Differential effects of AGS3 expression on D_{2L} dopamine receptor-mediated adenylyl cyclase signaling

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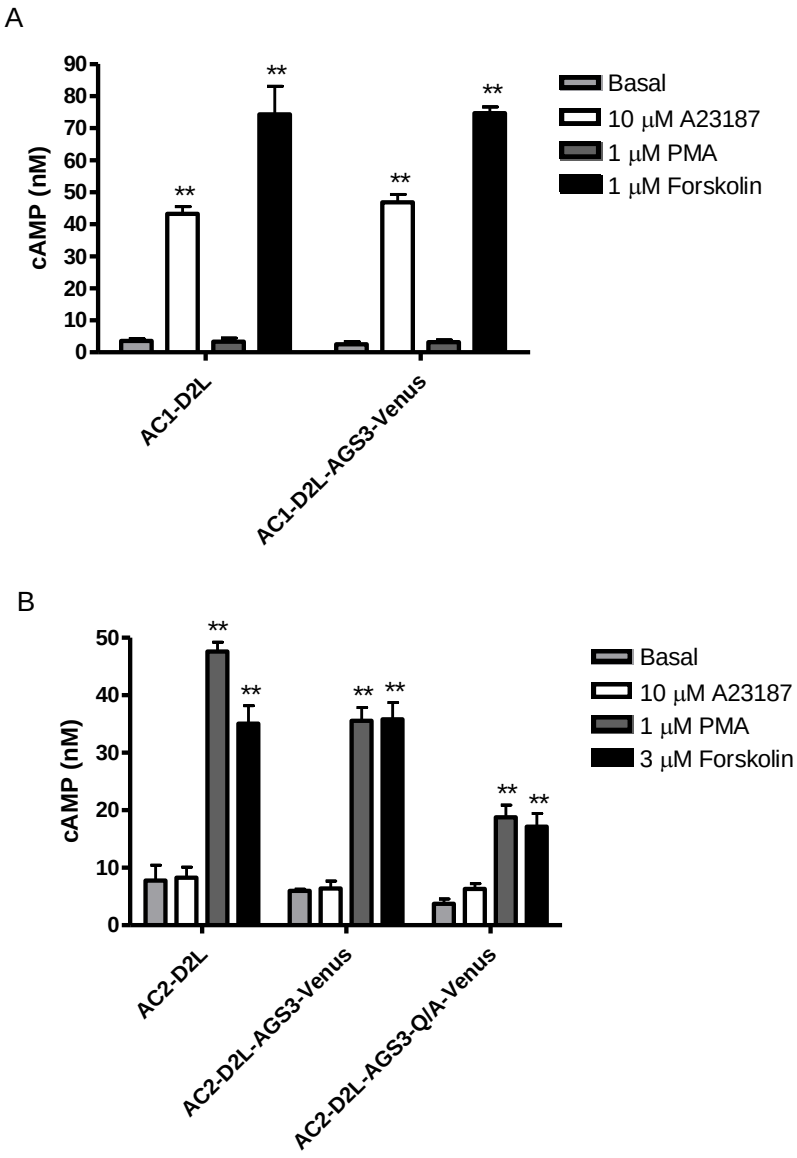
Online Resource 1 Selective activation of recombinant AC1 by the calcium ionophore, A23187 and recombinant AC2 by the phorbol ester, PMA. **A.** Cell lines expressing recombinant AC1 or **B.** recombinant AC2 were incubated under the indicated conditions and cAMP accumulation was measured using a Cisbio homogenous time-resolved fluorescent (HTRF) cAMP assay kit according to the manufacturer's protocol. Data represent the mean ± S.E.M. of three experiments. ** $p < 0.01$, compared to the basal condition within each cell line (one way ANOVA, followed by Dunnett's post hoc test).



Online Resource 2 D_{2L}DR expression levels in cellular models used for functional studies. [^{3}H]-Methylspiperone saturation binding assays and analysis was performed (as described in Przybyla and Watts, 2010) to measure the D_{2L}DR expression level in each cell line. Data represent the mean ± S.E.M. of four independent experiments

Cell Line	B_{max} *(pmol/mg protein)*	K_d *(nM)*
HEK-AC1-D_{2L}	2.5±0.10	0.10±0.02
HEK-AC1-D_{2L}-AGS3-Venus	4.0±0.27	0.11±0.02
HEK-AC2-D_{2L}	9.1±2.5	0.11±0.01
HEK-AC2-D_{2L}-AGS3-Venus	15±3.0	0.17±0.04
HEK-AC2-D_{2L}-AGS3-Q/A-Venus	8.9±2.1	0.10±0.01

Online Resource 3 AGS3-Venus expression by confocal microscopy. Representative fields were imaged for cell lines expressing AGS3 fused to the Venus fluorescent protein at 20X magnification using a Nikon A1 confocal system when excited with the 488 nm laser and filtered for detection of Venus fluorescence (Emission: 525/50)