Engineering AKAP-selective regulatory subunits of PKA through structure-based phage selection (Gold et al.): **Supplementary Information**

Supplementary Methods

Protein purification- 16 DNA sequences coding for ~ 60 amino acid regions containing RII-anchoring helices of sixteen different AKAPs were synthetically amplified with codons optimized for *E. coli* expression. The sixteen AKAPs comprise AKAPs 1 through 14 as designated by the HUGO gene nomenclature committee, MAP2 and WAVE1. The construct boundaries were designed to avoid interrupting predicted secondary structure elements. The AKAP fragments were ligated into pGEX6P1 for expression as fusions to the C-terminus of GST *(Supplementary Table S1)* in *BL21* DE3 STAR cells (Invitrogen). Expression and purification of each GST-AKAP fragment was attempted as follows: Cells were pelleted 3 h after induction of 1.2 L cultures incubated at 37 °C with 0.5 mM IPTG. All purification steps were performed at 4 °C. Cells were lysed by resuspension in 100 mL lysis buffer (25 mM Tris pH 7.5, 0.5 M NaCl, 2 mM DTT, 0.5 mM EDTA, 1 mM benzamidine, one EDTA-free protease inhibitor tablet (Roche), 0.1 μg/mL mg lysozyme) and the lysate was cleared by 30 min centrifugation (20,000 g) following sonication (3 x 5 s). The cleared lysate was incubated for 1 h with 2 mL Glutathione Sepharose (GE Healthcare) beads, which were washed with 3 x 10 mL wash buffer (20 mM Na HEPES pH 7.5, 200 mM NaCl, 2 mM DTT, 0.5 mM EDTA). Purified GST-AKAP fragments were eluted in wash buffer supplemented with 10 mM L-Glutathione prior to gel filtation using a Superdex S200 column (GE Healthcare) with 25 mM Tris pH 7.5, 200 mM NaCl, 2 mM DTT, 0.5 mM EDTA). Peak fractions were collected and flash frozen in liquid N_2. Full length RII and R_{Select}AKAP18 were expressed in the same way, and bacterial lysis was performed in Lysis Buffer supplemented with 10 % glycerol. Following incubation with 2 mL Glutathione Sepharose, full length regulatory subunits were released by overnight incubation with 0.1 mg PreScission protease at

4°C, and the purification was completed using gel filtration as for the GST-AKAP baits. R_{Select} D/D domains (residues 1-45) were expressed with an N-terminal PreScission-cleavable 6xHis-tag and C-terminal V5 tag in pET28m. R_{Select} protein lysates were prepared as for GST-AKAP fragments except cells were resuspended in 100 mL nickel lysis buffer (25 mM Tris pH 8.0, 0.5 M NaCl, 10 mM imidazole, 1 mM benzamidine, one EDTA-free protease inhibitor tablet (Roche), 0.1 μg/mL mg lysozyme). Cleared lysates were incubated with 1 mL Ni-NTA agarose (Qiagen) for 1 h prior to washing in (25 mM Tris pH 8.0, 0.5 M NaCl, 10 mM imizadole) and elution in (25 mM Tris pH 7.5, 0.5 M NaCl, 0.3 M imidazole). Eluted R_{Select} D/D-V5 protein was buffer exchanged to remove imidazole, incubated for 3 h with PreScission protease to remove the nickel tag, and incubated for a second time with Ni-NTA agarose for 10 min. The flow-through was collected and applied to a Superdex S200 column to complete purification in the same way as the GST-AKAP fragments. Overlays were performed with 1 : 10,000 w/v RII/R_{Select} subunits in TBS-T supplemented with 10 % milk. RII/R_{Select} binding was detected with 1 : 5,000 w/v anti-V5-HRP conjugate antibody (Invitrogen).

Mutant RII-phage library generation- RII D/D – phage were generated using the T7 select system (Novagen). The first 45 amino acids of PKA RIIα with the ten-amino acid N-terminal linker sequence SGSGSSGGSG were inserted after residue Asn351 of the T7 capsid protein by ligation into T7Select10-3b EcoR1/HindIII vector arms (Novagen). Forward and reverse primers *(Supplementary Table 3)* were used to amplify the following template DNA sequence prior to ligation:

GTTCTTCTGGTGGTTCTGGTATGTCTCAC**ATC**CAG**ATC**CCGCCGGGTCTG**ACC**GAAC

TGCTG**CAG**GGTTAC*ACC*GTTGAAGTTCTGCGTCAGCAGCCGCCGG

In the central template oligonucleotide, the bases underlined in italics code for RII residue 17: These were replaced with 'TGG' to generate Thr17Trp mutant RII phage. The bases underlined in bold code for RII positions 3, 5, 10 and 14: The template oligonucletide was synthesized with a random mix (25 % of each nucleotide) at these 12 positions (Trilink Biosciences) to generate the RII mutant phage library used to screen AKAP-selective RII sequences. All PCR reactions were performed with 50 ng of central template oligonucletide. Following ligation of the RII inserts into T7Select 10-3b vector arms, packaging into T7 bacteriophage was performed with the T7Select phage display kit following the manufacturer's instructions (Novagen/EMD Chemicals). Since there are 4^{12} total DNA variants, the library coverage = $1 - (16777215/16777216)^{n}$, where *n* is the packaging number. 8.8 x 10^{7} inserts were packaged into the phage, so ~ 99.5 % of all possible DNA variants are represented in the library. Given the redundancy of the genetic code, the coverage of total protein variants is approaching 100 %. Phage amplification and titering was performed in BLT5403 cells in LB supplemented with 50 μg/mL carbenicillin.

Mutant RII-phage selection- All steps were performed in Phage Wash Buffer (PWB): 25 mM Tris pH 7.2, 150 mM NaCl, 0.05 % Tween-20, 2 mM DTT, 0.5 mM EDTA. Prior to each round of selection with high competitive pressure, 0.125 μL Glutathione Magnetic beads (Pierce) were incubated with 0.1 μg of a single GST-AKAP fusion protein. After washing in 3 x 1 mL PWB, ~ 1 x10^{10} RII-phage were input in each round of selection in the presence of 40 μg peptide aliquots corresponding to the anchoring helices of the fifteen AKAPs that were not immobilized by fusion to GST *(Supplementary Table S1)*. Although new AKAPs are still being identified, we reasoned that this comprehensive list of competitors would drive the selection towards the most selective RII mutant sequence for each AKAP. The concentrations of bait and competitors in the screen correspond to a 5, 000-fold molar excess of each competitor AKAP relative to the bait AKAP. Following washing in 6 x 1 mL PWB, phage associated with the GST-AKAP were eluted by 2 h

incubation with 0.5 μg PreScission protease (GE healthcare) in 100 μL PWB. Eluted proteins were incubated for 10 min with 2 μL Glutathione Magnetic beads to remove residual PreScission protease, and the eluate was amplified to serve as the input in the next round of selection. The mutant RII sequence of phage present in eight plaques from the sixth round of selection were amplified by PCR and subjected to Sanger sequencing (Supplementary Table S2). Two further rounds were necessary for emergence of a predominant sequence when screening with either GST-AKAP18 or GST-AKAP-Lbc as the bait (Supplementary Table S2). For screens with medium selection pressure, the quantity of each free AKAP competitor peptide was reduced to 2 μg. 1 μg of GST-AKAP bound to 0.5 uL Magnetic Glutathione Phage was used as the bait when screening in the absence of competitors. Piloting experiments were performed in quadruple by preincubating 1 μL Magnetic Glutathione beads with 10 μg GST or GST-AKAP79 (335-427) in PWB. After washing in PWB, the beads were incubated with 2 x 10^9 phage in 1 mL PWB. The input phage mixture for Supplementary Figure 1*C*-1*F* contained a 1000 : 1 ratio of RII (1-45) Thr10Trp to wild-type phage. After washing in 6 x 1 mL PWB, phage were eluted by addition of 1 μg PreScission protease prior to titering and amplification.

Cell culture, pull-down and imaging experiments- 2FLAG-AKAP18δ, AKAP2-V5, 2FLAG-MAP2 and RII-RFP were cloned into pcDNA3.1 (Invitrogen). RII (1-45), R_{Select}AKAP18 (1-45) and R_{Select}AKAP2 (1-45) were fused to the N-terminus of YFP or mCherry by overlap extension PCR prior to ligation into pcDNA3.1/V5-His for expression of R_{Select}-fluorescent fusion proteins bearing C-terminal V5 tags. HEK293 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % (v/v) fetal bovine serum and penicillin/streptomycin. Cells were transfected overnight using Transit-LT1 (Mirus). HEK293 cells were cultured on glass coverslips (no. 1.5). For pull-down experiments, cells co-transfected with 2FLAG-AKAP18δ and AKAP2-V5 were harvested 2 d after transfection in (50 mM Tris pH 7.5, 150 mM NaCl, 1 %

Igepal CA-630, 1 mM EDTA, 1 mM benzamidine; 3 mL per 10 cm dish) and immunoprecipitation was performed with 2 μg rabbit anti-FLAG antibody, rabbit anti-V5 antibody or rabbit IgG (Sigma) per 1 mL lysate supplemented with 0.1 μg RII and 0.5 μg C subunit (New England Biolabs) and 20 μL protein A agarose. Pull-downs were supplemented with 0.4 μg R_{Select}AKAP18 and 10 μM Ht31 peptide as appropriate prior to washing with 3 x 1 mL ATP-wash buffer (30 mM Tris pH 7.4, 150 mM NaCl, 1 % Igepal-630, 1 mM DTT, 1 mM $MgCl_2$, 50 μM ATP). Finally, PKA C subunits were released by incubation with ATP-wash buffer supplemented with 1 mM cAMP. FLAG-AKAP18δ was detected using αFLAG-HRP conjugate (Sigma); AKAP2-V5 using αV5-conjugate (Invitrogen); and PKA C subunits using mouse αC antibody (BD Biosciences).

Prior to imaging, cells were washed in 2 x 1 mL PBS and fixed to coverslips by 10 min incubation with 400 μL 4 % PFA in PBS. Fixed cells were permeabilized by 10 min incubation in 0.1 % Tx-100/PBS. Staining of AKAP18 and AKAP2 was performed by initially blocking by 2 h incubation with 0.1 % Tx-100 & 1 % BSA/ PBS, followed by incubation with rabbit anti-V5 and mouse anti-FLAG antibodies diluted 1:200 in blocking buffer for 2 h. Following 3 x 1 mL washes with 0.1% Tx-100/PBS, cells were incubated for 1 h in blocking buffer with 1:500 Alexa Fluor 568 donkey anti-rabbit and Alexa Fluor 488 goat anti-mouse secondary antibodies (Invitrogen). Cells were washed once more and mounted on glass slides using ProLong antifade media (Invitrogen). Imaging was performed with a Zeiss LSM 510 META. All steps were performed at room temperature.

FRET measurements- HEK293 cells were grown on 1.5 glass coverslips and transiently transfected as described with appropriate donor CFP-tagged and acceptor YFP-tagged constructs. Coverslips were placed in a Luden chamber and covered with a HEPES buffered saline solution (25mM HEPES at pH 7.4, 114mM NaCl, 2.2mM KCl, 2 mM $CaCl_2$, 2mM $MgCl_2$, 22mM $NaHCO_3$, 1.1 mM NaH_2PO_4 and 2 mM glucose) supplemented with 10% FBS (Gibco) for

imaging. Wide-field intermolecular FRET microscopy was performed using a 40 x oil immersion (numerical aperture=1.4) immersion Fluor lens on a Leica DMI6000B inverted microscope (Leica) with an EL6000 (fluorescent light source, filter wheel, ultra fast shutter, Leica) controlled by Metamorph 7.6.4 (Molecular Devices). Dual emission FRET images were obtained using a Dual-View image splitter (Photometrics) with S470/30 and S535/30 emission filters and 505 dcxr dichroic mirror (Chroma). Metamorph imaging software was used to quantify the FRET images using the fully specified bleed-through correction method. Corrected FRET (cFRET) was calculated using the equation cFRET = Raw FRET - [mYFP-(DiA x mCFP)] x [AiF]-[mCFP-(AiD x mYFP)] x [DiF] where mCFP, mYFP and Raw FRET correspond to background corrected images obtained from the CFP, YFP and FRET channels respectively. DiA and DiF refer to correction values for donor (CFP) bleed through into the acceptor and FRET channels respectively, while AiD and AiF refer to correction values for acceptor (YFP) bleed through into the donor and FRET channels respectively.

Structural modeling- Models of R_{Select} – AKAP complexes were generated using the RII D/D – AKAP-*is* structure[5] as a template. Positions 3, 5, 10 and 14 in RII were mutated according to the R_{Select} subunit, and the residues in AKAP-*is* were mutated on the basis of alignments between AKAP-*is,* AKAP2, AKAP150 and AKAP18 using the invariant small aliphatic residues to anchor the alignment. The mutated protein complex structures were manually refined using the $2F_o - F_c$ electron density map of the RII D/D – AKAP-*is* crystal structure as a reference in Coot[36]. Cartoon representations were generated using PyMol (DeLano Scientific).

Illumina library preparation, sequencing and quality filtration- Phage library DNA was isolated by phenol-chloroform extraction and ethanol precipitation. 50 ng of phage DNA was amplified using the Phusion HF system and the DF-97_PCR_long_p1F and DF-154_PCR_p1R primers (Supplementary Table S3) for 15 cycles according to the manufacturer's instructions (New

England Biolabs). The product was isolated using the Qiaquick PCR purification kit (Qiagen) and purified using the Ampure reagent (Beckman-Coulter). Concentrations were determined using the Quant-IT dsDNA HS kit according to the manufacturer's instructions (Invitrogen). Libraries prepared from the fourth round of selection of RII phage with AKAP18 in the absence of competitors, the third round of selection in the presence of a high concentration of competitors and the input phage were sequenced. From each library, 0.36 fmol total DNA was loaded into its own lane and sequenced using a HiSeq 2000 (Illumina) with the DF-154_SEQ_F and DF-154_SEQ_R primers (Supplementary Table S3).

High-throughput sequencing data analysis- Sequencing data analysis was carried out using the Enrich software package[25,37]. Standard Enrich output was generated using the following configuration parameters:

<wtDNA>ATGTCTCACATCCAGATCCCGCCGGGTCTGACCGAACTGCTGCAGGGTTAC
ACCGTTGAAGTTCTGCGT</wtDNA>

<wtPRO>MSHIQIPPGLTELLQGYTVEVLR</wtPRO>
<index_mismatch_threshold>1</index_mismatch_threshold>
<index_mode>index</index_mode>
<index_sequence>AATTCTGG</index_sequence>
<run_filter>y</run_filter>
<fuser_mode>B</fuser_mode>
<include_nonoverlap_region>n</include_nonoverlap_region>
<paired_mismatch_threshold>0</paired_mismatch_threshold>
<read1_overlap_end>68</read1_overlap_end>
<read1_overlap_start>0</read1_overlap_start>
<read2_overlap_end>100</read2_overlap_end>

<read2_overlap_start>32</read2_overlap_start>
<run_aligner>n</run_aligner>
<Ncount_max>0</Ncount_max>
<avg_quality>20</avg_quality>
<chaste>y</chaste>
<gap_max>0</gap_max>
<maximum_mutation_run>3</maximum_mutation_run>
<unresolvable_max>0</unresolvable_max>
<unlink_modes>counts,wild_counts</unlink_modes>

Briefly, an average Illumina quality score was calculated for each read in a given set of paired end reads, and pairs that had an average Phred quality score of less than 20 for either read were removed. Read pairs were merged into a single sequence, with disagreements between the read pairs being resolved in favor of the higher-quality base. Read pairs with disagreements and equal quality scores were discarded. DNA sequences and their amino acid translations were aligned with the wild-type RII domain sequence. Sequences with more than three consecutive mutations on the DNA level were discarded, as were sequences containing 'N' characters. Unique sequences (variants) from each library were identified and their frequency computed. Variants supported by less than ten reads were removed. Log-transformed variant enrichment ratios (E) were calculated as described[37]. Unlinked mutation scores for the i^{th} position and the j^{th} amino acid ($U_{i,j}$) were calculated as follows:

$$U_{i,j} = \sum F_{i,j}$$

These were divided by the sum of all unlinked mutation scores to the produce unlinked amino acid frequency for the i^{th} position and the j^{th} amino acid ($Fu_{i,j}$):

$$Fu_{i,j} = \frac{U_{i,j}}{\sum U}$$

The unlinked enrichment ratio for the i^{th} position and the j^{th} amino acid between the input library and the X^{th} round ($E_{unlinked,i,j,X}$) was calculated as follows:

$$E_{unlinked,i,j,X} = \frac{Fu_{i,j,X}}{Fu_{i,j,input}}$$

The selectivity index was calculated for the i^{th} position and the j^{th} amino acid between the AKAP18 library in the presence or absence of AKAP competitor peptide ($SI_{i,j}$) as follows:

$$SI_{i,j} = E_{unlinked,i,j,+competitor} - E_{unlinked,i,j,-competitor}$$

Supplementary Figure Legends and Supplementary Table Legends

Supplementary Figure S1. RII phage pilot experiments (A) RII (1-45) D/D was displayed using the T7Select10-3b system, which is expected to result in the expression of approximately ten copies of capsid-RII D/D on the surface of each phage. (B) RII D/D – phage were incubated with either GST or GST-AKAP79 (residues 335-427, PKA anchoring helix). After pulldown with magnetic Glutathione Sepharose beads, AKAP-associated phage were eluted by incubation with PreScission protease and tittered for plaque forming units. (C) Location of RII residue Thr17 at the base of the AKAP – RII interface (AKAP helix is shown in orange and the RII D/D dimer in grey). (D) To test whether selection of higher affinity RII mutants is possible, phage were generated displaying RII D/D containing the substitution Thr17Trp. A mixture of wild-type and Thr17Trp RII D/D phage, with the Thr17Trp mutant in a 1,000-fold excess, were used as the input into multiple rounds of enrichment to immobilized GST-AKAP79 (335-427). (E & F) Sanger sequencing of RII codon 17 in DNA extracted from input phage, and phage eluted from GST-AKAP79 (335-427) after the first two rounds of selection.

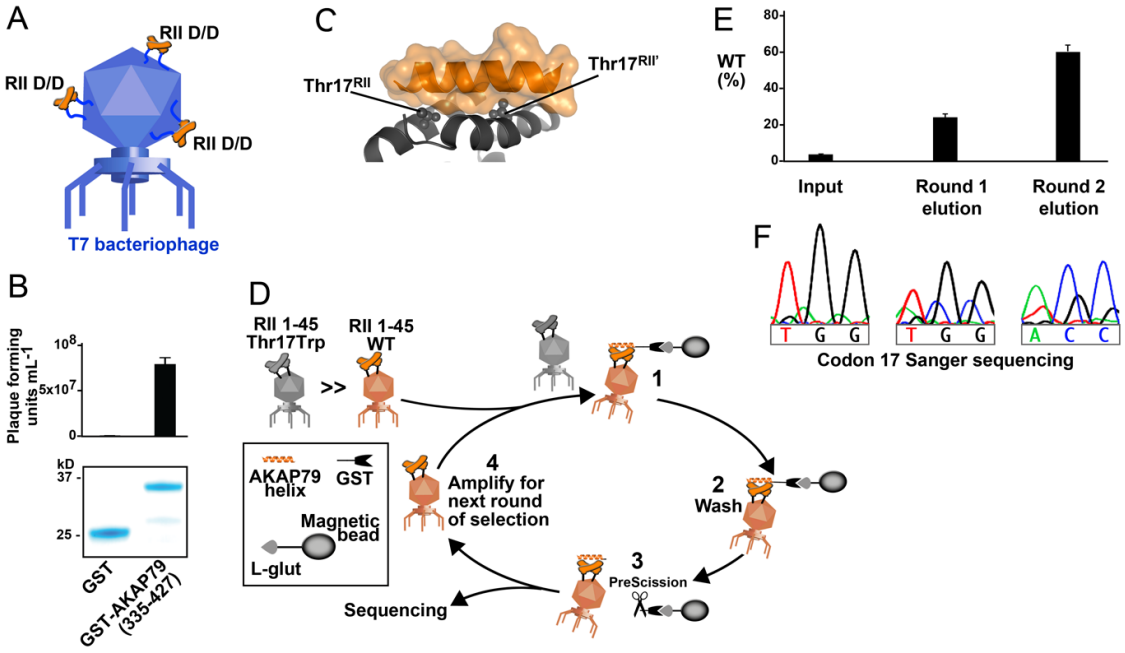
Supplementary Figure S2. Structural models of R_{Select}AKAP2 – AKAP2 and R_{Select}AKAP150 – AKAP150 interactions. (A) Alignment of AKAP anchoring helices. Variable residues in AKAP2 and AKAP150 that have been modeled to interact with side-chains in positions 3, 5, 10 or 14 in R_{Select} are colored red. (B-E) Cartoon representations of structural models are presented for the AKAP2 (grey)– R_{Select}AKAP2 (yellow-gold) complex (B & C), and the AKAP150 (grey)– R_{Select}AKAP150 (yellow-gold) complex (D & E).

Supplementary Table S1. AKAP bait and competitor sequences. Sequences are shown for AKAP constructs that were incorporated at the C-terminus of GST. These fusion proteins were used as bait in phage selections. The 22-amino acid peptides (underlined in red), corresponding

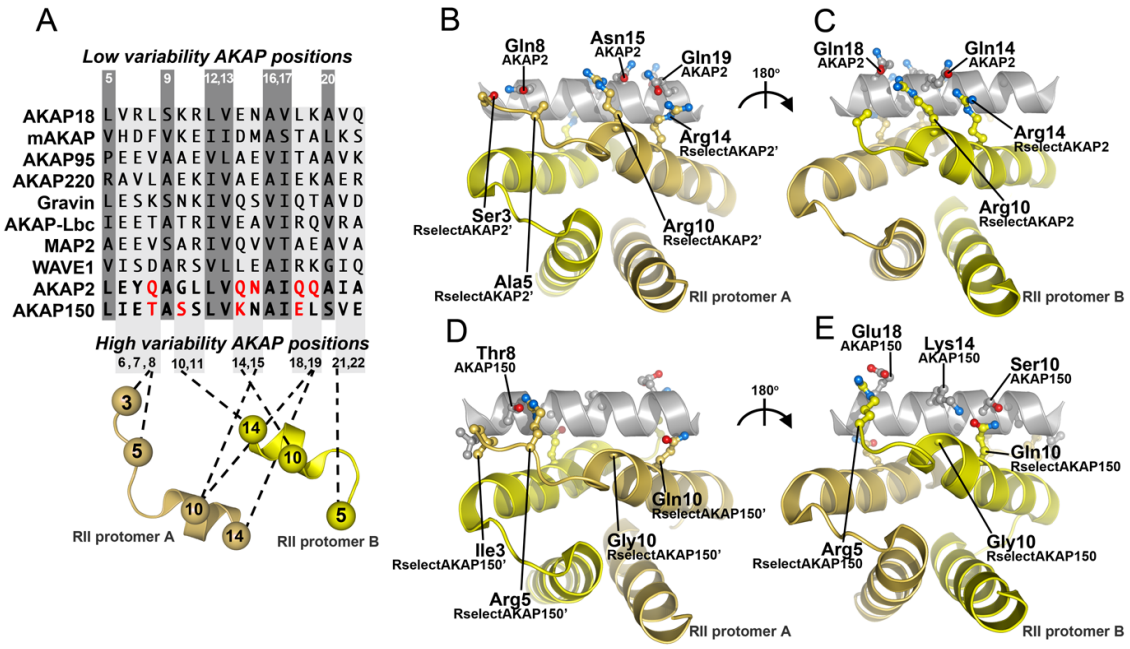
to the RII-binding aliphatic helix of each AKAP, that were used as free competitors in phage selections are shown.

Supplementary Table S2. Sanger sequencing results of phage selections. Sequences of eight plaques from the final round of selection with ten different AKAP baits are listed. Predominant sequences are in bold text; variable RII residues are colored red.

Supplementary Table S3. Oligonucleotide primers. Primer sequences are listed for construction and high-throughput sequencing of RII – phage libraries.



Supplementary Figure 1



Supplementary Figure 2

AKAP	Also Known As	Residues	Sequence
D-AKAP1	AKAP1 AKAP84	285-344	TSDRDLAGELDKDETVP**ENDQIKQAAFQLISQVILEATE**ELRATTVGKTVAQVHPISATQ
AKAP2	AKAP-KL	562-622	KSFSDHGFYSPSSTLGDSPS**VDDPLEYQAGLLVQNAIQQAIA**EQVDKAEAHTSKEGSEQQE
AKAP110	AKAP3	103-162	SHKENPSQGLISHVGNGG**SIDEVSFYANRLTNLVIAMARK**EINEKIHGAENKCVHQSLYM
AKAP82	AKAP4	194-253	PATKSPSNQRSVATPEGEC**SMDDLSFYVNRLSSLVIQMARK**EIKDKLEGGSKCLHHSMYT
AKAP150	AKAP5 AKAP79 AKAP75	684-745	MENEQVGVFANDSDFEGRTSEQ**YETLLIETASSLVKNAIELSVE**QLVNEMVSEDNQINTLFQ
mAKAP	AKAP6	2036-2096	KPDVFHQKDD**EDCSVHDFVKEIIDMASTALKS**KSQPESEVAAPTSLTQIKEKVLEHSHRPI
AKAP18	AKAP7 AKAP15	1-81 (α isoform)	MGQLCCFPFSRDEGKISEKNGGEP**DDAELVRLSKRLVENAVLKAVQ**QYLEETQNKNKPGEGS SVKTEAADQNGNDNENNRK
AKAP95	AKAP8	547-607	TADLETEGDENVGEE**KEETPEEVAAEVLAEVITAAVK**AVEGEGEPAAAHSDVLTEVEGPVD
Yotiao	AKAP9 AKAP450	2450-2510	DVELTQCREQTETIQEQAQSETDRLQKKLTD**LQRSLEKFAAALVSQVQMEAAQ**EYVPFHQE
D-AKAP2	AKAP10	597-657	FIRESEPEPDVKKSKGFMFSQAMKKWVQGNTDEA**QEELAWKIAKMIVSDVMQQAHH**DQPLE
AKAP220	AKAP11	1597-1657	SAKPSSRSKLSSIRQKSRIFHLDVPQIHV**NLDKRAVLAEKIVAEAIEKAER**ELSNTSLAAD
Gravin	AKAP12 AKAP250	1475-1529	PDAGPDAAGKESAAREKILRAEP**EILELESKSNKIVQSVIQTAVD**QFARTETAPE
AKAP-Lbc	AKAP13	1186-1246	DMKQVAQASIPAEESNATTVSTQAADVPT**RADSIEETATRIVEAVIRQVRA**SNALMAKVET
AKAP28	AKAP14	344-394	EIKQKAHVKDVKEKKIVPEVKEKKPVVVK**KKDGASEVARTVVDGVMAAAVE**MVEEARNPIK
MAP2	-	59-119 (short isoform)	GEHRSQGTYSDTKENGINGELTSA**DRETAEEVSARIVQVVTAEAVA**VLKGEQEKEAQYKDQ
WAVE1	-	483-542	EPKRHP**STLPVISDARSVLLEAIRKGIQ**LRKVEEQREQEAKHERIENDVATILSRRIAVE

Supplementary Table S1

Supplementary Table S2

AKAP	Selection Pressure	Rd. Selection	Sequences	Predominant Sequence
AKAP2	High	6	MSHGQPPPGLLELLGGYT	**MSHSQAPPGLRELLRGYT**
			MSHFQVPPGLSELLIGYT	
			MSHIQWPPGLPELLRGYT	
			MSHSQAPPGLRELLRGYT	
			MSHSQAPPGLRELLRGYT	
			MSHSQAPPGLRELLRGYT	
			MSHSQAPPGLRELLRGYT	
			MSHSQAPPGLRELLRGYT	
AKAP150	High	6	MSHFQPPPGLGELLRGYT	**MSHLQRPPGLGELLQGYT**
			MSHLQRPPGLGELLQGYT	
			MSHIQRPPGLMELLQGYT	
			MSHLQRPPGLGELLQGYT	
			MSHLQRPPGLGELLQGYT	
			MSHLQKPPGLPELLRGYT	
			MSHLQRPPGLGELLQGYT	
			MSHIQRPPGLPELLQGYT	
AKAP18	High	8	**MSHVQLPPGLDELLGGYT**	**MSHVQLPPGLDELLGGYT**
			MSHSQPPPGLDELLAGYT	
			MSHVQLPPGLDELLGGYT	
			MSHIQLPPGLDELLSGYT	
			MSHRQVPPGLDELLEGYT	
			MSHVQLPPGLDELLGGYT	
			MSHIQLPPGLDELLGGYT	
			MSHIQLPPGLDELLSGYT	
AKAP-Lbc	High	8	**MSHQQDPPGLGELLQGYT**	**MSHQQDPPGLGELLQGYT**
			MSHQQDPPGLGELLQGYT	
			MSHQQDPPGLGELLQGYT	
			MSHQQDPPGLGELLQGYT	
			MSHQQDPPGLGELLQGYT	
			MSHQQDPPGLGELLQGYT	
			MSHQQDPPGLGELLQGYT	
			MSHLQDPPGLNELLHGYT	
AKAP220	High	6	MSHSQRPPGLSELLKGYT	**MSHRQIPPGLFELLSGYT**
			MSHRQIPPGLIELLSGYT	
			MSHRQIPPGLFELLSGYT	
			MSHRQIPPGLFELLSGYT	
			MSHRQIPPGLFELLSGYT	
			MSHSQRPPGLSELLKGYT	
			MSHSQRPPGLSELLKGYT	
mAKAP	Medium	6	**MSHHQRPPGLKELLSGYT**	**MSHHQRPPGLKELLSGYT**
			MSHRQFPPGLRELLGGYT	
			MSHHQRPPGLKELLSGYT	
			MSHRQVPPGLAELLKSYT	
			MSHHQRPPGLKELLSGYT	

			MSHHQRPPGLKELLSGYT	
			MSHPQRPPGLRELLGGYT	
			MSHHQRPPGLKELLSGYT	
Gravin	Medium	6	**MSHGQAPPGLRELLGGYT**	**MSHGQAPPGLRELLGGYT**
			MSHGQAPPGLRELLGGYT	
			MSHTQTPPGLGELLNGYT	
			MSHVQEPPGLSELLGGYT	
			MSHTQTPPGLGELLNGYT	
			MSHDQGPPGLRELLNGYT	
			MSHDQGPPGLRELLNGYT	
			MSHGQAPPGLRELLGGYT	
MAP2	Medium	6	MSHSQSPPGLGELLRGYT	**MSHKQSPPGLGELLRGYT**
			MSHKQSPPGLGELLRGYT	
			MSHKQSPPGLGELLRGYT	
			MSHVQAPPGLNELLRGYT	
			MSHKQSPPGLGELLRGYT	
			MSHKQSPPGLGELLRGYT	
			MSHRQGPPGLQELLRGYT	
			MSHKQSPPGLGELLRGYT	
WAVE1	None	6	**MSHAQIPPGLEELLIGYT**	**MSHAQIPPGLEELLIGYT**
			MSHAQIPPGLEELLIGYT	
			MSHAQIPPGLEELLIGYT	
			MSHVQVPPGLEELLMGYT	
			MSHGQVPPGLEELLMGYT	
			MSHAQIPPGLEELLIGYT	
			MSHGQIPPGLEELLMGYT	
			MSHGQVPPGLEELLMGYT	
AKAP-95	None	6	**MSHRQVPPGLRELLLGYT**	**MSHRQVPPGLRELLLGYT**
			MSHRQVPPGLRELLLGYT	
			MSHRQVPPGLRELLLGYT	
			MSHRQVPPGLKELLVGYT	
			MSHVQIPPGLRELLLGYT	
			MSHVQVPPGLKELLFGYT	
			MSHCQVPPGLRELLLGYT	
			MSHRQVPPGLRELLLGYT	

Supplementary Table S3

Oligonucleotide name	Purpose	Sequence (5' – 3')	
EcoR1_Linker_RII_F	Amplification of bacteriophage insert	GGTTCATGTGCTCAGAATTCTGGTTCTGGTTCTTCTGGTGGTTCTGGT	
RII_stop_HindIII	Amplification of bacteriophage insert	CCGTGACACAGCAGAAGCTTTCATTACTAAGCACGAGCTTCACGCAGACGGGTGAAGTATTCAACAGCAAATTCAACCAGGTCCGGCGGCTGCTG	
DF-154_SEQ_F	Phage DNA amplification	CCGAATTCTGGTTCTGGTTCTTCTGGTGGTTCTGGT	
DF-154_SEQ_R	Phage DNA amplification	CAGGTCCGGCGGCTGCTG	
DF-97_PCR_Long_p1F	Phage DNA amplification	AATGATACGGCGACCACCGAGATCTACACGCAGGAGCTGTCGTATTCCAGTCAG*G	* = phosphorothioate
DF-154_PCR_p1R	Phage DNA amplification	CAAGCAGAAGACGGCATACGAGATTCATTACTAAGCACGAGCTTCACGCAGA*C	* = phosphorothioate