

SUPPLEMENTARY ONLINE DATA

Muscarinic receptors stimulate AC2 by novel phosphorylation sites, whereas $G\beta\gamma$ subunits exert opposing effects depending on the G-protein source

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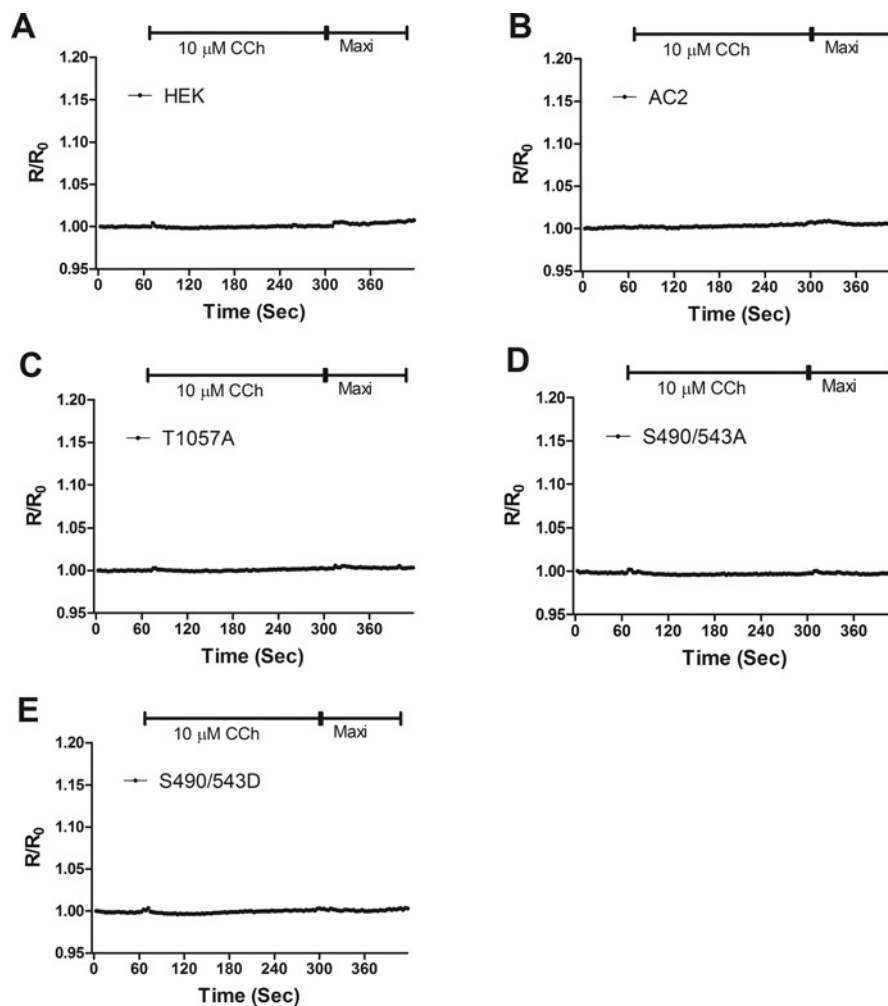


Figure S1 Changes in the CFP/YFP response of the cells

Changes in the CFP/YFP response (R/R_0) of HEK-293 cells (A) or the indicated AC2 constructs (B–E) stable cells transiently transfected with global ('dead') Epac2^{R297E} in response to 10 μM CCh. Maximal change in R/R_0 (Maxi) was sought using 100 μM IBMX, 10 μM FSK and 10 μM Iso at 300 s for 2 min. Results are means ± S.E.M.

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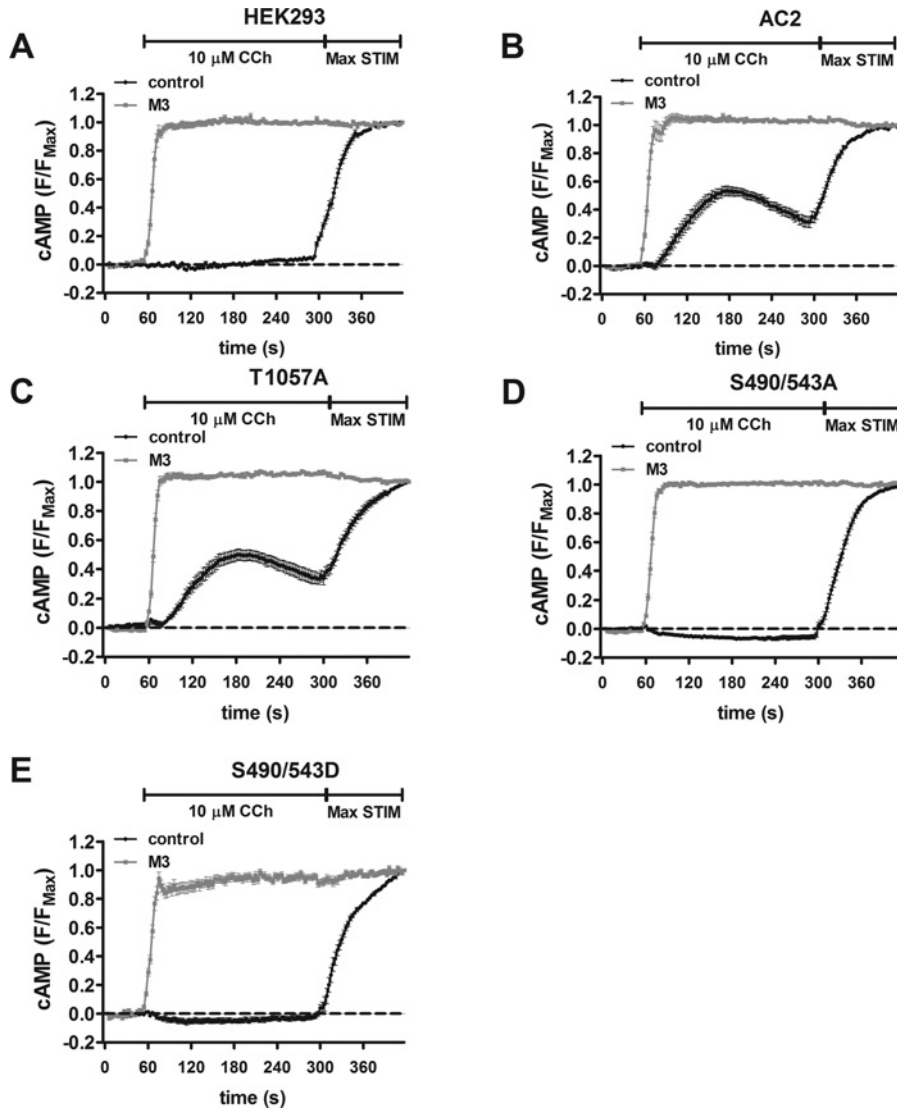


Figure S2 Effects of overexpressing the M3 receptor on CCh-mediated cAMP activity in single cells

Untransfected HEK-293 cells (A) and cells stably expressing wild-type AC2 (B), T1057A-AC2 (C), phosphorylation-deficient mutant S490/543A-AC2 (D) and phosphomimetic S490/543A-AC2 (E) transfected with or without the M3 receptor were stimulated with 10 μM CCh at 60 s for 4 min. Results are means ± S.E.M.

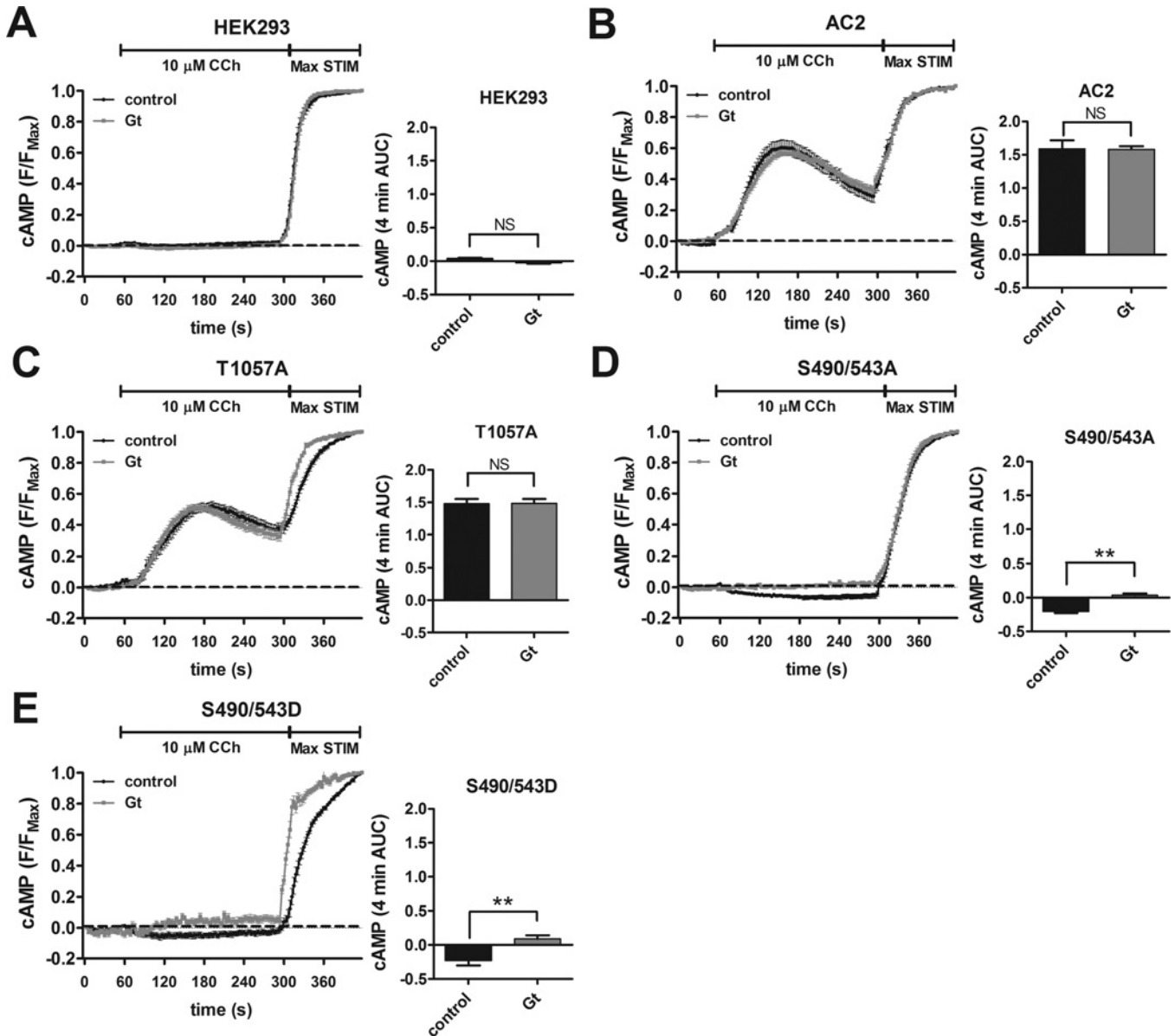


Figure S3 Effects of overexpressing $G_{i\alpha}$ (transducin) on CCh-mediated cAMP activity in single cells

Untransfected HEK-293 cells (**A**) and cells stably expressing wild-type AC2 (**B**), T1057A-AC2 (**C**), phosphorylation-deficient mutant S490/543A-AC2 (**D**) and phosphomimetic S490/543A-AC2 (**E**) transfected with or without $G_{i\alpha}$ were stimulated with 10 μ M CCh at 60 s for 4 min. The time course of cAMP production is shown in the left-hand panel and the 4 min AUC (calculated from the left-hand panel) is on the right-hand side. Results are means \pm S.D. * P < 0.05 and ** P < 0.01 using Student's t test. Max STIM, saturation of the sensor; NS, not significant.

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