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Supplementary Materials for

An Atypical CNG Channel Activated by a Single cGMP Molecule Controls Sperm Chemotaxis

Wolfgang Bönigk, Astrid Loogen, Reinhard Seifert, Nachiket Kashikar, Clementine Klemm, Eberhard Krause, Volker Hagen, Elisabeth Kremmer, Timo Strünker, U. Benjamin Kaupp*

*To whom correspondence should be addressed. E-mail: u.b.kaupp@caesar.de

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A


MAQRTVSLELEEPETGGSTDRATISSQPQACFLNPQNSRMRAWETLIIISILTTFSLDLF - 60
LAAFDSQVLHLWIIIYTCDIMFLTDVVMRFFLGYMKDGILISDSNKIKLHYLRTTFPLDL - 120
FTILPTDLLALVYPGLGSKNIWKWVATFRFANRLFRTLRINNYFDNRESKLGSNTALLRT - 180
LKYTVLAAMVIHSTACGWYYFACTGWHGSEPPRCHNGSWAKSFDLGQDMDQAKPGVSYVI - 240
ALYWSVATATSTGYGDISAVNEKEKWFSIIAMLLGIVVFFGMILGGMASMLTNFDGQRAR - 300
YTHRFNVIKDSLRDQSVEEELQKQVIGYYEYLWVRKKGVTDESLINALPLTFHAEVSLSG - 360
NKFILDKAPMFQGLSDGFLRMLSLEIKPSLYLPRQTIADRNEICHDMYFIQRGEIEILSA - 420
EDDDTPIERLNHGKLFGEVSLIFSMPRTNRIRAAAHCDLLVLDKADLQNVLHHYPEVAKK - 480
LQQIAEQRCDAAGLLEDRVRAGEGIIPASKSLSRHATPQTKRDFPINSYEAFDMEDVDSE - 540
WVTVHRGDVMMRTGRQLQILWDDIQSFFTDTVVMPNSRFAKTWELLVVLVTIVIAFLYPY - 600
CASFIAANQNRSQSPDPNEERVQENLILLTLMYLMDFVLVIDIVVRLRTAVSTPNGTFKD - 660
FHYIRDYYVRSWGFVADVLAILPLDLFCLLFPVGPKRTHALYLLALNRLIKCWRVPNYLQ - 720
NLEQNLEVNIGTIRFFKFVIYISLLSHWCSCLWYLAACPSGSARGTPAPAVSDPTAAVAN - 780
PVPWHEYIISLYWAAATMTSTGYGDISAHSTIGRAIALGAMLVGLLLYGYCLSSIAATLA - 840
NSDAPRVGFQEKLFAVQEFMKRHNLSPDLQQRVINYLSLVFRRHRGEAIPGGKRIMHDMP - 900
IQLQQDIAYQDVEATLSKVPLFKGCDANFLRMLALKLHVFIFMPGDVVVYQGDMGREMYF - 960
IRRGTCEVLSKDGKHVMSNIGPSQYFGEVGLIFGDYRTATVRAASYCELLMLKRADLDDV - 1020
LKHFPLIEKQFSDAAKNQGHLRELRDASKQKRDEPMVAGTVLQPEIQDVEEEDMSAYPEA - 1080
TSLRPVDISVKVFDTRSEDYTEPFQAISLPSRLVSKLLMSRSFIPSGTCFKRWEAIRVAV - 1140
AILAAFAVTLQAAFLHMNIGLWVVNYTLDFICFVDMYLKFHTAFYNENNVLVTHPLSTAK - 1200
HYLRTNFLIDVLACFPTELIAYAVIGHLSPESIHVYALVRINRLLQLYRVPLAFNYLESD - 1260
VQNLTGNIRMMKFFFYMVLFIHMLSCLWYMNACPPVFTFPISNDTFQDLILTNYHRCKNG - 1320
SWTSHTDNFYNLTITAQYLTSMYWASATGASVGYGDIHAKNISEMILALFSMIVGIVFFG - 1380
YIIASVAASLANADPHRARYQEKLTAIKRYLKDQGVEKSLAQHIDVYYNYMWLRTRGVEP - 1440
DSLFDGLPLALKADVSLNLYQGMINKVPLFHNTEIGFQKMLAMCIKPVYYLNKEYIVRKH - 1500
DFGKEMFFIHRGLVEVVSEDGSIVFDTMQSGRFFGEISLVFSCPRTASIRAENNVDMFVL - 1560
TKEDLDEVLTHYPSIKAQIYTVAEERISAVRKRSKAKVQASNTPNNSNNNSIKGSSINSI - 1620
EKSENSTASASSAGVPSSSSTAEASAPARPDDSQSTATAATRSNQGQGESTSVSSAVATT - 1680
PTSTIPPASPTPTPTPPTQENANSNANNNTGSGESSGNNNNQGGNNASSSQPDSTPATDG - 1740
ASATPHVPTRYPAPFCCVRRDKNYLEALLHANRFVLNPDSPVVRNLARLTCILAVATSWT - 1800
IMYQAFYQVQSTPFLIFSYLCECVFIFEIYIKFHVSCADEYGALEKDFTKIYNTYLRKWS - 1860
GFTQDFVPTVPIELLALCFSGETLFPVLSFLRFRQLLRFVRVSQFFDRWECELNINVLIV - 1920
RLIKFFVLLLIIIHLFASIWYTIACPLSVCHPGSWADSLNYNGSDPYIFYRYCDTIYWAV - 1980
ATLTSTGYGDIHAYSVPEIVFASMVMVFGKLLFGWVLGNIASTLANAESGRVSYEERLAA - 2040
VKDQMKDMRLSSKLRNRVISYFDYVWARNKGIDQSNLFRDAPFCLQTDLGLNVCGDHLLR - 2100
VSLFQEADESFHRALSLMLKPVLFMPSDLIVRQGDVGDEMYFISRGVVEEMEVNSNSRVA - 2160
RILESGEFFDDINLLYDVPRRTSFKARTHVDVKSLSVRDLRSVLEQYPNVEAQIRRIGKE - 2220
LYGDYAASINAPEHFPLRQTVV - 2242

B

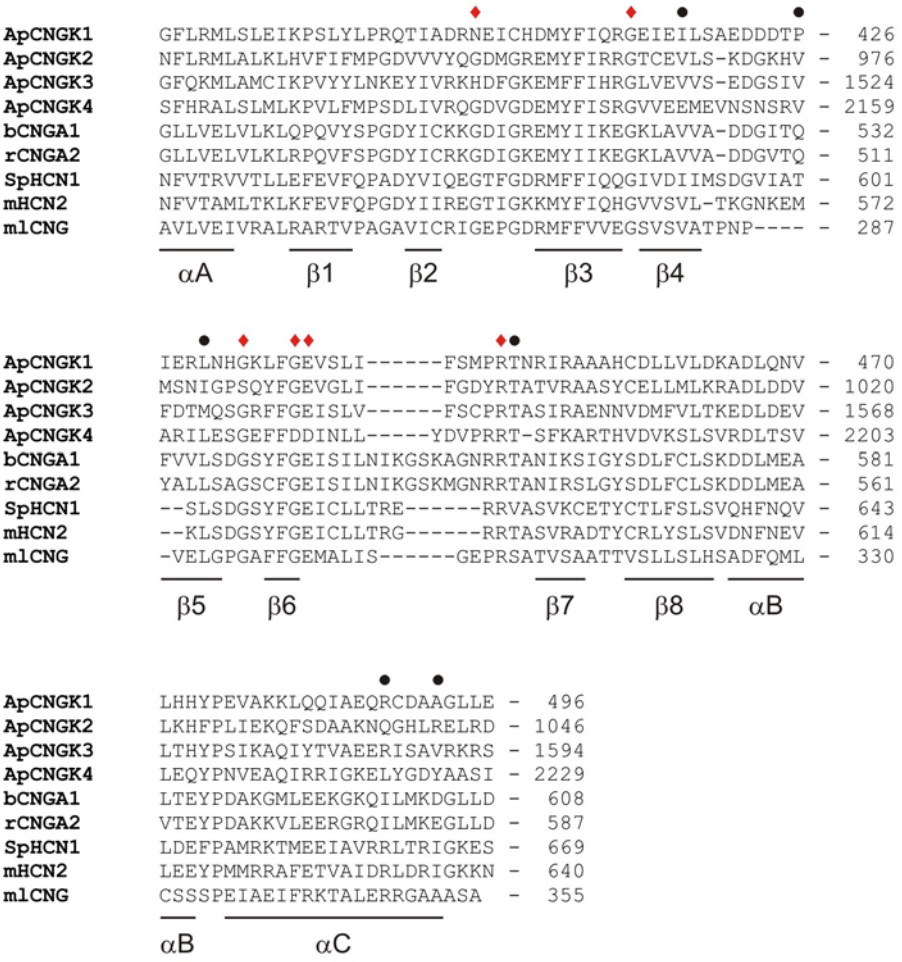

Fig. S1: Primary structure of the CNGK channel. (A) Peptides determined by mass spectrometry are shown as bars above the sequence. Red bars: peptides identified at around 50 to 70 kD; blue bars: peptides identified at around 170 kD. **(B)** Comparison of the cyclic nucleotide-binding domains of repeats 1-4 with those of CNG channels (bCNGA1, NP_776703; rCNGA2, NP_037060), HCN channels (SpHCN1, NP_999729; mHCN2, NP_032252), and a cyclic nucleotide-gated K^+ channel from *Mesorhizobium loti* (mlCNG, NP_104392). Amino-acid residues that are important for the structure of the cNMP-binding domains and for the binding of the ligand are indicated by red rhombs and black circles. Alpha helices (αA-C) and beta sheets (β1-8) of the secondary structure are shown below the sequences.

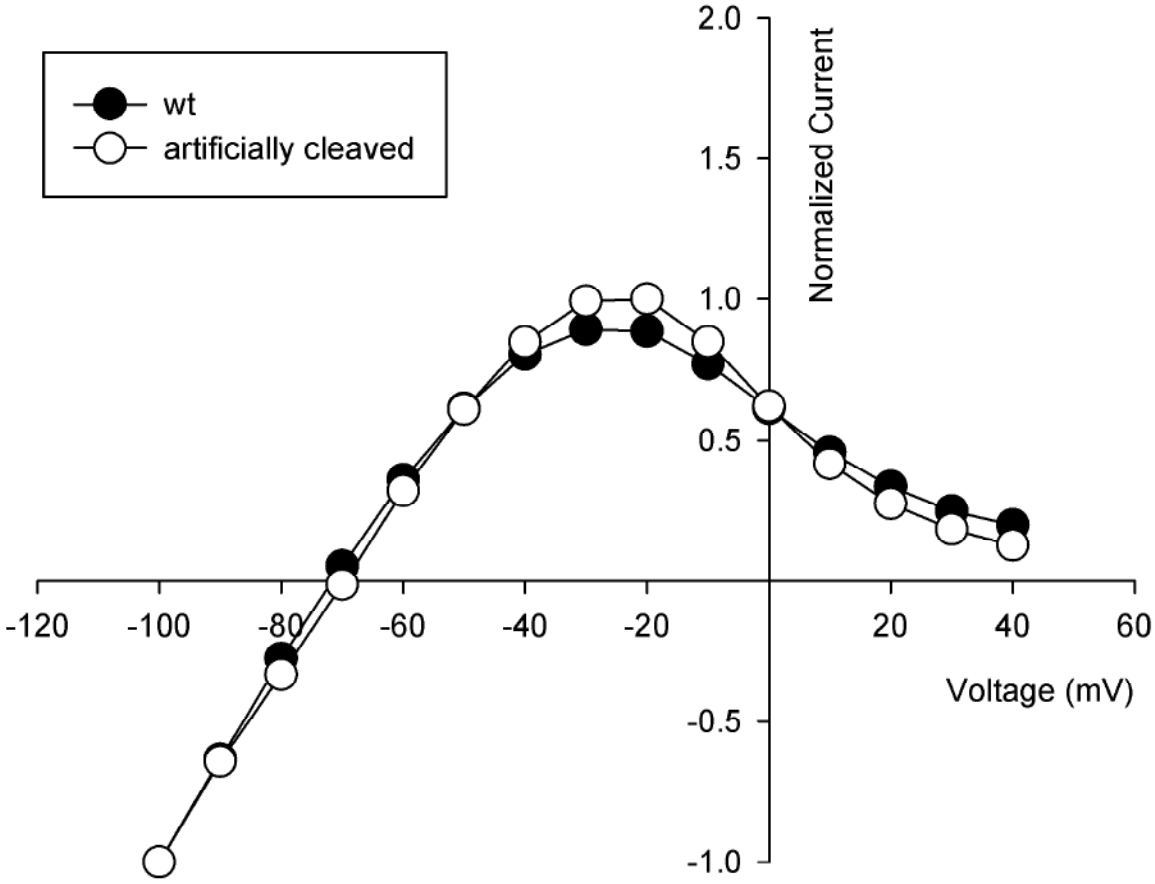

Fig. S2: Comparison of currents from wild-type and artificially cleaved CNGK channels. Current-voltage (IV) relation of mean whole-cell currents of wild-type channels (filled circles) and channels that formed during coexpression of constructs coding for the repeats 1-3 and repeat 4 (open circles). The pipette solution consisted of (in mM): KCl 140, EGTA 0.1, HEPES 10, cGMP 0.01, pH 7.4 (KOH).

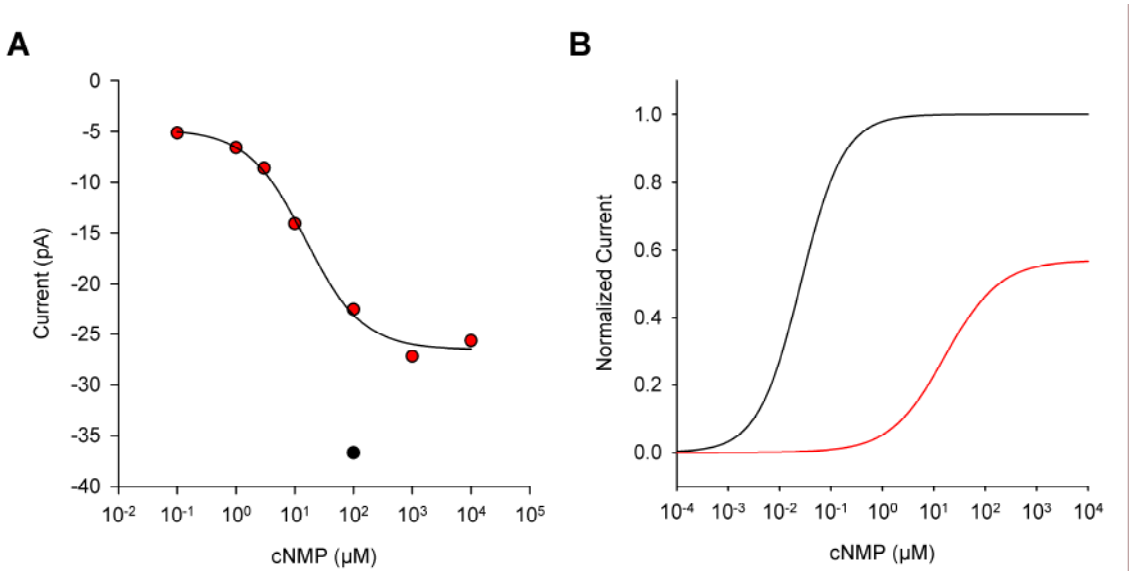

Fig. S3: Activation of CNGK channels by cAMP.

(A) Mean currents (at -80 mV) of an inside out patch activated by various concentrations of cAMP (red circles) or 100 µM cGMP (black circle). The solid line was calculated with the Hill equation $I = (I_{max} - I_{min}) * c^n / (c^n + K_{1/2}{}^n) + I_{min}$, with the following parameters: $K_{1/2}$ = 15.35 µM, n = 0.88, I_{max} = -26.5 pA, I_{min} = -4.8 pA. **(B)** Normalized mean activation curve of cGMP (black line) and cAMP (red line) calculated using mean $K_{1/2}$ and Hill coefficients (see text). Currents were normalized to the maximal currents in the presence of saturating concentrations of cGMP.