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online data

SUPPLEMENTARY ONLINE DATA

Interaction with receptor for activated C-kinase 1 (RACK 1) sensitizes the phosphodiesterase PDE4D5 towards hydrolysis of cAMP and activation by protein kinase C

Rebecca J. BIRD*, George S. BAILLIE† and Stephen J. YARWOOD*[1]

*Institute of Molecular Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K., and †Institute of Neuroscience and Psychology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

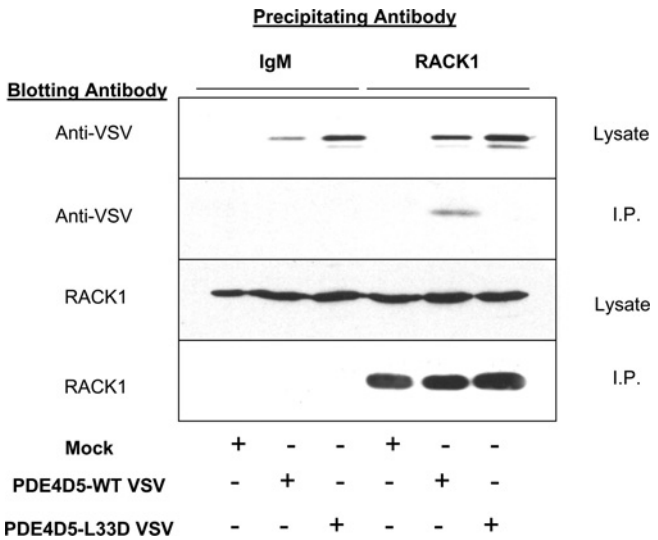

Figure S1 Wild-type PDE4D5, but not PDE4D5-L33D, associates with RACK1 in HEK-293 cells

Confluent HEK-293 cells were transiently transfected with VSV-tagged PDE4D5 wild-type (PDE4D5-WT) or VSV-tagged PDE4D5-L33D mutant (PDE4D5-L33D). Whole-cell lysates were then prepared and immunoprecipitated (I.P.) with control (IgM) antibody or anti-RACK1 antibody (IgM clone). Immunoprecipitates were then immunoblotted with anti-VSV antibodies, to detect PDE4D5 forms, or anti-RACK1 antibody. Results are representative of an experiment carried out on three separate occasions.

[1] To whom correspondence should be addressed (email stephen.yarwood@glasgow.ac.uk).

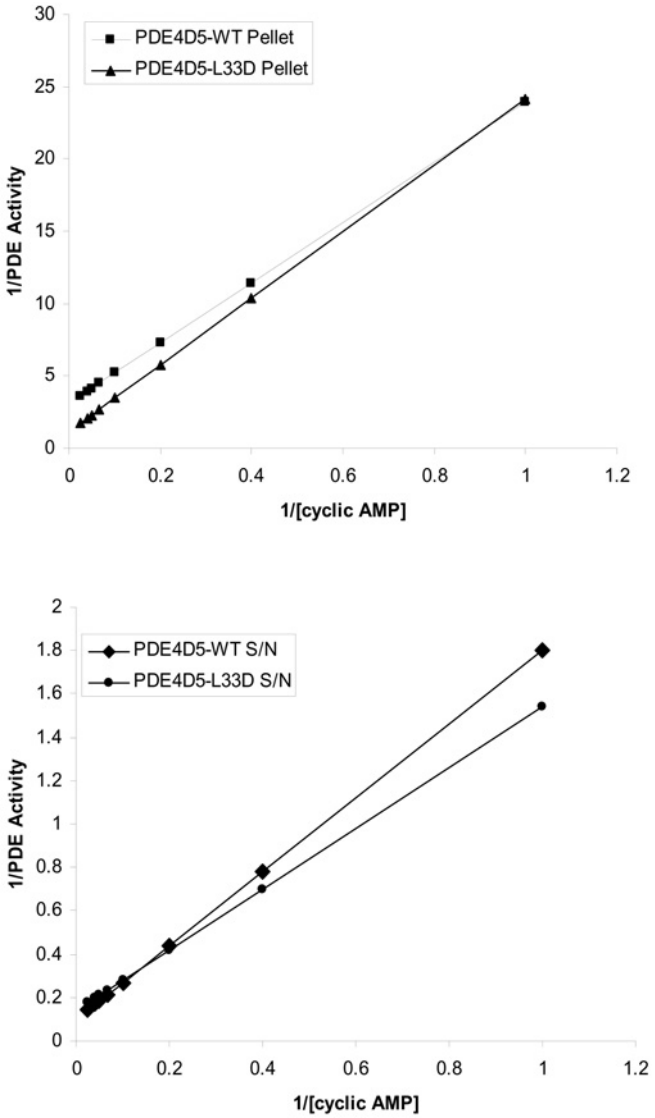

Figure S2 Lineweaver–Burk plots for PDE4D5-WT and PDE4D5-L33D

Particulate (pellet; upper panel) and soluble fractions (S/N; lower panel) from HEK-293 cells were transfected with cDNAs encoding PDE4D5-WT or PDE4D5-L33D and assayed for PDE activity in the presence of cAMP at concentrations between 1 μM and 40 μM. Mean PDE activities from three separate experiments were then used to produce Lineweaver–Burk plots for each fraction.

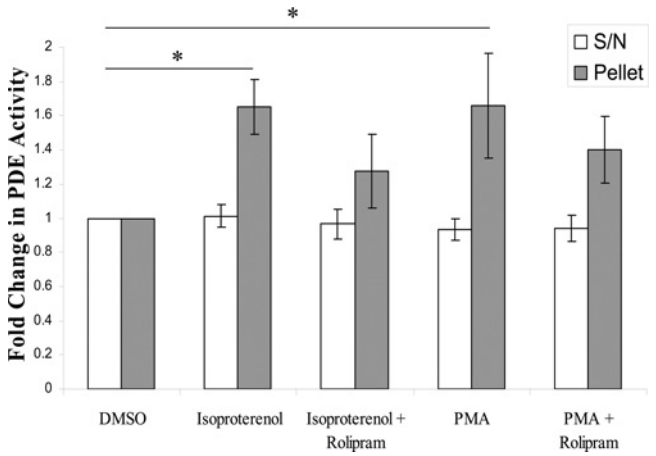

Figure S3 Isoproterenol and PMA stimulate PDE4 activity associated with RACK1 immunoprecipitates from HEK-293 cells

Confluent HEK-293 cells were stimulated with either 10 μM isoproterenol or 10 μM PMA and then fractionated into soluble (S/N) and particulate (pellet) fractions, which were then immunoprecipitated with anti-RACK1 antibodies. RACK1 immunoprecipitates were then assayed for associated PDE activity in the presence or absence of rolipram, as indicated. The results are means $\pm$ S.E.M. for three separate experiments and are plotted as the fold change relative to non-stimulated cells (DMSO). Significant differences relative to the control are indicated ($^*P < 0.01$).

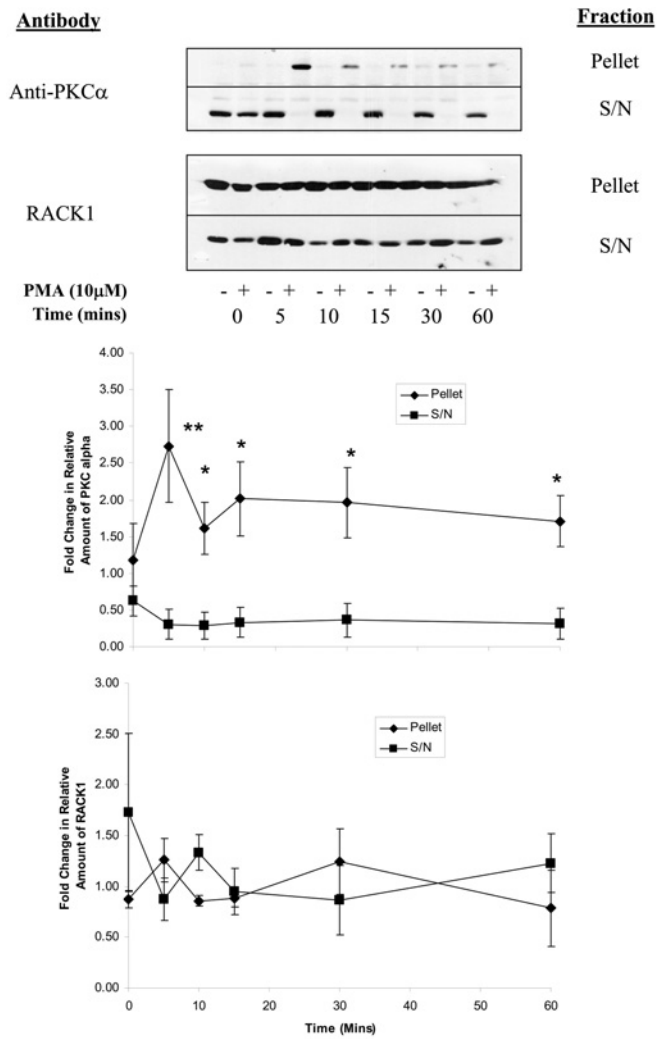

Figure S4 Stimulation of HEK-293 cells with PMA triggers intracellular translocation of PKCα, but not RACK1

HEK-293 cells were stimulated with 10 μM PMA for between 0 and 60 min, following which cells were fractionated into soluble (S/N) and particulate (pellet) fractions then immunoblotted with anti-RACK1 and anti-PKCα antibodies. The immunoblots show that PKCα translocates from the supernatant to the pellet fraction within 5 min of PMA stimulation. Densitometry was carried out on non-saturated immunoblots from three separate experiments and plotted as means $\pm$ S.E.M. Significant differences relative to non-stimulated control are indicated ($^{*}P < 0.05$ and $^{**}P < 0.01$).

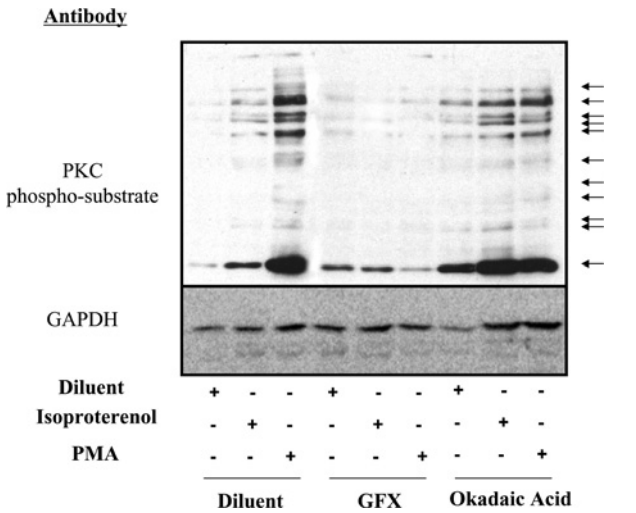

Figure S5 Stimulation of HEK-293 with ispoproterenol provokes serine phosphorylation of multiple intracellular proteins in a PKC-dependent manner

HEK-293 cells were stimulated for 15 min with either 10 μM isoproterenol or 10 μM PMA in the presence or absence of 10 μM GFX or 10 μM okadaic acid, as indicated. Cell lysates were then immunoblotted with an anti-PKC substrate phospho-specific antibody. The arrows on the right-hand side indicate immunoreactive protein species phosphorylated in response to isoproterenol that were both inhibited by GFX and enhanced by okadaic acid.

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