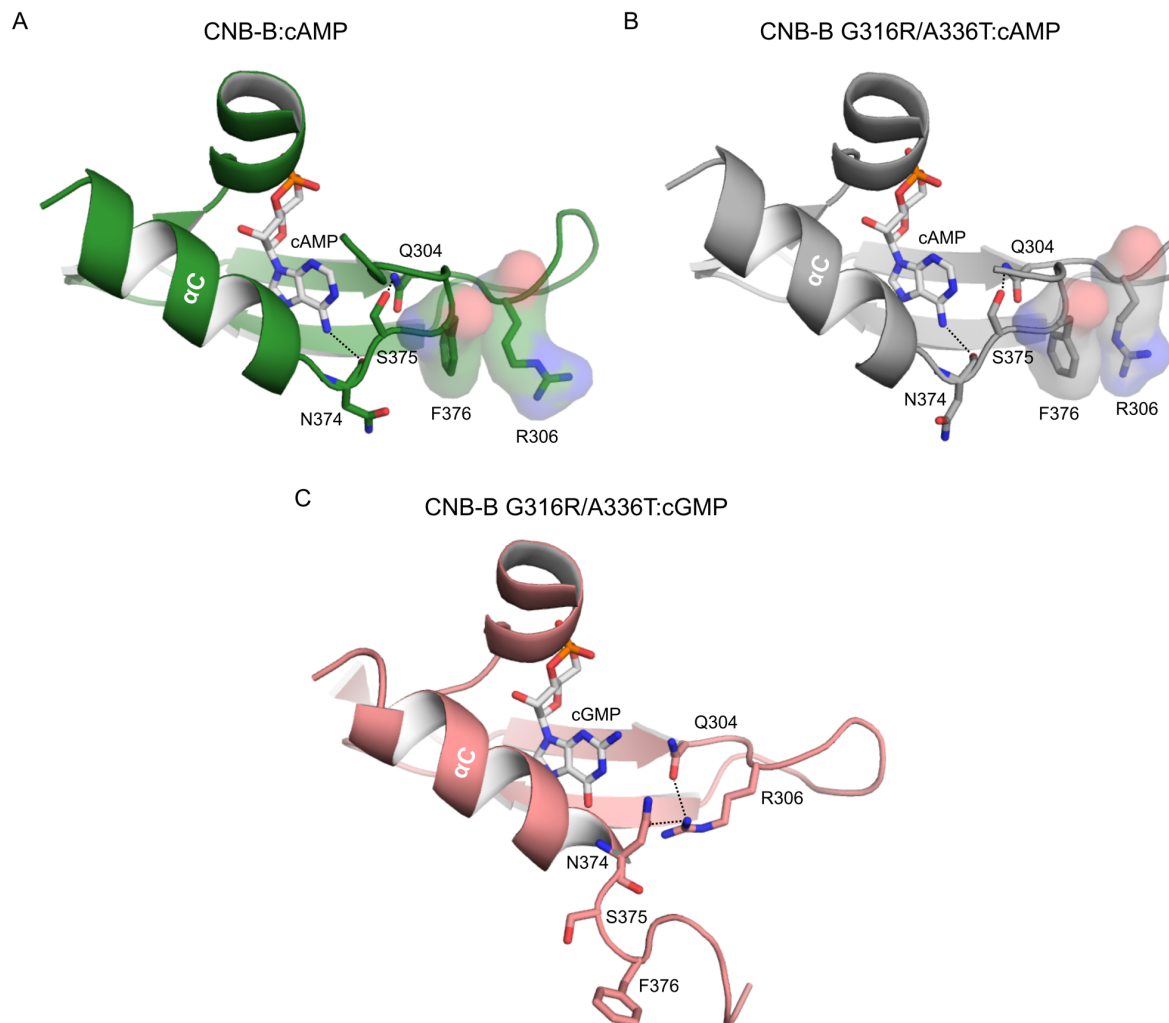


PKA RI α CNB-A	115	- - - - R K V I P K D Y K T M A A L A K A I E K N V	136
PKG I β CNB-A	92	S H V T L P F Y P K S P Q S K D L I K E A I L D N D	117
PKA RI α CNB-B	234	- - - - - I L M G S T L R K R K M Y E E F L S K V S	254
PKG I β CNB-B	219	- - - - - - - - - T G L I K H T E Y M E F L K S V P	235
PKA RI α CNB-A	137	L F S H L D D N E R S D I F D A M F S V S F I A G E	162
PKG I β CNB-A	118	F M K N L E L S Q I Q E I V D C M Y P V E Y G K D S	143
PKA RI α CNB-B	255	I L E S L D K W E R L T V A D A L E P V Q F E D G Q	280
PKG I β CNB-B	236	T F Q S L P E E I L S K L A D V L E E T H Y E N G E	261
PKA RI α CNB-A	163	T V I Q Q G D E G D N F Y V I D Q G E T D V Y V N -	187
PKG I β CNB-A	144	C I I K E G D V G S L V Y V M E D G K V E V T K E -	168
PKA RI α CNB-B	281	K I V V Q G E P G D E F F I I L E G S A A V L Q R R	306
PKG I β CNB-B	262	Y I I R Q G A R G D T F F I I S K G T V N V T R E D	287
PKA RI α CNB-A	188	- - - - - N E W A T S V G E G G S F G E L A L I Y G	208
PKG I β CNB-A	169	- - - - - G V K L C T M G P G K V F G E L A I L Y N	189
PKA RI α CNB-B	307	S E N E E F V E V G R L G P S D Y F G E I A L L M N	332
PKG I β CNB-B	288	S P S E D P V F L R T L G K G D W F G E K A L Q G E	313
PKA RI α CNB-A	209	T P R A A T V K A K T N V K L W G I D R D S Y R R I	234
PKG I β CNB-A	190	C T R T A T V K T L V N V K L W A I D R Q C F Q T I	215
PKA RI α CNB-B	333	R P R A A T V V A R G P L K C V K L D R P R F E R V	358
PKG I β CNB-B	314	D V R T A N V I A A E A V T C L V I D R D S F K H L	339
PKA RI α CNB-A	235	L M G S T L R K R K M Y E E F L S K V S I L E S L D	260
PKG I β CNB-A	216	M M R T G L I K H T E Y - - - - - - - - - - -	227
PKA RI α CNB-B	359	L G P C S D I L K R N I Q Q Y N S F V S L S V - - -	381
PKG I β CNB-B	340	I G G L D D V S N K A Y E D A E A K A K Y E A E A A	365
PKA RI α CNB-A	261	K W E R L T V A D A L E P V	274
PKG I β CNB-A		- - - - - - - - - - - - - - - - -	
PKA RI α CNB-B		- - - - - - - - - - - - - - - - -	
1 PKG I β CNB-B	366	F F A N - - - - - - - - - - -	369

2 **S1 Fig. Sequence alignment of CNB domain constructs used in this study.** Amino
3 sequences of the CNB domains of the human isoforms of PKA RI α and PKG I β were aligned
4 using Clustal Omega (54,55). Identical residues are shown in yellow. The three cGMP-specific
5 sites of the PKG I β CNB-B and the corresponding residues of PKA RI α are marked in red.
6 Capping residues are shown in blue.

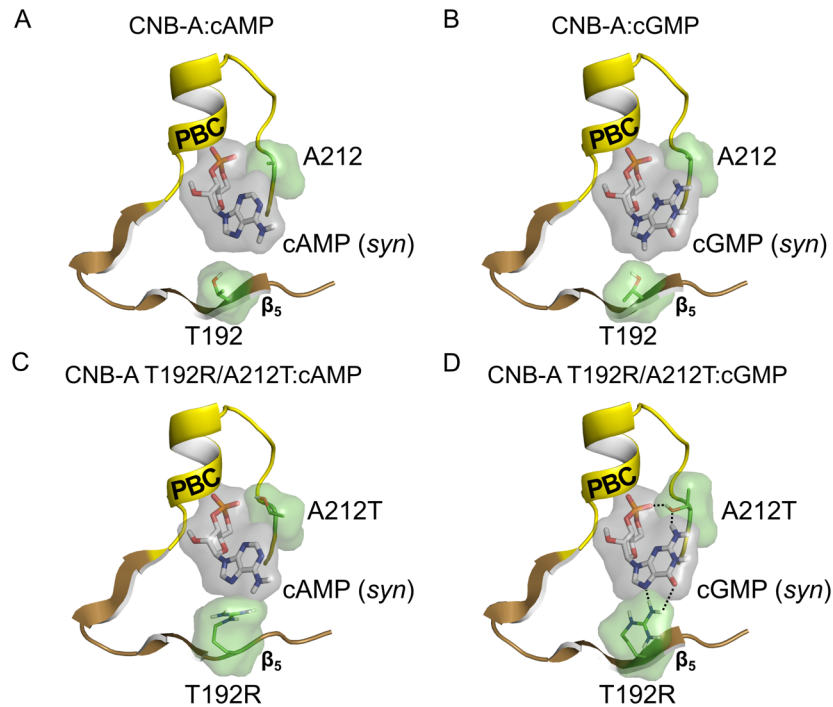
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10 **S2 Fig. Interaction networks of the CNB-B double mutant.** (A) In the co-crystal structure of
 11 the wild type CNB-B:cAMP, the C-terminal loop interacts with PBC and the β 4- β 5 loop thus
 12 blocking the binding pocket. In particular, the backbone carbonyl of N374 interacts with the N6
 13 amino group of the adenine ring, while S375 hydrogen bonds Q304. F376 interacts with R306
 14 through a hydrophobic interaction. (B) The co-crystal structure of the CNB-B
 15 G316R/A336T:cAMP shows the same conformation of the C-terminal loop as in the wild type
 16 CNB-B. (C) In the co-crystal structure of the CNB-B G316R/A336T:cGMP, N374 no longer
 17 interacts with the guanine moiety, but forms a hydrogen bond with R306. The side chain of the
 18 R306 additionally interacts with Q304. These changes in interactions displace the C-terminal
 19 loop from the binding pocket.



20

21 **S3 Fig. Structural model of PKA R1α CNB-A wild type and T192/A212T double mutant.** The

22 structural models (B-D) were generated with YASARA (40) using the structure of the bovine PKA

23 R1α as a template (PDB code 1RGS) (32). Residues 115-274 of human PKA R1α analogous to

24 114-273 in bovine PKA R1α. (A) a model of PKA R1α CNB-A binding pocket bound with cAMP

25 (B) a model of PKA R1α CNB-A Binding pocket bound with cGMP. (C) a model of PKA R1α CNB-

26 A double mutant T192R/A212T binding pocket bound with cAMP. Introducing an arginine

27 residue (T192R) at β5 leads to a steric clash with the position 6 amine of cAMP. (D) a model of

28 PKA R1α CNB-A double mutant T192R/A212T bound with cGMP bound. Both cGMP selective

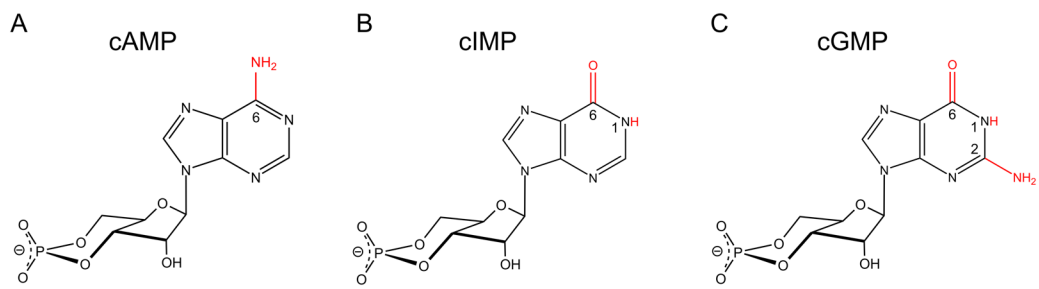
29 contact residues, T192R and A212T, form specific hydrogen bonds with cGMP. Residues 192

30 and 212 are shown as green sticks with transparent surface. cAMP and cGMP are colored by

31 atom type with a transparent surface.

35

36



37 **S4 Fig. 2D projection of cAMP, cIMP and cGMP.** The purine nucleotides differ in their
38 nucleobase. Differences between cAMP (A), cIMP (B) and cGMP (C) are marked in red.

39