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Supplementary Materials for

Rapgef2 Connects GPCR-Mediated cAMP Signals to ERK Activation in Neuronal and Endocrine Cells

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The PDF file includes:

Fig. S1

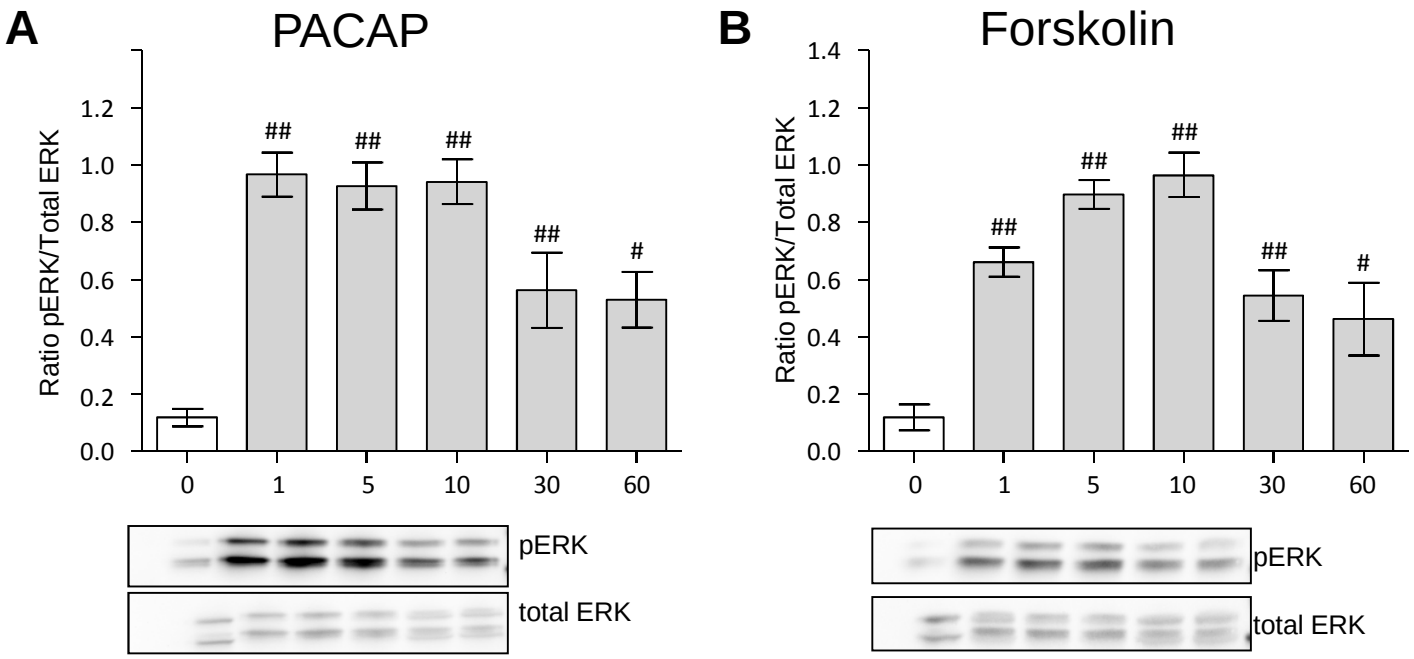

Fig. S1. Time course of cAMP-dependent ERK activation in bovine chromaffin cells. (**A** and **B**) Bovine chromaffin cells (BCCs) were treated for the indicated times (min) with either (A) 100 nM PACAP-38 or (B) 10 μM forskolin and then were analyzed by Western blotting with antibodies specific for phosphorylated ERK (pERK) and total ERK. Bar charts show data from the densitometric analysis of band intensities and are presented as mean ratios ± SEM of pERK abundance to that of total ERK protein from three experiments. #$P < 0.05$, ##$P < 0.01$, compared to time zero (Bonferroni-corrected multiple comparison tests). Western blots are representative of three experiments.

Fig. S2

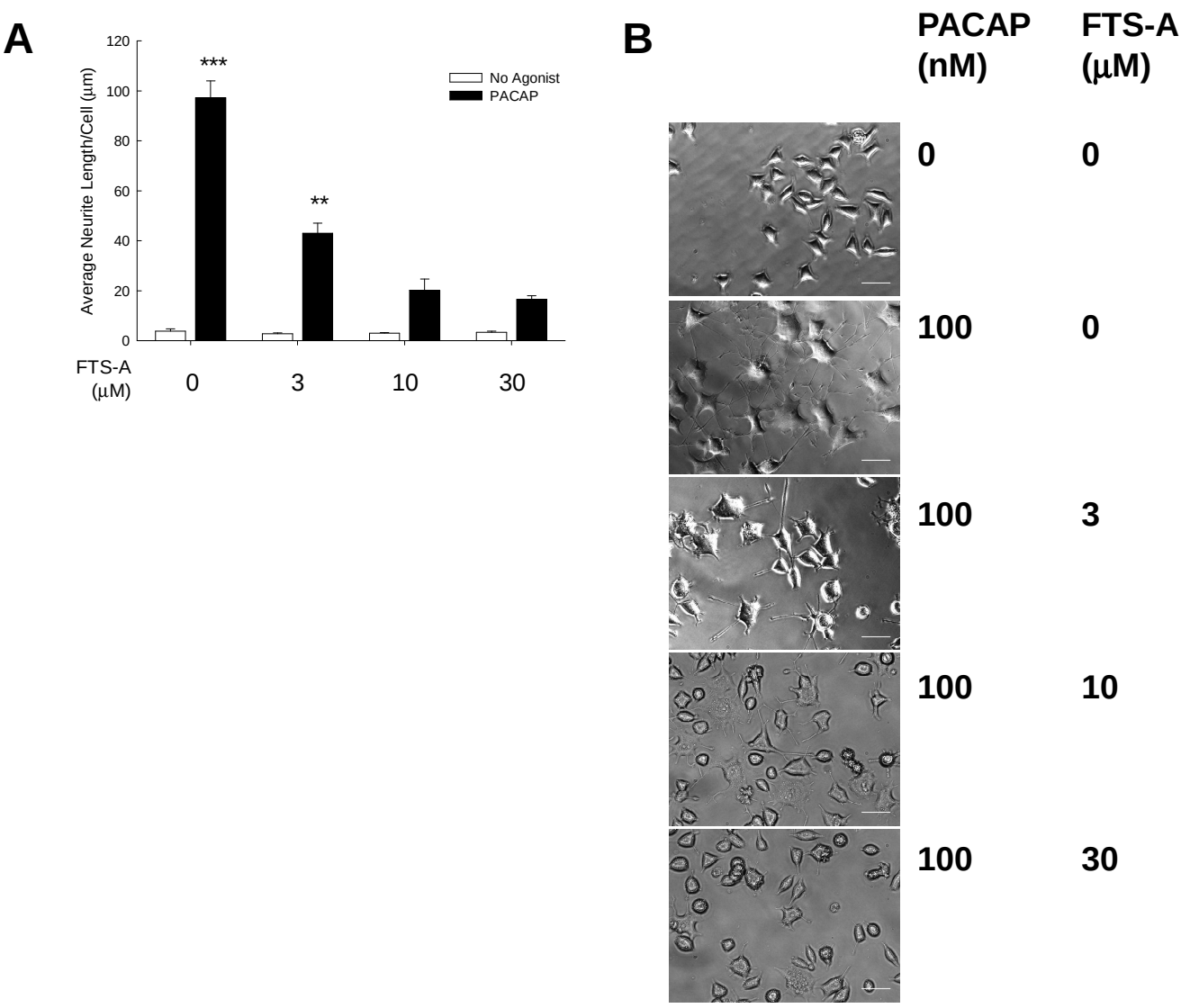

Fig. S2. PACAP-dependent neuritogenesis is sensitive to inhibition by FTS-A. (**A** and **B**) NS-1 cells were left untreated or were treated with 100 nM PACAP-38 for 48 hours in the absence or presence of the indicated concentrations of FTS-A. (A) Quantification of average neurite length from three neurite outgrowth assays. ***$P < 0.001$, **$P < 0.01$, (Bonferroni-corrected, pair-wise comparisons of cells treated with PACAP-38 in the presence of each concentration of FTS-A). (B) Representative photomicrographs from the neuritogenesis assays showing untreated cells and cells treated with the indicated concentrations of PACAP-38 and FTS-A. Scale bar: 50 μm.

Fig. S3

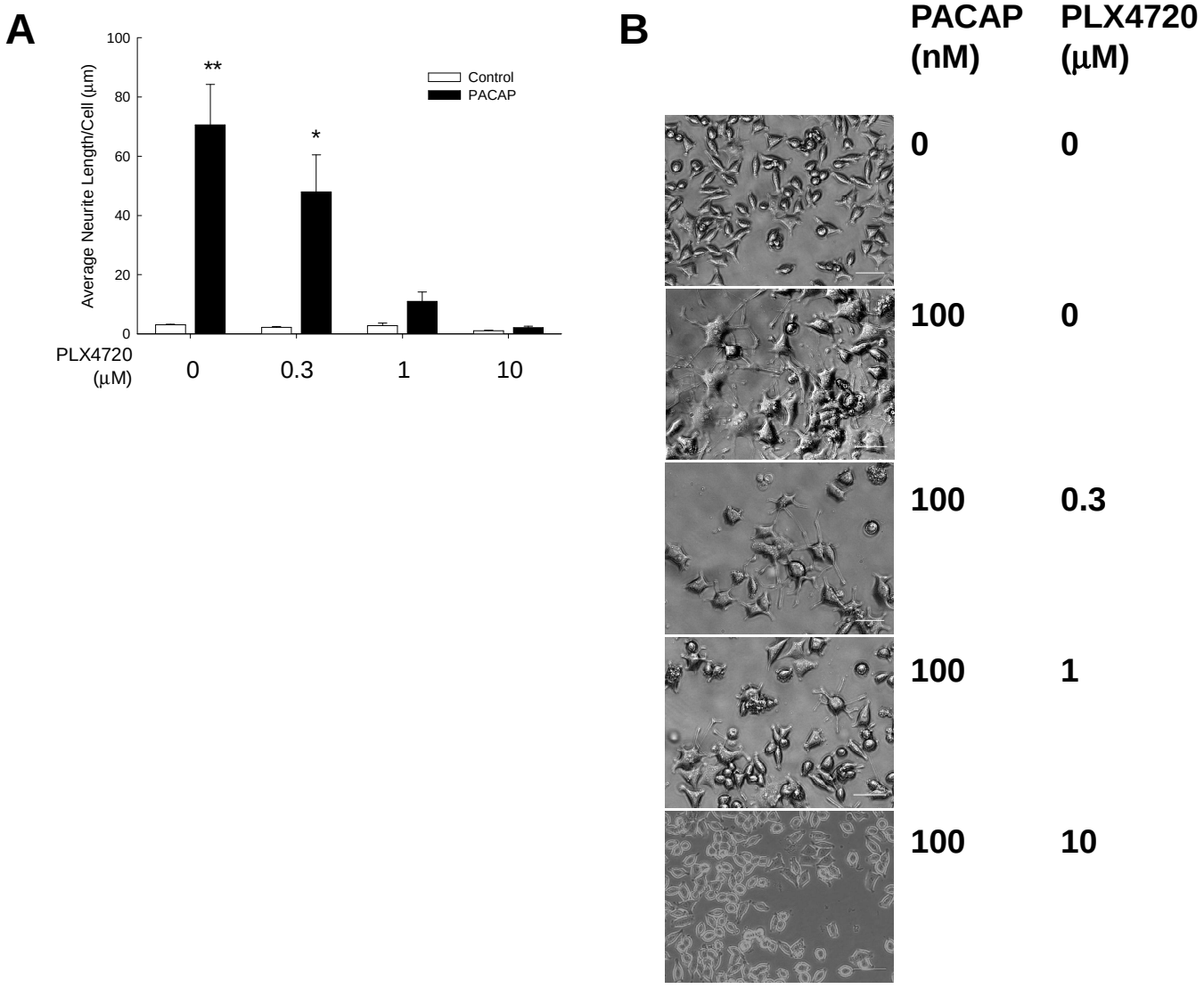

Fig. S3. PACAP-dependent neuritogenesis is PLX4720-sensitive. (**A** and **B**) NS-1 cells were left untreated or were treated with 100 nM PACAP-38 for 48 hours in the presence of the indicated concentrations of PLX4720. (A) Quantification of average neurite length from three neurite outgrowth assays. $^{**}P < 0.01$, $^{*}P < 0.05$ (Bonferroni-corrected, pair-wise comparisons of cells treated with PACAP-38 in the presence of each concentration of PLX4720). (B) Representative photomicrographs from neuritogenesis assays showing untreated cells and cells treated with the indicated concentrations of PACAP-38 and PLX4720. Scale bar: 50 μm.

Fig. S4

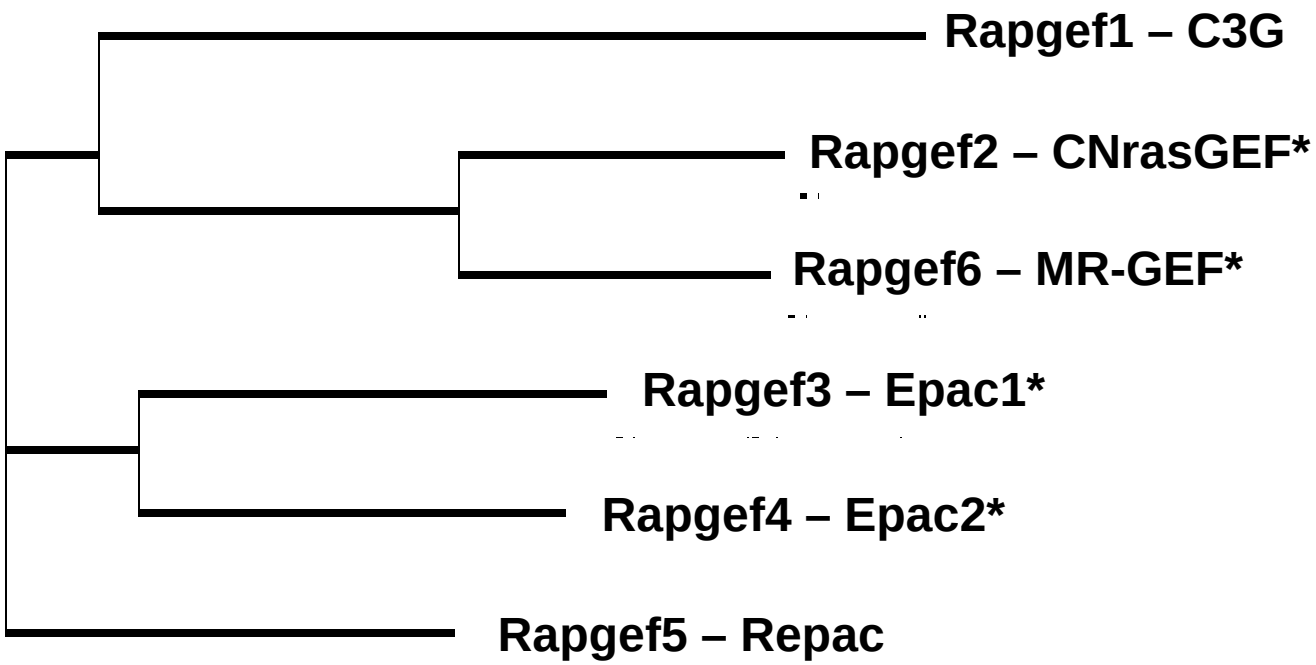

Fig. S4. Phylogenetic relationship of members of the human Rapgef protein family. Dendrogram from the ClustalW analysis of protein sequences of the indicated members of the human Rapgef family. *, protein sequences that contain a predicted cAMP-binding motif.

Fig. S5

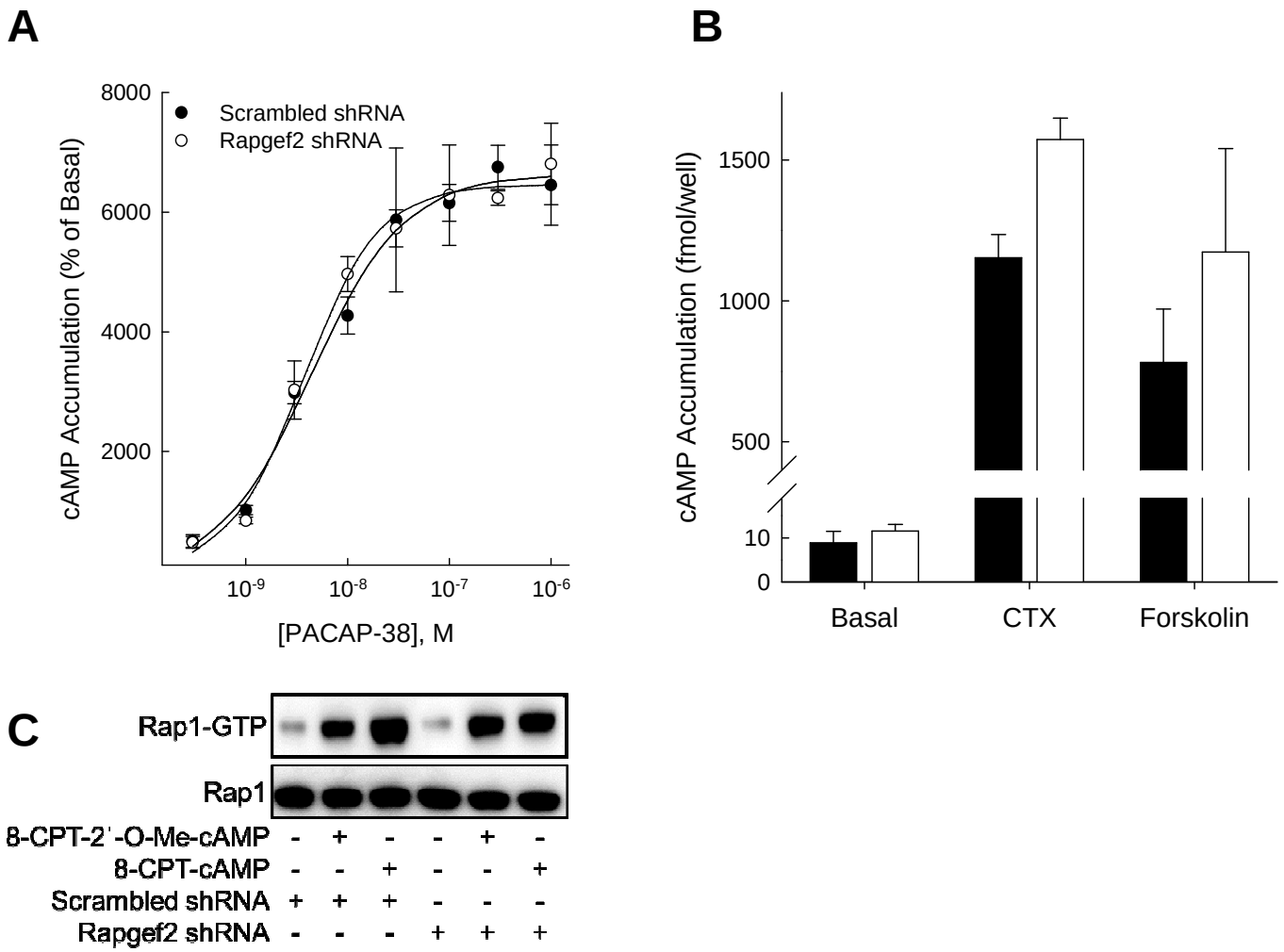

Fig. S5. Silencing of Rapgef2 does not affect cAMP accumulation or Epac activity in NS-1 cells. (**A** and **B**) Comparison of the extent of stimulus-dependent increases in cAMP concentration in NS-1 cells stably expressing scrambled shRNA or Rapgef2-specific shRNA. Cells were pretreated with 0.5 mM 3-isobutyl-1-methylxanthine for 30 min and then stimulated with (A) the indicated concentrations of PACAP-38 or (B) either CTX (50 μg/ml) or 25 μM forskolin. (**C**) Rap1 activation assays were performed with NS-1 cells expressing either scrambled shRNA or Rapgef2-specific shRNA. Cells were treated with either 100 μM 8-CPT-cAMP or 100 μM 8-CPT-2'-O-Me-cAMP for 10 min. Rapgef2-deficient cells exhibited similar amounts of Epac-dependent Rap1 activation relative to control cells expressing scrambled shRNA. Western blotting data are representative of four experiments with similar results.

Fig. S6

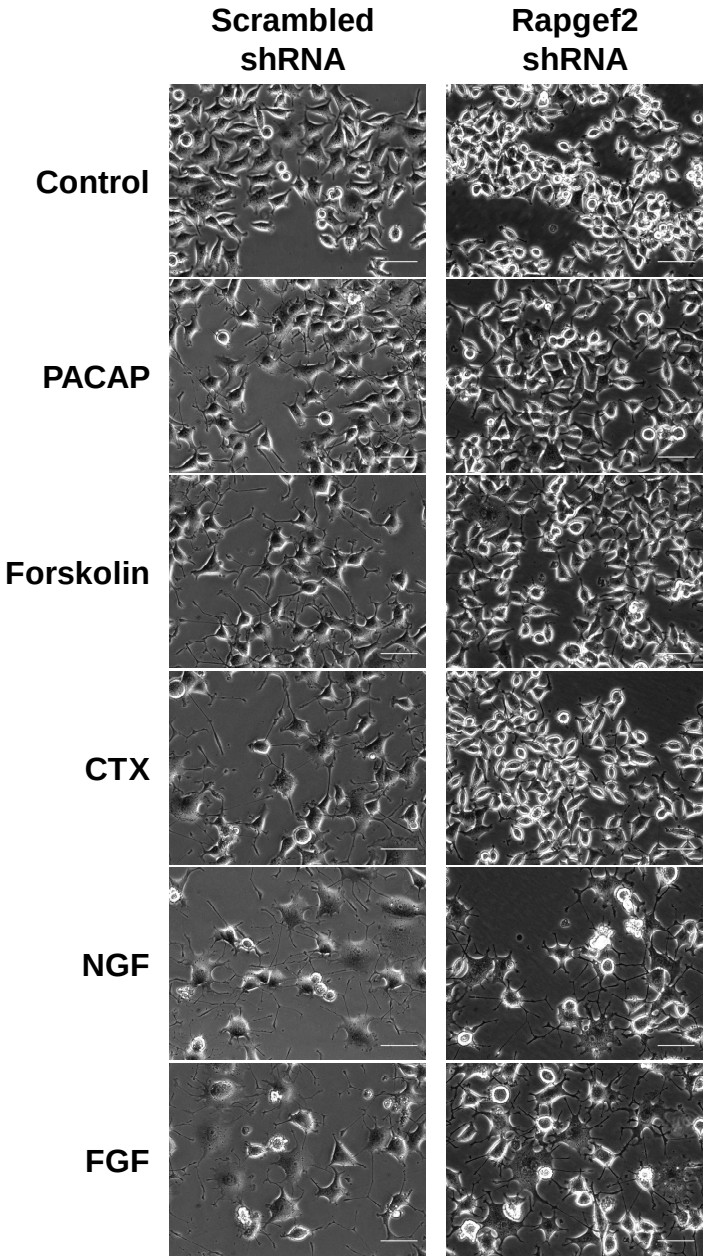

Fig. S6. Rapgef2 is required for cAMP-dependent neuritogenesis in NS-1 cells. Representative photomicrographs from neurite outgrowth assays. NS-1 cells stably expressing either scrambled shRNA or Rapgef2-specific shRNA were treated for 48 hours with 100 nM PACAP-38, 25 μM forskolin, CTX (50 μg/ml), NGF (100 ng/ml), or FGF (100 ng/ml). Scale bar: 50 μm. These images are representative of the experiments presented in Fig. 3C.

Fig. S7

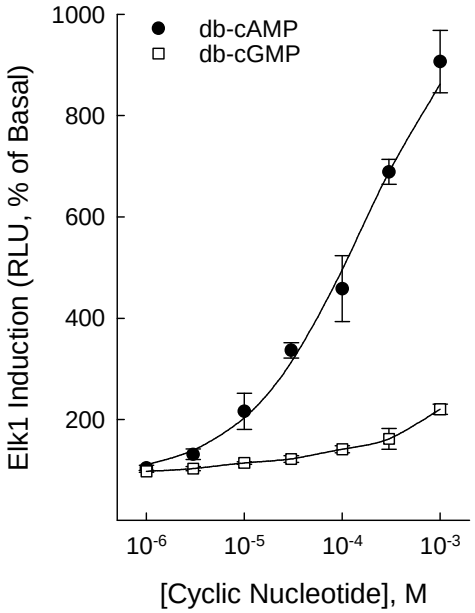

Fig. S7. MAPK -dependent Rapgef2 signaling is initiated by cAMP, but not cGMP. NS-1 cells expressing a Gal4-Elk1 fusion protein and a corresponding reporter gene were treated for 6 hours with varying concentrations of dibutyryl-cAMP (db-cAMP, filled circles) or dibutyryl-cGMP (db-cGMP, open squares). Data were normalized to the mean basal response of untreated cells, and data points represent means from three experiments with error bars corresponding to the SEM. Curves were fit to data points with 4-parameter logistic regressions.

Table S1. Efficacy of GPCR agonists and the AC activator forskolin in stimulating a CRE reporter gene. HEK 293T cells stably expressing CRE-luciferase were seeded in 96-well plates and treated as indicated. Luciferase activity was measured after 4 hours. All agents tested statistically significantly activated CRE-luciferase. ***$P < 0.001$, as determined by Bonferroni corrected *t*-tests comparing treated cells to untreated controls (basal). There was no statistically significant variance between the effects of the different agents tested as determined by Bonferroni-corrected *t*-tests comparing each condition.

CRE-luciferase					
Agonist	**μM**	**Response (RLU)**			***P***
		Mean	**SEM**	***N***	
Basal	-	275	22	3	
PACAP	0.1	34132	457	3	***
Isoproterenol	30	34963	1222	3	***
Forskolin	30	28860	838	3	***