

Supplementary Materials for

**The Complex of G Protein Regulator RGS9-2 and G $\beta_5$  Controls Sensitization and Signaling Kinetics of Type 5 Adenylyl Cyclase in the Striatum**

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Published 28 August 2012, *Sci. Signal.* **5**, ra63 (2012)  
DOI: 10.1126/scisignal.2002922

**The PDF file includes:**

Fig. S1. The N364H mutation abolishes GTPase activity of RGS9-2 and its effects on G protein subunit reassociation.

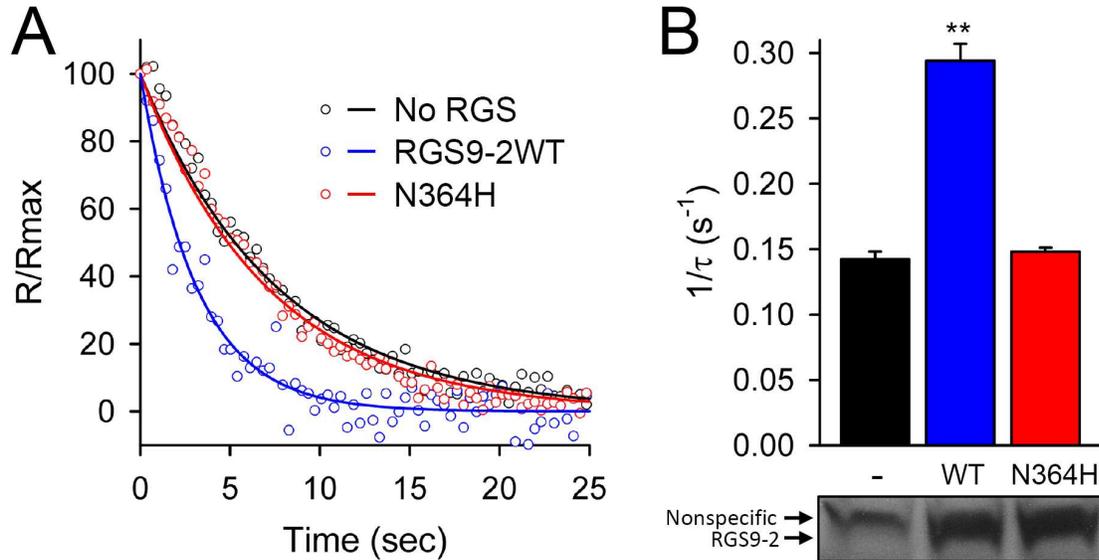
Fig. S2. Absence of AC superactivation by chronic stimulation of  $\mu$ -ORs in HEK293T cells lacking AC5.

Fig. S3. Equal expression of wild-type RGS9-2 and its N364H mutant upon expression in transfected HEK293 cells.

Fig. S4. Absence of a specific BRET signal in cells not transfected with  $\mu$ -OR or G $\alpha$ .

Fig. S5. Uniform expression of G $\alpha$  subunits in the BRET assay system.

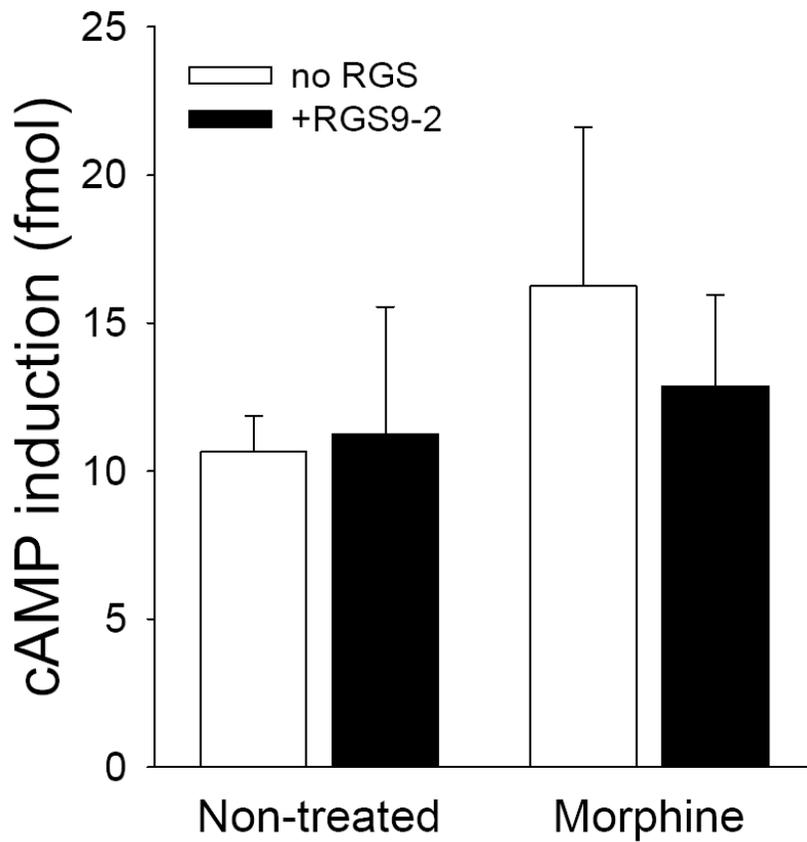
Fig. S6. Time course of changes in cAMP concentration induced by isoproterenol in transfected HEK293T cells.



**Supplemental Figure S1. The N364H mutation abolishes GTPase activity of RGS9-2 and its effects on G protein subunit reassociation.**

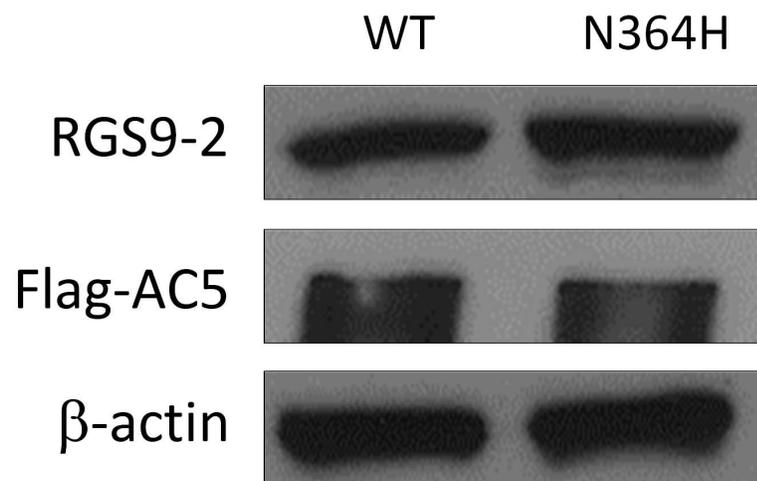
**A**, Effect of wild type and N364H mutant of RGS9-2/Gβ5/R7BP on deactivation kinetics of the Gβγ-mediated signaling triggered by MOR. Cells were transfected with MOR, Gβγ and GRK reporter constructs, as well as Gαo subunits, with or without RGS9-2/Gβ5/R7BP complexes. BRET signal following termination of MOR activation by adding antagonist naloxone averaged from 6 experiments is plotted as individual data points. Solid lines show fits of the deactivation phases by exponential function. The experiment is performed as described in Materials and Methods. For additional details refer to Fig. 5

**B**, Quantification of the deactivation time constant. Exponential fits of the data shown in panel A were used to derive time constant  $\tau$ . \*\*,  $p < 0.01$  comparing with no RGS group (one-way ANOVA followed by *post hoc* Dunnett's test. n=6).

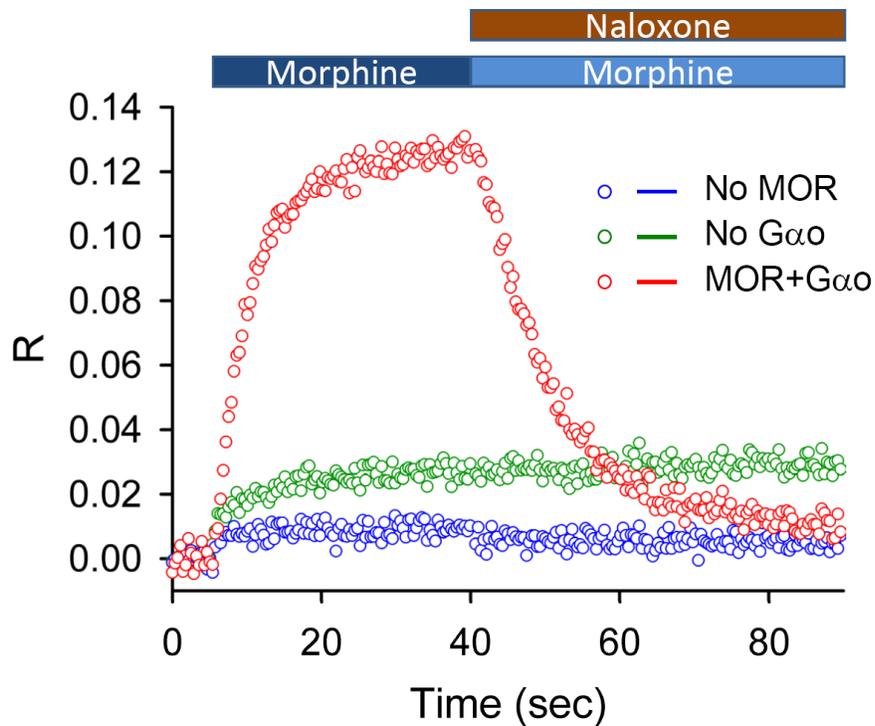


**Supplemental Figure S2. Absence of AC superactivation by chronic stimulation of  $\mu$ -ORs in HEK293T cells lacking AC5.**

HEK293T cells were transfected with MOR with or without RGS9-2/G $\beta$ 5/R7BP complex and treated with 1  $\mu$ M morphine overnight. The next day, media was changed and 5 minutes later the cells were harvested for the determination of the cAMP concentration.

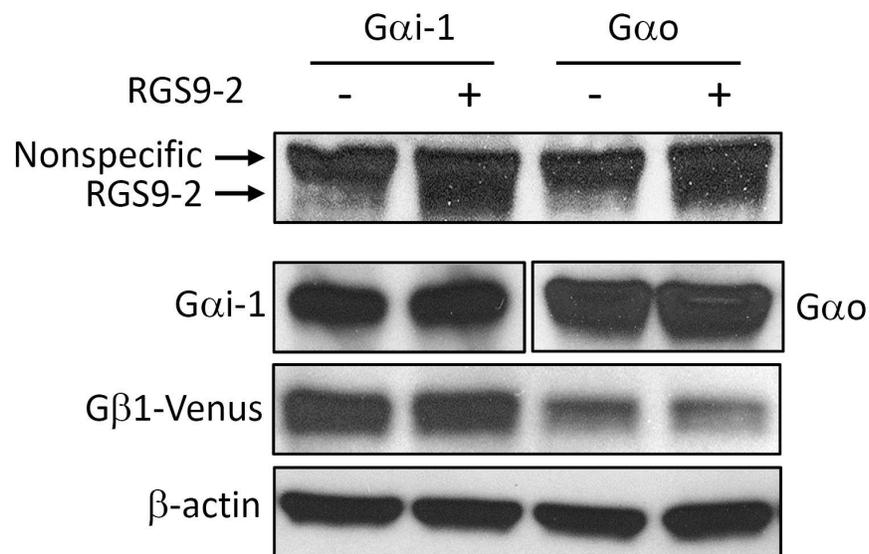


**Supplemental Figure S3. Equal expression of wild-type RGS9-2 and its N364H mutant upon expression in transfected HEK293 cells.** Cells were transfected with  $\mu$ -OR, Flag-AC5, G $\beta$ 5, R7BP and wild type (WT) or mutant (N364H) RGS9-2. Cell lysates were subjected to Western blotting with specific antibodies. N=3 experiments.



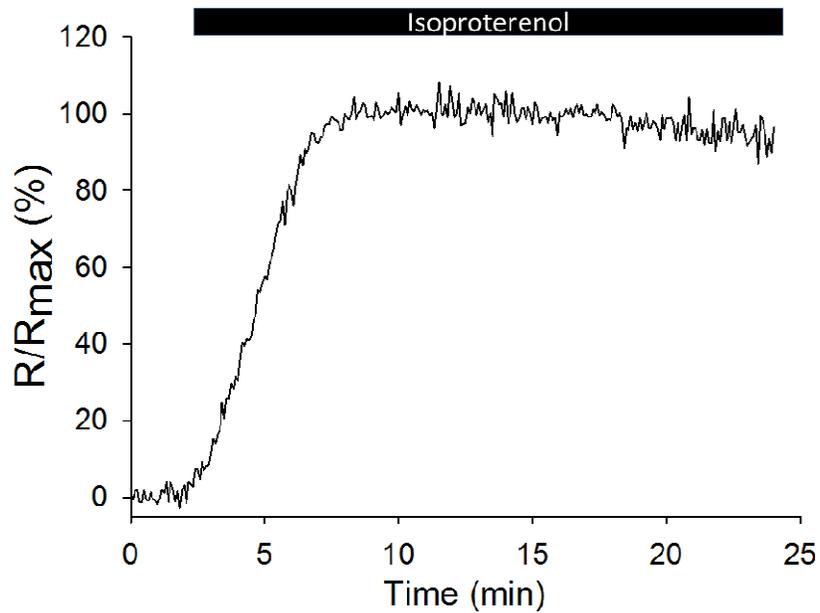
**Supplemental Figure S4. Absence of a specific BRET signal in cells not transfected with  $\mu$ -OR or  $G\alpha_o$ .**

HEK293T Cells were transfected with reporter constructs with or without MOR and/or  $G\alpha_o$  as indicated. BRET response was triggered by stimulation of MOR by 100 nM morphine followed by termination by adding 200  $\mu$ M antagonist naloxone. Response averaged from 4 experiments is plotted as individual data points.



**Supplemental Figure S5. Uniform expression of G $\alpha$  subunits in the BRET assay system.**

Cells were transfected with MOR, G $\beta\gamma$  and GRK reporter constructs, and indicated G $\alpha$  subunits, with or without RGS9-2/G $\beta$ 5/R7BP complex. Cell lysates were subjected to Western blotting with specific antibodies.



**Supplemental Figure S6. Time course of changes in cAMP concentration induced by isoproterenol in transfected HEK293T cells.**

FRET cAMP sensor, MOR, AC5, G $\alpha$ i-2, G $\beta$ 1, and G $\gamma$ 2 were transfected into HEK293T cells. Signal was monitored by the ratiometric imaging as described in the Materials and Methods. Isoproterenol was applied when indicated by the upper bar and consistently perfused throughout the experiment. Response before isoproterenol was set as 0 and cAMP changes were then normalized to the percentage of maximal response.