

SUPPLEMENTAL DATA

Supplemental Table 1. **Quantitation of FRET by three different methods.** Values are given as mean \pm S.E.M.; *, $p < 0.05$ versus AKAP79 and NKA α 1 control; ^s, $p < 0.05$ versus the corresponding deletion (AC5 and AKAP79 Δ B or PKA RII and AKAP79 Δ PKA).

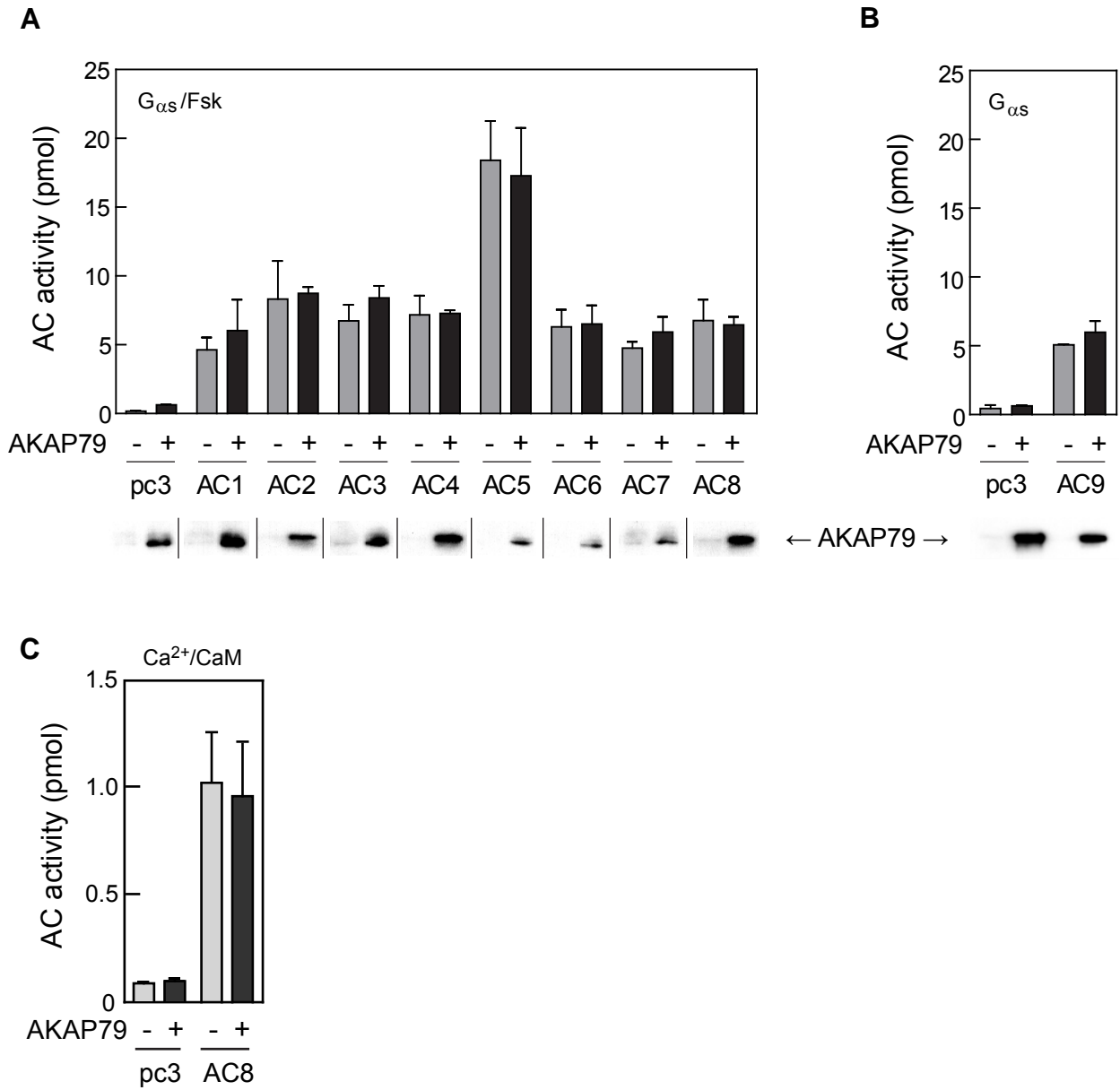
Supplemental Figure 1. **Total AC activity associated with overexpression of AC isoforms 1-9.** HEK293 cells were transfected with AC isoforms 1 through 9 +/- AKAP79-FLAG. Samples were immunoprecipitated with anti-FLAG agarose. The starting extracts (Sup. Fig. 1) and immunoprecipitations (shown in Fig. 1A) were assayed for AC activity stimulated with 50 nM G α s and 100 μ M forskolin (**A**) or 200 nM G α s (**B**). The overexpression of each AC isoform was confirmed by the increase in AC activity compared to vector alone. AKAP79 expression was confirmed by Western blotting.

Supplemental Figure 2. **FLIM-FRET of AKAP79 Δ PKA-CFP and PKA RII-YFP.** HEK293 cells expressing AKAP79 Δ PKA-CFP alone or with PKA RII-YFP were imaged in the frequency domain in a wide-field FLIM microscope. Deletion of the PKA binding domain (AKAP79 Δ PKA-CFP) abolishes a change in lifetime when co-expressed with PKA RII-YFP. Graphical quantitation of CFP-tagged protein lifetimes is shown (mean \pm S.E.M.; $n = 3$; 60-120 cells).

Supplemental Table 1

Donor Protein	Acceptor Protein	FRET ^N	N _{FRET}	FRET ^C /I _{CFP}
AKAP79	NKA α 1	0.028 \pm 0.005	0.430 \pm 0.023	0.788 \pm 0.112
AKAP79	AC5	0.067 \pm 0.012* ^{\$}	1.003 \pm 0.102* ^{\$}	1.812 \pm 0.283* ^{\$}
AKAP79 Δ B	AC5	0.037 \pm 0.006	0.576 \pm 0.053*	1.013 \pm 0.124
AKAP79	PKA RII	0.076 \pm 0.012* ^{\$}	1.179 \pm 0.087* ^{\$}	2.092 \pm 0.250* ^{\$}
AKAP79 Δ PKA	PKA RII	0.034 \pm 0.005	0.521 \pm 0.032*	0.932 \pm 0.107

Supplemental Figure 1



Supplemental Figure 2

