LEGEND TO SUPPLEMENTARY FIGURES:

Supplementary Figure S1.: Identification of S/Ts within the cytosolic regions of GIRK4, important for phosphorylation by PKA in-vitro.

Figure S1A.: Statistical analysis of ^{32}P incorporation into N-T, C-T and truncations of C-T of GIRK4. Numbers in upper case confer to the aminoacid range of the truncations within the hGIRK4 sequence (see also Figure 5). The number of individual phosphorylation experiments is shown in parenthesis above each bar. **, (***): the mean value deviates statistically significant from zero at the $p<0.01$ (0.001) level.

Figure S1B.: relative contribution of individual S/Ts to the total ^{32}P incorporation into GIRK4 N- and C-termini. Numbers confer to single aminoacid positions within the hGIRK4 sequence. The number of individual phosphorylation experiments is shown in parenthesis above each bar.

Supplementary Figure S2: Role of selected S/Ts in the heterologous facilitation of homooligomeric GIRK4* channels.

Figure S2A: Effect of mutation of single S/Ts on the ratio agonist vs. basal current ($\frac{I_{ACh}}{I_{HK}}$). 7-15 oocytes from three different batches were used for calculation of mean values. GIRK4* denotes the homooligomeric construct. ***: the mean value deviates significantly from GIRK4* at the $p<0.001$ level.

Figure S2B: Effect of mutation of single S/Ts on the ratio of I_{cAMP} *vs.* I_{ACh} (cAMP was injected during agonist application). Since mutation of S75, S191, T199, S412 and S418 exhibited profound and differential effects on the ratio I_{ACh}/I_{HK} (see panel S2A), cAMP effects were normalized to the size of I_{ACh} of the corresponding mutation and oocyte, in order make the results better comparable. Same oocytes as in S2A. *: the mean value deviates significantly from GIRK4* at the $p<0.05$ level.

SUPPLEMENTARY TABLES:

Supplementary Table S1.:

Primer	Sequence
S57C_f	5‘-CATGGAGAAGTGCGGCAAGTGCAACGTGCAGCACGGC-3‘
S57C_r	5‘-GCCGTGCTGCACGTTGCACTTGCCGCACTTCTCCATG-3‘
S75C_f	5‘-GACCTACCGGTATCTGTGTGACCTCTTCACCACCC-3‘
S75C_r	5‘-GGGTGGTGAAGAGGTCACACAGATACCGGTAGGTC-3‘
S191C_f	5‘-GTCAAGATCTGCCAGCCCAAGAAGAGAGC-3‘
S191C_r	5‘-GCTCTCTTCTTGGGCTGGCAGATCTTGAC-3‘
T199C_f	5‘-AAGAGAGCGGAATGCCTCATGTTTTCCAAC-3‘
T199C_r	5‘-GTTGGAAAACATGAGGCATTCCGCTCTCTT-3‘
S227C_f	5‘-GGTGGGCGACCTACGTAACTGCCACATCGTGGAGGCC-3‘
S277C_r	5‘-GGCCTCCACGATGTGGCAGTTACGTAGGTCGCCCACC-3‘
T244C_f	5‘-CAAGTCCCGGCAGTGCAAAGAGGGGGAATTCATCCCCC-3‘
T244C_r	5‘-GGGGGATGAATTCCCCCTCTTTGCACTGCCGGGACTTG-3‘
T255C_f	5‘-CTGAACCAGTGTGGATATCAACGTGG-3‘
T255C_r	5‘-CCACGTTGATATCCACACTGGTTCAG-3‘
S277C_f	5‘-GCCTCTTCCTGGTGTGTCCTCTAATCATCTCCC-3‘
S277C_r	5‘-GGGAGATGATTAGAGGACACACCAGGAAGAGGC-3‘
S284A_f	5‘-GATCAACGAGAGGGCCCCTTTCTGGGAGATG-3‘
S284A_r	5‘-CATCTCCCAGAAAGGGGCCCTCTCGTTGATC-3‘
S321C_f	5‘-GCCAAGCCCGGAGCTGCTACATGGATACAGAGG-3‘
S321C_r	5‘-CCTCTGTATCCATGTAGCAGCTCCGGGCTTGGC-3‘
T334C_f	5‘-CTGGGGCCACCGATTCTGCCCAGTCCTCACCTTGG-3‘
T334C_r	5‘-CCAAGGTGAGGACTGGGCAGAATCGGTGGCCCCAG-3‘
C382A_f	5‘-CCGGCTCCTCCAGTACCTCCCCGCCCCACCACTGCTG-3‘
C382A_r	5‘-CAGCAGTGGTGGGGCGGGGAGGTACTGGAGGAGCCGG-3‘
S412C_f	5‘-GCTGGGTGGGTGCAGGGTGGCCAGGG-3‘
S412C_r	5‘-CCCTGGCCACCCTGCACCCACCAGC-3‘
S418C_pGEX_f	5‘-GGGTCCAGGAGGCCCGGGGCTGCGTGTGAGCTCGAGCGGCCG-3‘
S418C_pGEX_r	5‘-CGGCCGCTCGAGCTCACACGCAGCCCCGGGCCTCCTGGACCC-3‘
S418A_mxt_f	5‘-GAGGCCAGGGGCGCCGTGTGAATCACTCAGC-3‘
S418A_mxt_r	5‘-GCTGAGTGATTCACACGGCGCCCCTGGCCTC-3‘

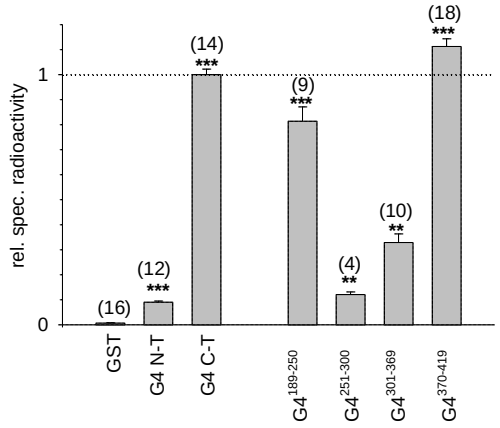
Sequence of the primers used for site directed mutagenisis. For S418 two different pairs of primers were used, depending whether the insert was located in pGEX-4T1 or the pMXT vector.

Supplementary Table S2.:

Sequence of the primers used for generation of truncated GST fusion protein encoding inserts.

Primer	Sequence
GIRK4^{NT}_f	5‘-ATAGGATCCATGGCTGGCGATTCTAGGAATG-3‘
GIRK4^{NT}_r	5‘-TTACTCGAGGTTGAAGCGCCACTTGAGGTCCAC-3‘
GIRK4$^{189-250}$_f	5‘-ATAGGATCCGTCAAGATCAGCCAGCC-3‘
GIRK4$^{189-250}$_r	5‘-TTACTCGAGGGATGAACTCCCCCTCTT-3‘
GIRK4$^{251-300}$_f	5‘-ATAGGATCCCTGAACCAGACAGACATCAA-3’C
GIRK4$^{251-300}$_r	5‘-TTACTCGAGACCACAACTTCAAACTCTTC-3‘
GIRK4$^{301-369}$_f	5‘-ATAGGATCCCTAGAAGGGATGGTGGAAGC-3‘
GIRK4$^{301-369}$_r	5‘-TTACTCGAGCTGGGTGTGTTGGTCTCATA-3‘
GIRK4$^{370-419}$_f	5‘-ATAGGATCCTGCTGTGCCAAGGAGCTGGCAGA-3‘
GIRK4$^{370-419}$_r	5‘-TTACTCGAGCTCACACCGAGCCCCTGGCCTCC-3‘

A



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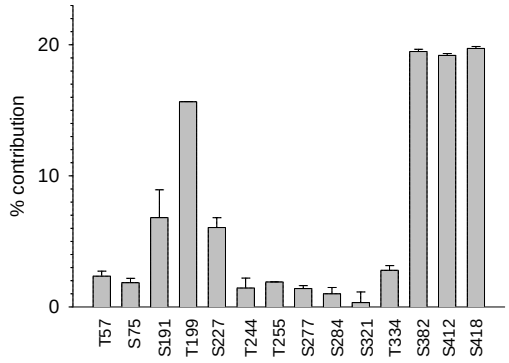

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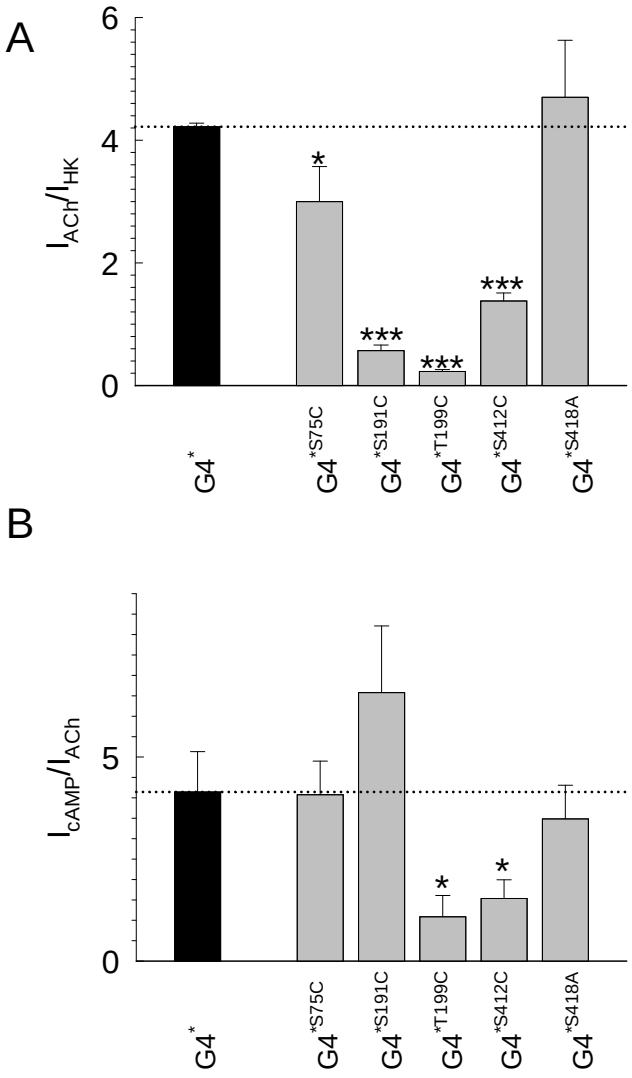

Treiber et al., suppl. Figure S2