nature cell biology

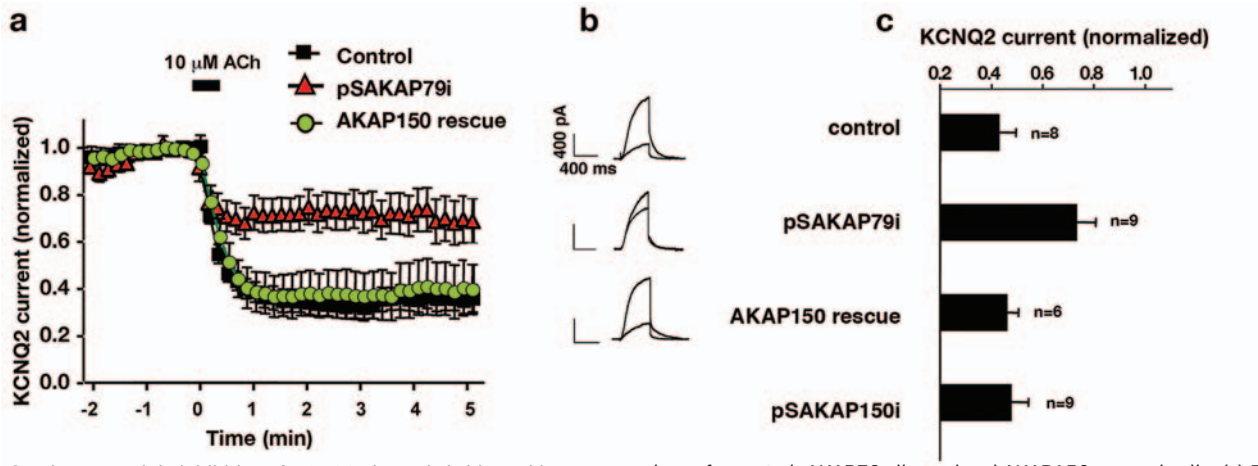

Figure S1 The muscarinic inhibition of KCNQ2 channels is blunted in AKAP79 gene-silenced HEK293 cells. RNAi knockdown and cell selection was as described in Fig. 1B. Cells were then transfected with KCNQ2 and GFP, plus or minus AKAP150. (a) Electrophysio-logical recording of the KCNQ2 response to 10 mM acetylcholine. Control cells show muscarinic inhibition of KCNQ2 (black squares, n = 8), which is ablated in AKAP79 silenced cells (red triangles, n = 9). Inhibition of the response is rescued in AKAP150 expressing cells (green circle, n = 6). (b) Representative traces at time 0 and 1 minute after the application of 10 μM acetylcholine are shown for control, AKAP79 silenced and AKAP150 rescued cells. (c) For all experimental conditions the amalgamated data depicting normalized current (relative to time 0) taken 1 minute after the application of agonist is shown. An additional control group of cells transfected with the pSAKAP79i construct specific for the murine ortholog is also shown (n=9). The statistical significance of electrophysiology data shown in this figure part c was calculated using ANOVA followed by two-tailed Student's t-test. Statistical significance of pSAKAP79i vs control has P value=0.011. Error bars indicate S.E.M.

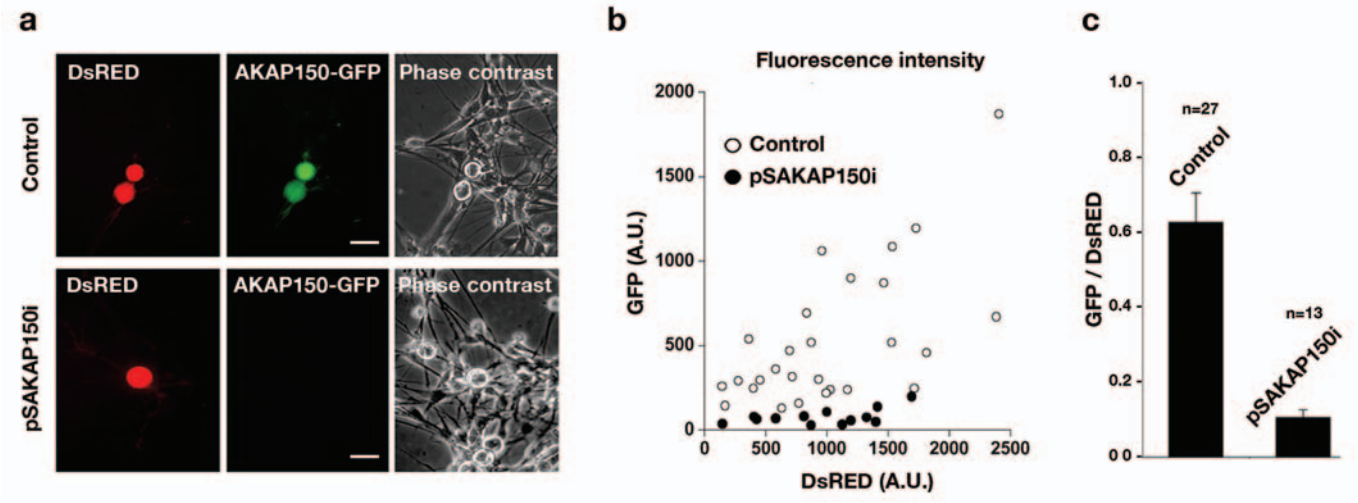

Figure S2 Gene silencing of AKAP150 in SCG. Cultured superior cervical ganglion (SCG) neurons nuclear injected with AKAP150-GFP and DsRED2 expression constructs were coinjected either withpSAKAP150i or without pSAKAP150i (control). (a)Three days after injection, images of the cells were collected using a Zeiss Axiovert135TV and MetaMorph 4.6 (scale bar equals 20 μm). (b) The fluorescent intensity of DsRED and AKAP150-GFP within a defined region of interest in each cell body was measured and plotted. The GFP fluorescence in the AKAP150 silenced neurons was minimal regardless of the injection volume as detected by DsRED expression levels. (c) Amalgamated data from (b) presented as the ratio of AKAP150-GFP/DsRED fluorescent intensity shows gene silencing of AKAP150 in pSAKAP150i injected neurons. (P<0.0001, two-tailed Student's t-test)

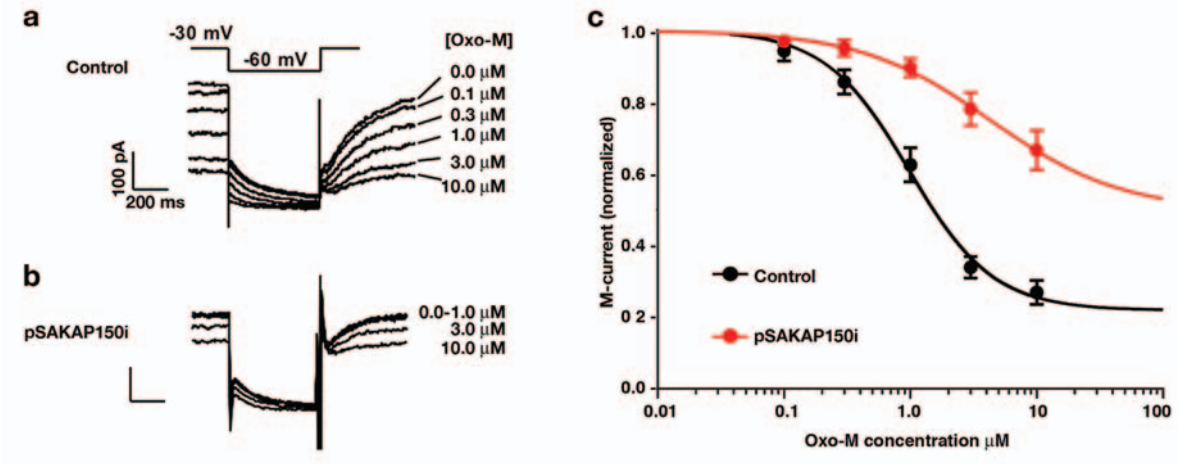

Figure S3 AKAP150 is required for Oxo-M suppression of M current. Muscarinicinhibition of M-current in SCG neurons was recorded three days after injection with pSAKAP150i. Recordings were taken at 1 minute intervals following each step of the sequential application of 0.0, 0.1, 0.3 1.0, 3.0, and 10 μM Oxo-M. Representative traces of this dose-response in (a) control and (b) AKAP150 silenced neurons are shown. (c) Amalgamated data from control (n = 7) and pSAKAP150i (n = 13) experiments show blunting of the muscarinic inhibition of M current in the AKAP150 silenced cells when compared to controls. Error bars indicate S.E.M. Curves show the best fit to Hill equation,

$$y = 1 - R_{\max} \frac{[L]^n}{EC_{50}{}^n + [L]^n}$$

where y is the normalized response, R_{max} is fraction of maximal response, [L] is the ligand concentration, n is the Hill coefficient. The fitted values are as follows; EC_{50} = 0.97 μM, n = 1.3, R_{max} = 0.78 for control and EC_{50} = 4.3 μM, n = 0.84, R_{max}= 0.5 for AKAP150 silenced cells. Knockdown of AKAP150 reduced the population of M currents that can respond to Oxo-M, suggesting that endogenous AKAP150 does not merely increase the sensitivity of the response in SCG but is required for the muscarinic inhibition of M current.

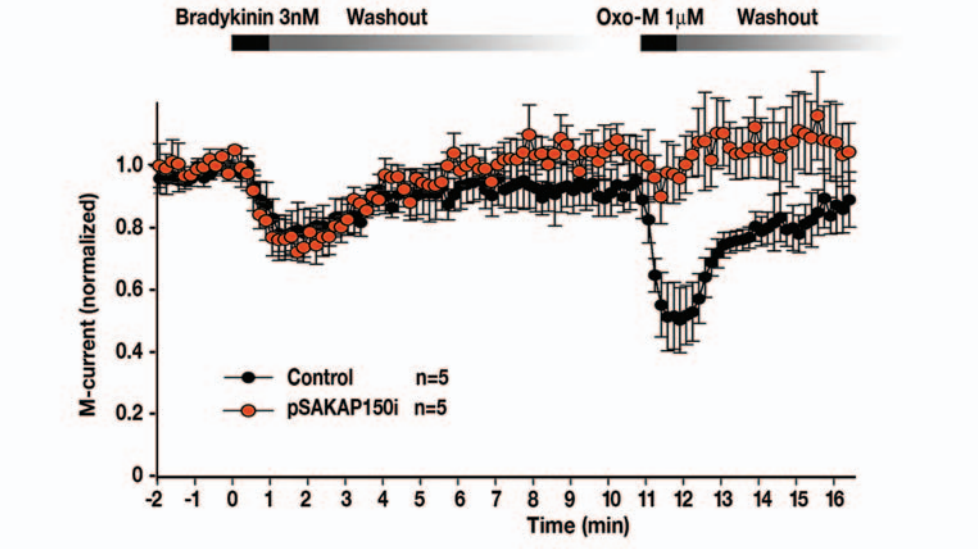

Figure S4 The M current inhibition that is induced by 3 nM bradykinin mirrors the response with a higher dose. M currents recorded from control (black circles, n = 5) and AKAP150 silenced (red circles, n = 5) SCG neurons. Cells were treated with 3 nM bradykinin at time 0 for 1 min. After a recovery period of 10 min a second train of agonist, Oxo-M (1 M, 1 min) was applied to the same cells. Responses to this concentration of bradykinin which is near its EC_{50} (~ 1 nM) were essentially the same as those presented in Fig. 6e. This data provides additional evidence that AKAP150-silenced neurons are responsive to physiological concentrations of bradykinin and that this agonist suppresses M currents via an AKAP150 independent pathway. Error bars indicate S.E.M.