells using the Bac-to-Bac Baculovirus System (Invitrogen). Insect cells were harvested after 72 h in medium containing 10% fetal bovine serum.

The protocols for purification of both the AKAP79–D/D and AK_DPC (AKAP79, $PKA_{D/D}$, PP2B, CaM) complexes are initially identical. In the absence of prior association to RII D/D, AKAP79 accumulated into soluble aggregates, as evidenced by gel filtration. Therefore, in both cases, affinity of AKAP79 to GST–D/D was performed as the first purification step. The pellet from 1.5 L expression of D/D was lysed in 100 mL lysis buffer A [25 mM Tris pH 7.5, 0.5 M NaCl, 2 mM DTT, 0.5 mM EDTA, one EDTA-free protease inhibitor tablet (Roche), 5 mg DNase 1, 10 mg lysozyme]; the lysate was cleared by centrifugation following sonication (4 × 8 s) before incubation with 5 mL glutathione sepharose (GE Healthcare) for 1 h. The beads were washed with 3 × 10 mL wash buffer (25 mM Tris pH 7.5, 0.5 M NaCl, 2 mM DTT, 0.5 mM EDTA), prior to immediate addition of lysate containing AKAP79. AKAP79 lysate was prepared by homogenization of frozen pellet from 6 L culture in 200 mL (25 mM Tris pH 7.5, 0.3 M NaCl, 2 mM DTT, 0.5 mM EDTA, one EDTA-free protease inhibitor tablet) prior to centrifugation. After 1 h, the beads were washed with 3 × 10 mL low-salt wash buffer (as before with 300 mM NaCl). For AK_DPC reconstitution only, lysate from 12 L culture of PP2B was added. PP2B lysate was prepared as for D/D using 200 mL modified lysis buffer A (containing 0.3 not 0.5 M NaCl). After 1 h incubation, the beads were washed with 3 × 10 mL low-salt wash buffer. In both cases, the beads were incubated with 1 mg PreScission protease in 6 mL low-salt wash buffer overnight.

In both cases, the eluate was collected in 16 mL low-salt wash buffer and desalted into nickel buffer A (25 mM Tris pH 8, 0.3 M NaCl, 15 mM imidazole) prior to 1 h incubation with 5 mL nickel-NTA beads (Qiagen). The beads were washed with 3 × 8 mL nickel buffer A, and AKAP79 and associated proteins were eluted with 25 mM Tris pH 7.5, 0.3 M NaCl, 300 mM imidazole. Prior to concentration for AK_DPC only, an excess of pure CaM (approximately 4 mg) was added to the eluate, and the buffer was supplemented with 0.2 mM $CaCl_2$. CaM was purified in advance from 6 L culture by glutathione affinity as for D/D, followed by overnight PreScission cleavage and gel filtration in 25 mM Tris pH 7.5, 0.5 M NaCl, 2 mM DTT, 0.5 mM EDTA. Finally, the eluates were concentrated to 2 mL and applied to a Superdex S200 gel filtration column with a 5 mL GSTtrap (both GE Healthcare). Gel filtration of AKAP79–D/D was performed in 20 mM Na Hepes pH 7.5, 200 mM NaCl—for AK_DPC, the buffer was supplemented with 0.2 mM $CaCl_2$. The peak fractions of AK_DPC (64–68 mL) were pooled and applied once more to the gel filtration column to reduce background contributed by aggregated protein in the void volume and excess PP2B (peak elution volume approximately 75 mL). All steps were performed at 4 °C.

Chemical Cross-Linking. N-terminal Strep(II)-tagged human AKAP79 was expressed in *Hi5* insect cells using the Bac-to-Bac Baculovirus System (Invitrogen). After baculoviral infection, *Hi5* cells were grown at 26 °C for approximately 72 h, then lysed by sonication in 50 mM Tris, pH 8.0, 500 mM NaCl, 2 mM DTT, 1 mM EDTA, 1 mM benzamidine. Strep–AKAP79 was purified by affinity to streptactin resin (GE). Following elution with 2.5 mM desthiobiotin, the protein was buffer exchanged into 20 mM HEPES, 250 mM NaCl (pH 7.5) prior to cross-linking. A 100 mM stock of disuccinimidyl suberate (DSS) (Pierce Biotechnology Inc.) was prepared in anhydrous dimethylsulfoxide. The DSS stock was serially diluted and added to 0.5 mg/mL purified Strep–AKAP79 with a highest final concentration of 1 mM DSS and allowed to react at room temperature for 30 min. Disulfide bonds were reduced through addition of tris(2-carboxyethyl)phosphine (Pierce Biotechnology Inc.) to achieve a 5 mM final concentration and incubated at 56 °C for 1 h. Cysteine residues were blocked by adding iodoacetamide (Sigma) to a final concentration of 10 mM and incubated at 37 °C for 30 min. Sequencing grade trypsin (Promega) was added at 1:250 (wt/wt) and allowed to react overnight. The digestion reaction was quenched by acidification using 0.1% TFA (Sigma-Aldrich). The sample was desalted using C18 Sepak (Waters Corporation). The sample was dried using a speed-vacuum device (Thermo Scientific). Strong-cation exchange (SCX) separation of the peptides was performed using SCX spin columns (Nest Group). Mobile phase A was composed of 25% acetonitrile, 0.1% formic acid. Mobile phase B consisted of 1 M ammonium acetate (Sigma), 25% acetonitrile, and 0.1% formic acid. Peptides were loaded in 100% mobile phase A and washed with mobile phase A. Eluted fractions include 3, 5, and 100% mobile phase B.

Liquid Chromatography and Mass Spectrometry of Cross-Linked AKAP79. Liquid chromatography was performed using a Waters NanoAcquity UPLC (Waters Corporation). Pulled tip columns were constructed in-house using a laser-pulling device (Sutter). A column of 30 cm in length was constructed with 75 μm ID× 360 μm OD fused silica capillary. The packing material used for peptide separation was 100-Å C18 magic beads (Microm Bioresources). A fused silica trap column was constructed from 100 μm ID × 360 μm OD fused silica capillary. The frit was made on one end of the trap with Kasil (PQ Corporation) to contain C18 packing material. The packing material used in the trap was 200-Å C18 magic beads (Microm Bioresources). A binary solvent gradient was used for peptide separation. Mobile phase A consisted of 99.9% water with 0.1% formic acid. Mobile phase B consisted of 95% acetonitrile with 0.1% formic acid and 4.9% water. The gradient was set up as follows: 5–60% B in 120 min. Column washing was done with 80% B for 20 min, followed by reequilibration for 20 min using 5% B.

MS was performed using a dual linear ion trap Fourier transform ion cyclotron resonance mass spectrometer, the Velos-FT, which is similar in performance to the Velos-Orbitrap (Thermo Scientific). A precursor scan was acquired at 50,000 resolving power followed by 20 data-dependent tandem MS (MSMS) scans. Dynamic exclusion parameters were two repeat counts followed by 15-s repeat duration. An exclusion list size of 500 species was used with exclusion of 30 s. Charge state screening was applied to exclude unassigned species, 1+, and 2+ ions. Raw data was converted using trans-proteomic pipeline tools (2) to Mascot generic format.

Database Searching, Cross-Linked Peptide Identification, and Accurate Mass Conformation. Data was searched using xQuest. A mass tolerance of 10 ppm was used for precursor m/z. The database

searched included only the AKAP79 amino acid sequence in FASTA format. For MSMS data, a tolerance of 0.1 Da was used. All hits were filtered using accurate mass and peptide MSMS data. Homodimeric peptides are the only unambiguous cross-linked peptides useful for demonstration of protein–protein proximity in the absence of other structural information about the AKAP79 such as a crystal structure. Therefore, data was filtered for homodimeric peptide cross-links yielding three candidates. Manual inspection of accurate mass, charge state, and fragmentation patterns allowed one site to be eliminated. In samples of low complexity, accurate mass has been shown to allow unambiguous elemental composition, determination, and simplified identification (3, 4). Here, we show that when AKAP79 was digested in silico with up to two allowed missed cleavages, and all peptide cross-linked combinations were considered within 10 ppm. The only cross-linked peptide candidates possible in the sample based on accurate mass measurements (Table S2) coincided with identifications obtained using the xQuest algorithm (Fig. S4). The xQuest search results reported are within acceptable limits for positive cross-linked peptide identification (5).

Protein Coimmunoprecipitation in HEK293 Cells. Two pcDNA3.1 vectors were constructed enabling transient expression of AKAP79, with either a FLAG or V5 tag at its N terminus, in HEK293 cells. The cells were harvested 2 d after transfection in 50 mM Tris pH 7.5, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM benzamidine, 2 μg/mL LeuPeptin, and FLAG–AKAP79 was imunoprecipitated using 4 μg mouse anti-FLAG antibody (Sigma). Immunprecipitation with 4 μg mouse IgG was also attempted as a control. FLAG–AKAP79 was detected using αFLAG–HRP conjugate (Sigma); V5–AKAP79 was detected using αV5–HRP conjugate (Invitrogen).

Pull-Down Assays. pGEX vectors were constructed for bacterial expression of GST fused to either AKAP79WT, AKAP79Δ337–343, AKAP79 1–153 ("N"), AKAP79 154–296 ("M") or AKAP79 297–427 ("C"). The fusion proteins were expressed in *E. coli* BL21 cells by induction with 0.5 mM IPTG after the bacteria had reached $OD_{600\,nm}$ of 0.5. After 3 h, 1.8 L bacterial culture was pelleted for each GST fusion protein and lysed in 100 mL lysis buffer A. The lysates were sufficient to saturate 300 μL glutathione sepharose beads. A FLAG tag was inserted at the C terminus of the A subunit of PP2B by site-directed mutagenesis in the bicistronic pGEX6P1 vector described earlier, and the tagged protein was purified as before.

In each pull-down experiment, 40 μL of glutathione sepharose beads saturated with GST–AKAP79 fusion protein were used to pull-down 2 μg PP2B–FLAG in 200 μL radioimmunoprecipitation assay (RIPA) buffer. When testing the effect of Ca^{2+}/CaM, 3 mM $CaCl_2$ and 10 μg purified CaM was included. Following 2 h of incubation, the beads were washed in 5 × 1 mL RIPA buffer. RIPA buffer was supplemented with 3 mM $CaCl_2$ when testing the effect of Ca^{2+}/CaM on pull-down. Beads were resuspended in SDS-PAGE loading buffer and pull-down of PP2B–FLAG was assessed by anti-FLAG western blot. For EDTA-mediated release experiments, 50 μL of RIPA buffer supplemented with 3 mM EDTA and 3 mM EGTA was added, and after 30 min of incubation, the supernatant fraction was separated from the sepharose resin by centrifugation in a spin column.

Pull-Down Protein Phosphatase Assays. GST–PP2B was expressed from pGEX6P1 as for the AK_DPC complex. Following enrichment by affinity to glutathione sepharose, PP2B was released by incubation with PreScission protease, further purified by affinity to calmodulin sepharose, and finally purified using a Superdex S200 gel filtration column (pure untagged PP2B is shown in Fig. S5*B*). Pull-down of 2 μg purified PP2B was attempted with 40 μL of glutathione sepharose beads saturated with either GST, GST–AKAP79FL, or GST–AKAP79FLΔ337–343 in 250 μL RIPA buffer supplemented with 3 mM $CaCl_2$ and 25 μg purified CaM. Following 2 h of incubation, pull-downs were washed in 4 × 1 mL RIPA supplemented with 3 mM $CaCl_2$. In one case, CaM was incubated with beads bearing GST–AKAP79FL for 2 h, and CaM not associated with AKAP79 was removed by washing with 4 × 1 mL RIPA (+3 mM $CaCl_2$) prior to the PP2B incubation step (Fig. 4*D*, lane 4). The beads were subsequently washed for a second time in the normal way with 4 × 1 mL RIPA (+3 mM $CaCl_2$) to remove unbound PP2B. In another case, a single wash step with 1 mL RIPA buffer supplemented with 3 mM EDTA and 3 mM EGTA was included following the final 4 × 1 mL washes in RIPA +3 mM $CaCl_2$ (Fig. 4*D*, lane 4). In every case, the beads were subject to a final wash in 50 mM imidazole pH 7.2, 50 mM $MgCl_2$, 5 mM $NiCl_2$, 1 mM $CaCl_2$ prior to phosphatase assay.

Phosphatase activity was measured using the Serine/Threonine Phosphatase Assay System (Promega). Phosphate release was quantified following 30 min incubation of pull-downs with 100 μM RRA(pT)VA phosphopeptide in 50 mM imidazole pH 7.2, 50 mM $MgCl_2$, 5 mM $NiCl_2$, 1 mM $CaCl_2$.

Electrospray MS. Prior to MS analysis, AKAP79 complexes were dialyzed into 50 mM ammonium acetate pH 7.5 ± 0.1 M $CaCl_2$. Mass spectra were obtained on a Q-Tof 2 or a Q-Tof 2B (Waters/Micromass), modified for high-mass operation (6), using a previously described protocol to preserve noncovalent interactions (7). In brief, volatile aqueous buffered solutions were used with nanoelectrospray to ionize the complex and effect its transfer into the gas phase intact. Additionally, careful optimization of instrument conditions, particularly the pressure and accelerating voltages, throughout the mass spectrometer was applied. Specifically, to avoid defocussing of ions during their traversal through the vacuum, higher than standard pressures were applied to bring about collisional focusing. The following experimental parameters were used: capillary voltage 1.5 kV, sample cone 80–200 V, extractor cone 5 V, cone gas 100 L/h, ion transfer stage pressure 5×10^{-3}–1.5×10^{-2} mbar, quadrupole analyzer pressure 1.0×10^{-5} mbar, and TOF analyzer pressure 8.1×10^{-6} mbar. The accelerating voltage into the collision cell was as low as 5 V for nonactivated MS spectra and was increased to 150 V for MSMS experiments, with the collision cell pressurized to 35 μbar (argon). Spectra were externally calibrated using a 33 mg/mL aqueous solution of cesium iodide (Sigma). Data were acquired and processed with MassLynx software (Waters) and are shown with minimal smoothing. For denaturing spectra, 50% acenonitrile (vol/vol) and 0.1% formic acid (vol/vol) were added to the purified samples

Structural Representation. Cartoon representations were generated using PyMol (DeLano Scientific), and the electrostatic surface of PP2B was rendered using CCP4MG (8).

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3. Smith RD, et al. (2002) An accurate mass tag strategy for quantitative and high-throughput proteome measurements. *Proteomics* 2:513–523.
4. Spengler B (2004) De novo sequencing, peptide composition analysis, and composition-based sequencing: a new strategy employing accurate mass determination by fourier transform ion cyclotron resonance mass spectrometry. *J Am Soc Mass Spectrom* 15:703–714.
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9. Cole C, Barber JD, Barton GJ (2008) The Jpred 3 secondary structure prediction server. *Nucleic Acids Res* 36:W197–W201.

A

AKAP79-binding molecule	Proposed basis of AKAP79 interaction	Binding site within AKAP79
PKA	Regulatory subunit D/D domain	Amphipathic helix (387-406)
PP2B	Strand β14 of PP2B A subunit	337PIAIIIT motif and surrounding region (315-360)
PKC	Kinase domain substrate-binding site	PKC pseudosubstrate sequence (31-52)
CaM	Similar to MARCKS	IQ motif (31-52)
PIP_2	Inositol 4,5-bisphosphates	Membrane-binding basic regions (MBBRs) (52-153)

B

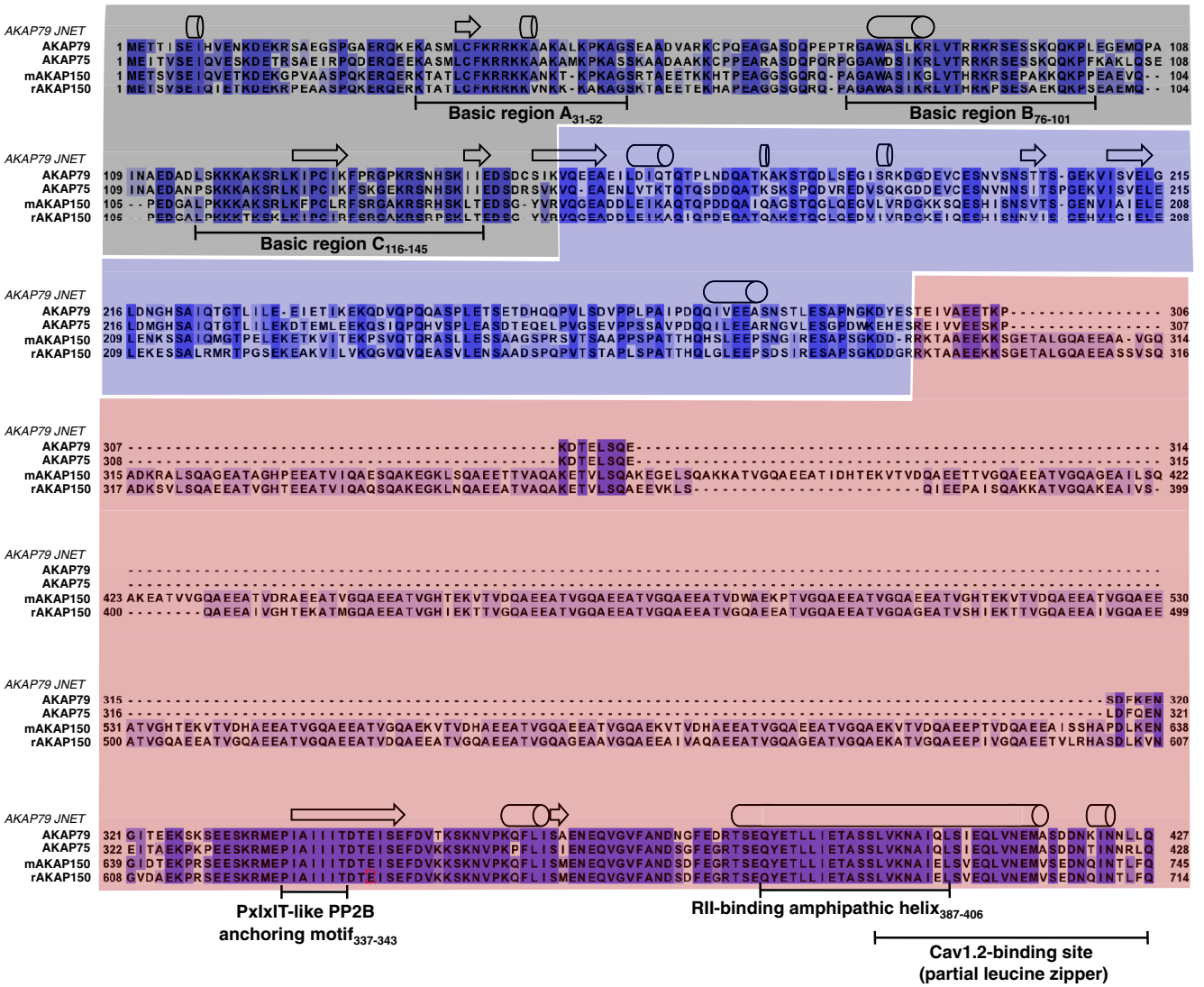

Fig. S1. AKAP79 topology and annotated sequence alignment. (*A*) AKAP79 topology with color-matched table of binding partners. (*B*) Multiple sequence alignment of the human, bovine, mouse, and rat homologs of AKAP79 is shown. Above the alignment is a secondary structure prediction for AKAP79 generated by JPRED (9) [arrows indicate regions of β-sheet secondary structure; cylinders indicate regions of helical secondary structure]. Previously mapped interaction sites are indicated below the alignment. The three constructs used in this study, which were divided on the basis of previously mapped interaction modules and sequence conservation, are designated by gray (N, 1–153), blue (M, 154–296), and red (C, 297–427) boxes.

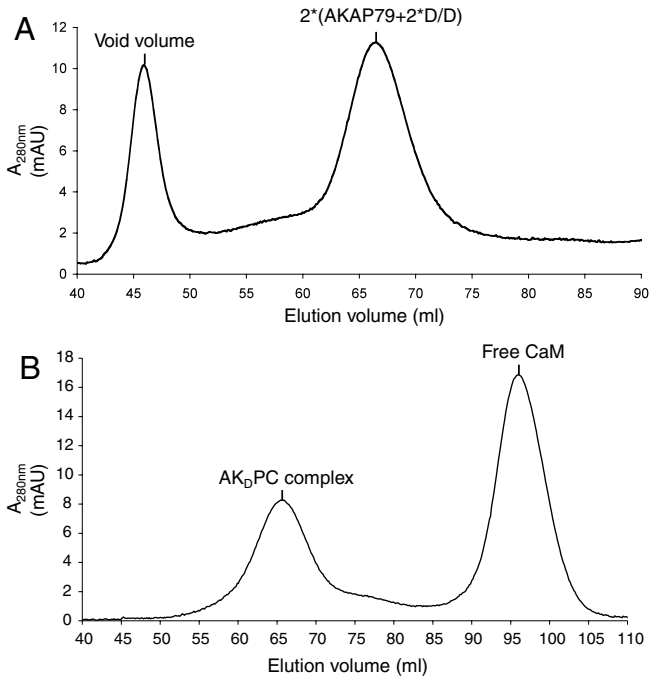

Fig. S2. Gel filtration of AKAP79 complexes. Elution profile absorbances at 280 nm for AKAP79 complex purifications using a Superdex S200 gel filtration column are shown. (*A*) AKAP79–D/D complex elution. The peak at 45.7 mL corresponds to protein aggregates eluting in the void volume; the peak at 66.6 mL is the AKAP79–D/D complex (*B*) AK_DPC complex elution. The second peak at 96 mL corresponds to excess CaM.

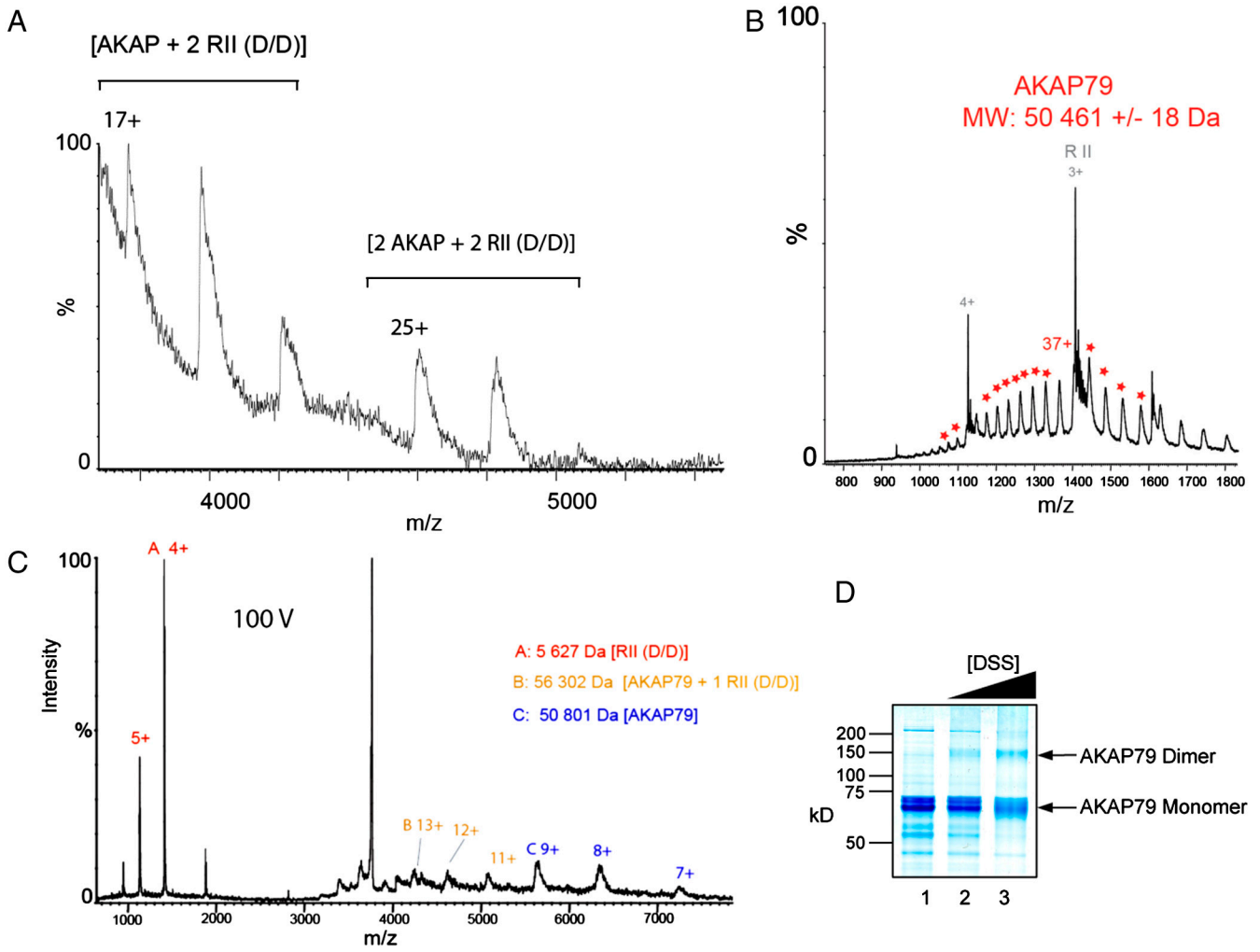

Fig. S3. MS and MSMS spectra of AKAP79–D/D complexes. (*A*) The MS spectrum of the AKAP79–D/D complex at very low activation energies, which minimize dissociation, is shown. Peak series for the [AKAP79 + 2 * D/D] and [2 * AKAP79 + 2 * D/D] complexes used for MSMS are indicated. (*B*) MS spectrum of the AKAP79–D/D complex under denaturing conditions. This approach enabled a highly accurate mass to be calculated for AKAP79. (*C*) MSMS spectrum of AKAP79–D/D where ions at 3,640 *m/z* were selected and activated by collision-induced dissociation. Activation resulted in the dissociation of a D/D subunit with charge states from 3+ to 6+ and the formation of the corresponding stripped complex of 56,302 Da [1 * AKAP79 + 1 * D/D] (gold). Upon further activation, this complex expelled a second D/D subunit to form a stripped complex of 50,801 Da [monomeric AKAP79] (blue). Thus, the original precursor ion can be assigned to the 17+ charge state of a complex composed of [1 * AKAP79 + 2 * D/D], one AKAP79 molecule binding to two D/D subunits. (*D*) Strep–AKAP79 dimer cross-linking with 0 (lane 1), 50 (lane 2), and 500 (lane 3) μM DSS, as shown by Coomassie staining.

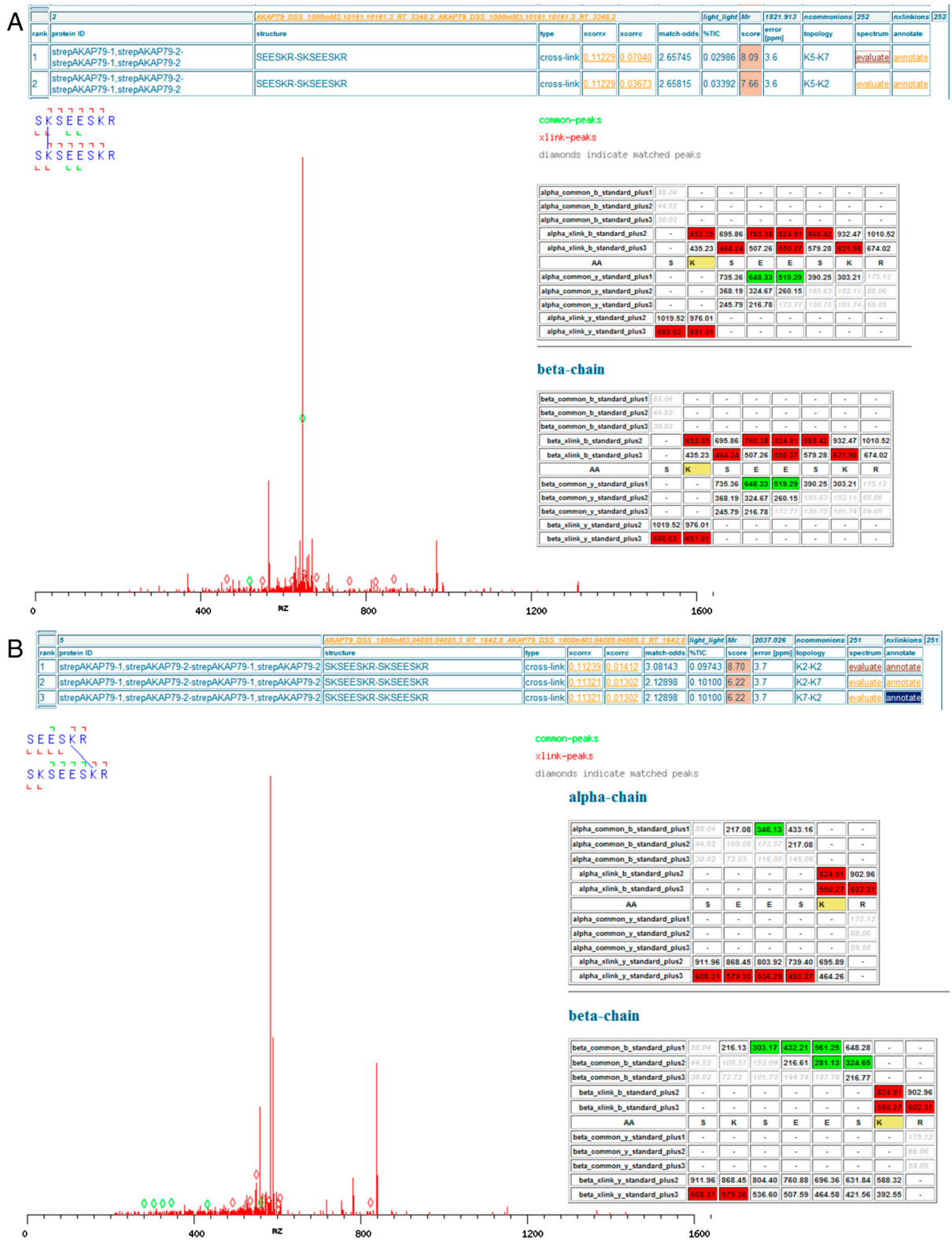

Fig. S4. Identification of dimeric AKAP79 cross-linking sites with xQuest. The xQuest scoring data for peptides containing (*A*) K328–K328 cross-links and (*B*) K333–K333 cross-links are shown. Reported scores ≥8 are generally considered to be significant.

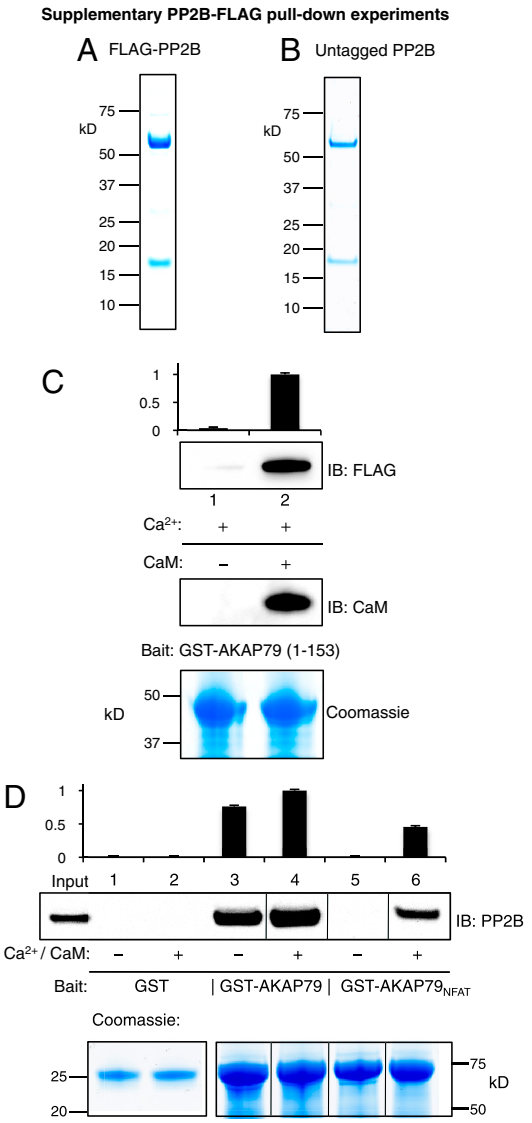

Fig. S5. PP2B preparations and additional PP2B–FLAG pull-down experiments. Coomassie-stained 4–12% SDS-PAGE of (*A*) 4 μg purified PP2B–FLAG and (*B*) 2 μg untagged PP2B. (*C*) Anti-FLAG immunoblot shows PP2B–FLAG pulled-down with GST–AKAP79 (1–153) and either 3 mM Ca^{2+} alone (lane 1) or 3 mM Ca^{2+} in the presence of 10 μg CaM (lane 2). Relative densitometry analysis ($n = 3$, *Top*), anti-calmodulin IB (*Middle*) and pull-down Coomassie (*Bottom*) are shown. These data demonstrate that PP2B interaction with the AKAP79 N-terminal site requires CaM. (*D*) Pull-down of PP2B–FLAG was attempted ±Ca^{2+}/CaM with either GST, GST–AKAP79, or GST fused to a mutant of AKAP79 in which the PIAIIIT motif was replaced with the sequence PRIEIT "AKAP79NFAT." The inability of AKAP79NFAT to bind PP2B in the absence of Ca^{2+}/CaM suggests that the PIAIIIT motif cooperates with other sequence determinants in the C-terminus of AKAP79 to anchor PP2B. Specifically, switching the anchor residues Pro337 and Thr343 to opposite sides of the anchoring-sheet may be incompatible with other binding motifs in the C-terminus of AKAP79.

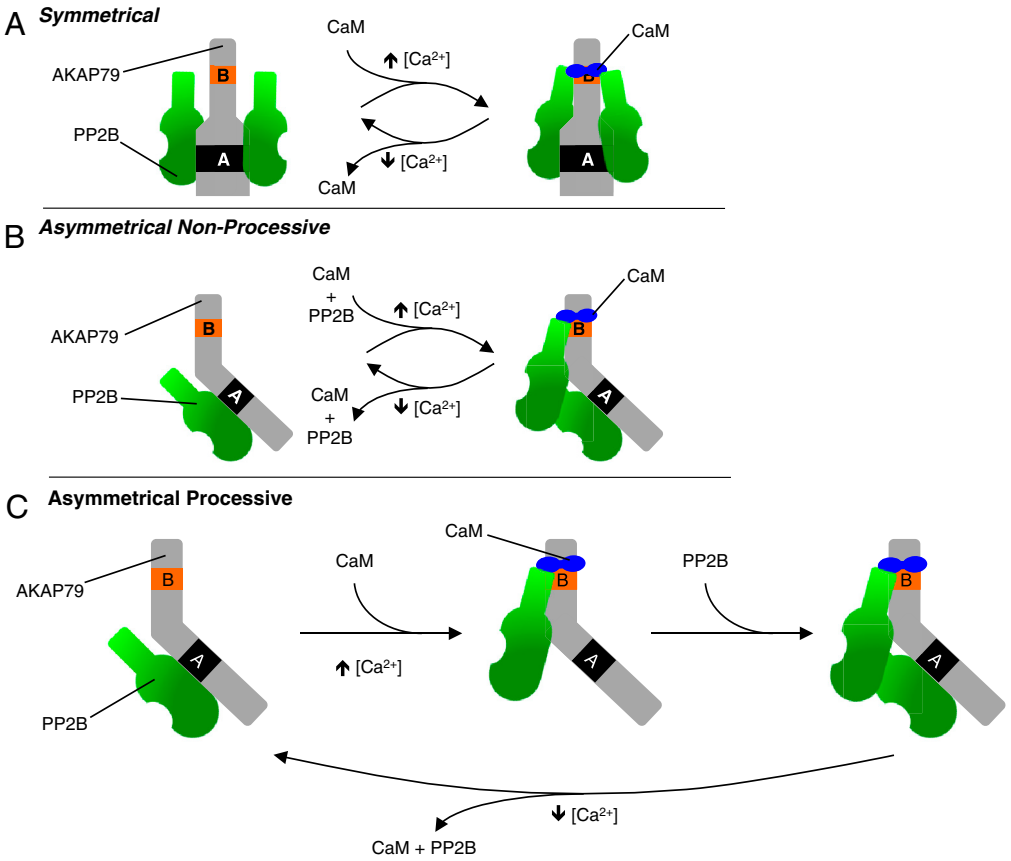

Fig. S6. Models of AKAP79–PP2B anchoring. In each model, site A corresponds to the PIAIIIT motif, and site B corresponds to the N-terminal Ca^{2+}/CaM-sensitive site. (*A*) Symmetrical. In this model two PP2B heterodimers associate in parallel on either side of the PIAIIIT motif of site A. Upon CaM activation, elements in the PP2B B subunits and/or P2B A subunit C-terminal regions of each anchored heterodimer simultaneously engage AKAP79. (*B*) Asymmetrical nonprocessive. In this model, the A site is constitutively occupied by one PP2B heterodimer, whereas another PP2B heterodimer can associate and dissociate with site B under the influence of Ca^{2+}/CaM. (*C*) In this model, similar to the asymmetrical nonprocessive model, AKAP79 contains two separate PP2B binding sites. In the absence of Ca^{2+}/CaM, one PP2B heterodimer associates with site A, where it is primed for translocation to site B upon CaM activation. Upon Ca^{2+}/CaM-mediated translocation of PP2B from site A to site B, site A becomes unoccupied, allowing association with a second PP2B heterodimer. Once cellular [Ca^{2+}] decreases sufficiently, both CaM and the PP2B heterodimer associated with the B site will dissociate, returning the signaling complex to its ground state with one PP2B heterodimer bound to the AKAP79 A site. It is important to note that for each of these models only one protomer of the AKAP79 dimer is described for the sake of clarity. The assumption is that each protomer in the AKAP79 dimer anchors PP2B in the same way.

Table S1. Theoretical and measured masses of AKAP79 signaling complexes and substituent proteins

Component protein, s	Theoretical mass, Da	Experimental mass Da	Deviation, %
RIIα D/D	5,624	5,627	0.05
PP2B A subunit	58,070	58,072	0.003
PP2B B subunit (5–170)	18,915	18,915	0
AKAP79	50,460	50,461	0.002
CaM	17,249	17,321	0.42
AKAP79 + D/D	56,084	56,302	0.39
AKAP79 + 2 * D/D	61,708	61,929	0.36
2 * AKAP79	100,920	101,036	0.11
2 * AKAP79 + 1 * D/D	106,544	106,820	0.26
2 * AKAP79 + 2 * D/D	112,168	112,447	0.25
AK_DPC complex 2 * [AKAP79 + 2 * PP2B(A + B) + 2 * D/D + CaM]	465,854	466,051	0.04

Predicted masses and masses recorded by MS of AKAP79 complex-associated proteins are listed.

Table S2. DSS cross-linked peptide candidates

Peptide 1	Peptide 2	Neutral mass (peptide1 + peptide2 + 138.0680796	Measured mass	PPM error
			1,821.91507	
SKSEESK	GAWASLKR	1,818.947578		1,628.78
SKNVPK	GPKRSNHSK	1,819.00643		1,596.47
AAKALKPK	FPRGPKR	1,820.114858		988.09
SKNVPK	ASMLCFKR	1,820.964096		521.96
SEESKR	**SKSEESKR**	**1,821.906835**		**4.52**
KAAK	EKASMLCFKR	1,822.979746		−584.37
KAAK	LKIPCIKFPR	1,825.101182		−1748.77
SEESKR	KAAKALKPK	1,826.062548		−2276.44
			2,037.02797	
GAWASLKR	GPKRSNHSK	2,035.107541		942.76
SKSEESKR	**SKSEESKR**	**2,037.033826**		**−2.87**
GAWASLKR	ASMLCFKR	2,037.065207		−18.28
KAAK	ALKPKAGSEAADVAR	2,037.158239		−63.95
QKEK	QKEKASMLCFK	2,038.059119		−506.20
LKIPCIK	IPCIKFPR	2,038.183532		−567.28
KAKSR	IPCIKFPRGPK	2,038.187371		−569.16

AKAP79 was digested with trypsin in silico with up to two allowed missed cleavages. Potential cross-linked AKAP79 peptide sequences within 10 ppm of two measured masses are listed. The best solutions are in bold.

Table S3. The best stoichiometric assignments for the 466-kDa AK_DPC complex

Stoichiometric assignment*	Estimated mass, Da
(R_II_(1–45))4_(CaM)2_(PP2B_B)4_(AKAP_79)2_(PP2B_A_)4	**465,854**
(R_II_(1–45))4_(CaM)11_(PP2B_B)5_(AKAP_79)2_(PP2B_A_)	465,800
(R_II_(1–45))4_(CaM)3_(AKAP_79)2_(PP2B_A_)5	465,513
(R_II_(1–45))4_(CaM)12_(PP2B_B)_(AKAP_79)2_(PP2B_A_)2	465,459
(R_II_(1–45))4_(PP2B_B)15_(AKAP_79)2_(PP2B_A_)	465,211
(R_II_(1–45))4_CaM_(PP2B_B)11_(AKAP_79)2_(PP2B_A_)2	464,870
(R_II_(1–45))4_(CaM)2_(PP2B_B)7_(AKAP_79)2_(PP2B_A_)3	464,529
(R_II_(1–45))4_(CaM)11_(PP2B_B)8_(AKAP_79)2	464,475
(R_II_(1–45))4_(CaM)3_(PP2B_B)3_(AKAP_79)2_(PP2B_A_)4	464,188
(R_II_(1–45))4_(CaM)12_(PP2B_B)4_(AKAP_79)2_(PP2B_A_)	464,134
(R_II_(1–45))4_(PP2B_B)18_(AKAP_79)2	463,886
(R_II_(1–45))4_(CaM)13_(AKAP_79)2_(PP2B_A_)2	463,793

To determine the most likely stoichiometry of the 466,051-Da AK_DPC complex, masses were calculated for all potential combinations of interactions between AKAP79 signaling complex component proteins, based on their theoretically predicted masses (see Table S1), using software developed by ref. 1. We assumed that an AKAP79 dimer associated with $4 * D/D$ is the basis of the complex (refer to Fig. 1). Solutions within 0.5% of the measured mass are shown; the closest solution to the measured mass is in bold. With the exception of the best solution, $2 * [AKAP79 + 2PP2B(A + B) + 2RII(D/D) + CaM]$, the stoichiometric solutions do not conform to either the known 1:1 ratio of the PP2B heterodimer, or to liberal ratios of $[n * CaM \leq 3n * AKAP79 + n * PP2B]$.

*Assuming error margins of 0.5% and AKAP: RII D/D stoichiometry of 2:4 based on MSMS data.

1 Rinner O, et al. (2008) Identification of cross-linked peptides from large sequence databases. *Nat Methods* 5:315–318.