Fig 1: Construction/function of ras/rap chimeras

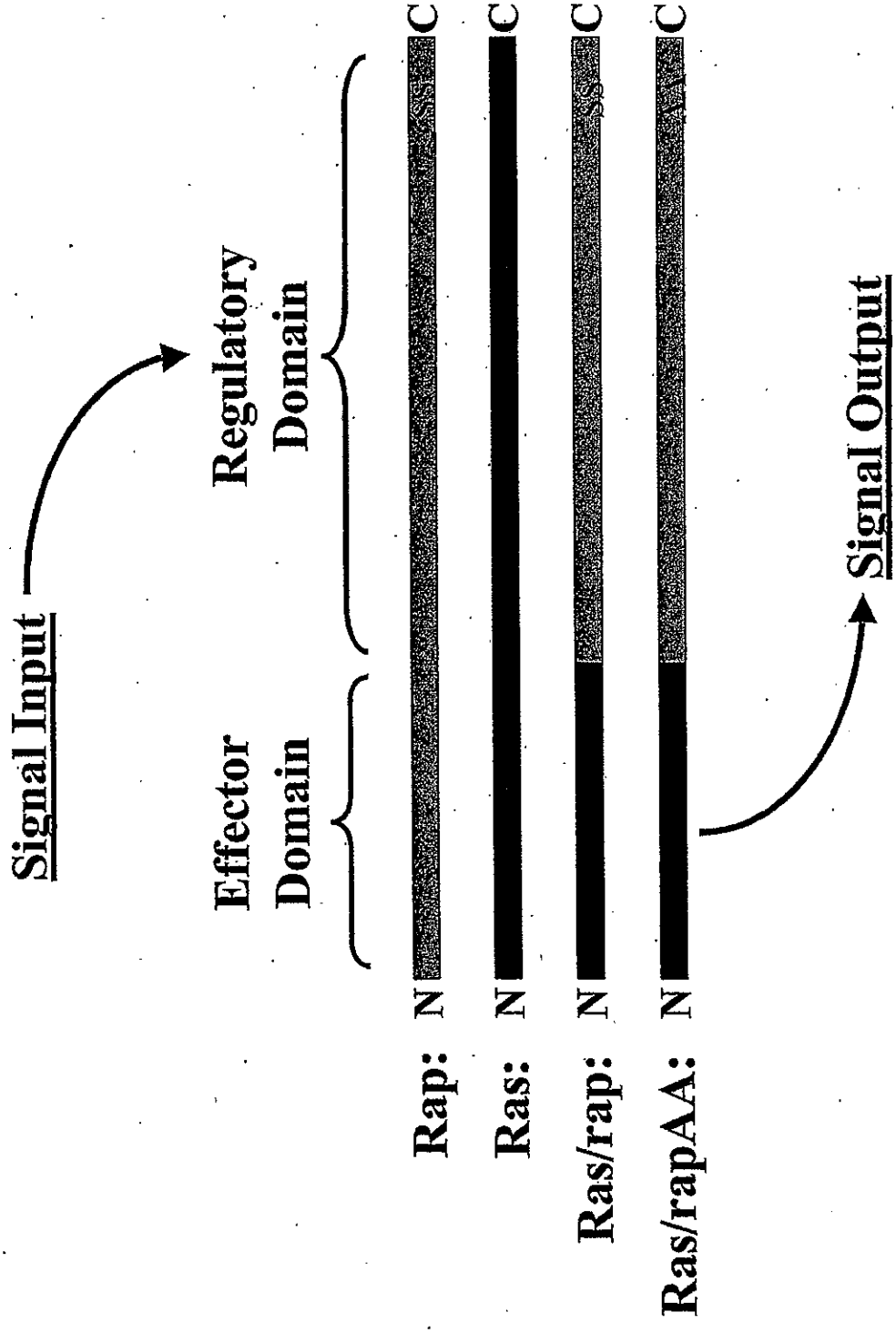


Figure 2A. Activation of cellular proliferation by ras/rap chimera.

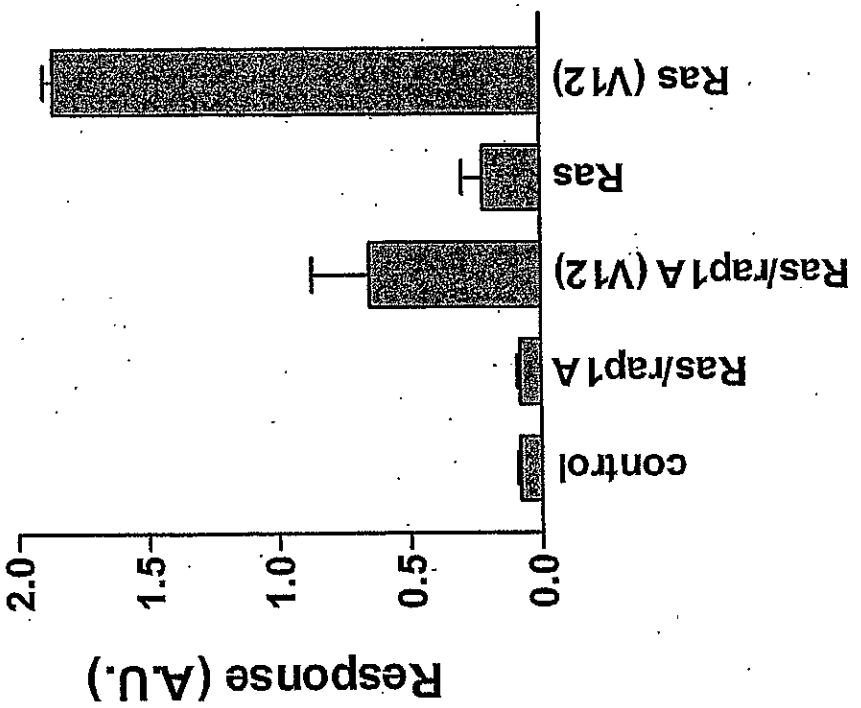


Figure 2B, 2C. Rap selective exchange factors activate cellular proliferation in a ras/rap chimera dependent manner

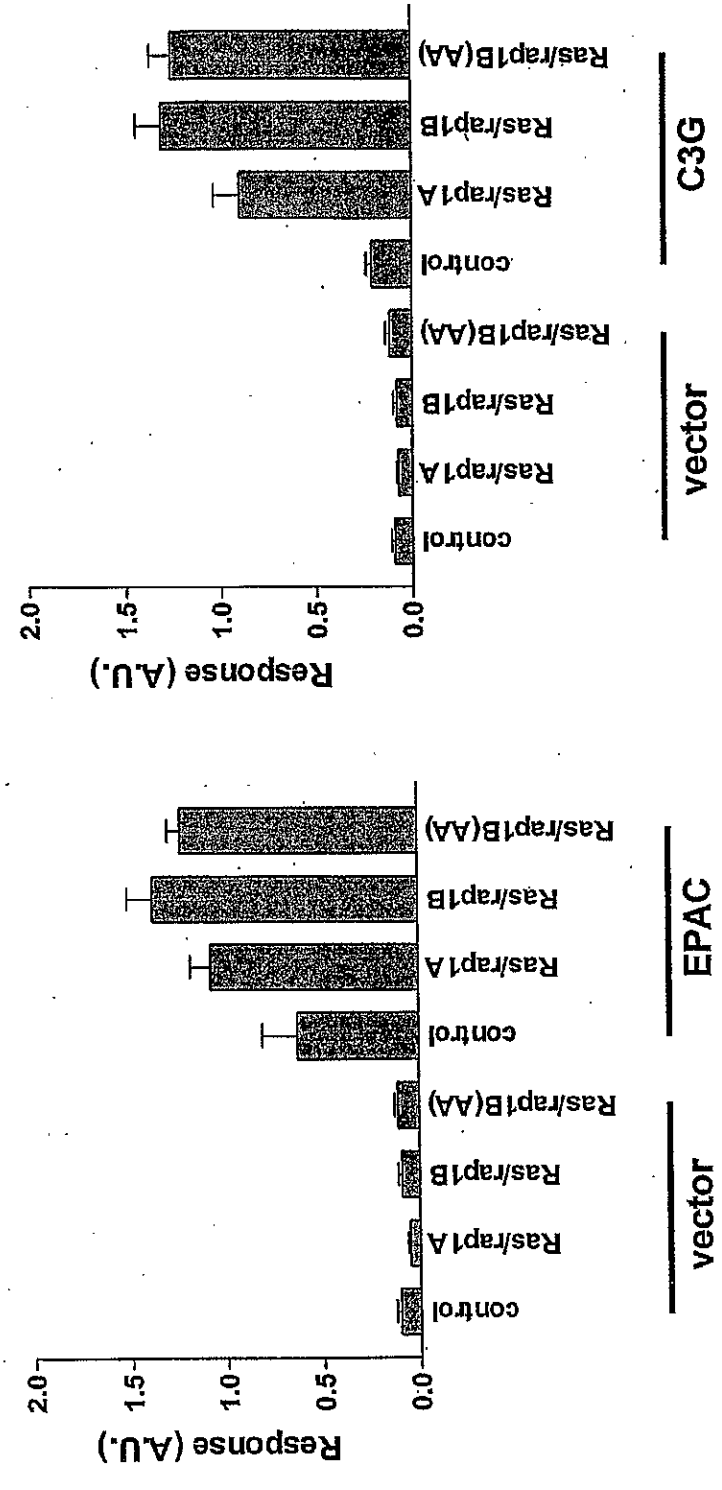


Figure 2D: Activation of EPAC by 8-Br cAMP

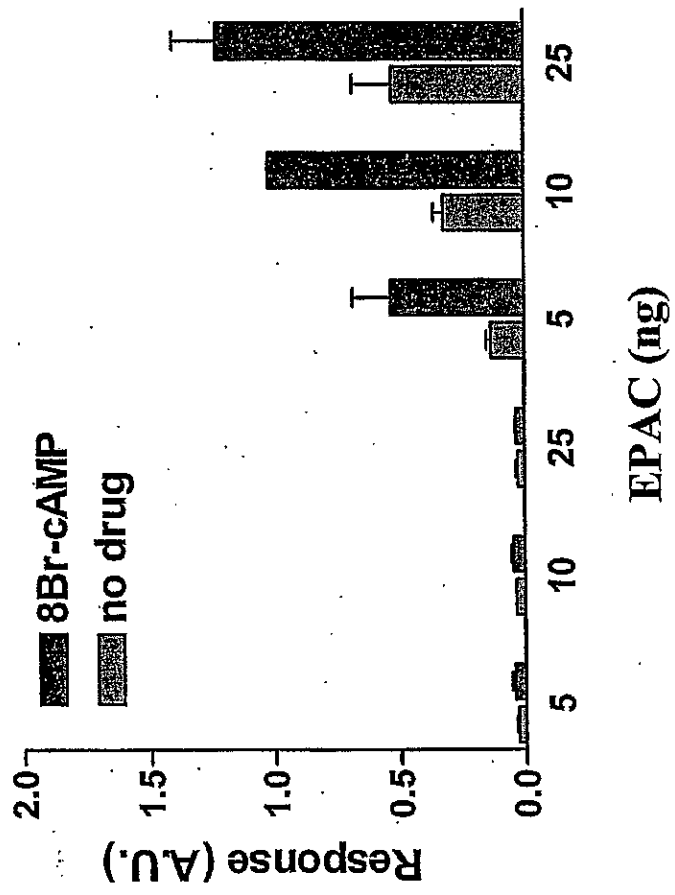


Figure 2E. Rap exchange factors are selective for ras-rap chimeras.

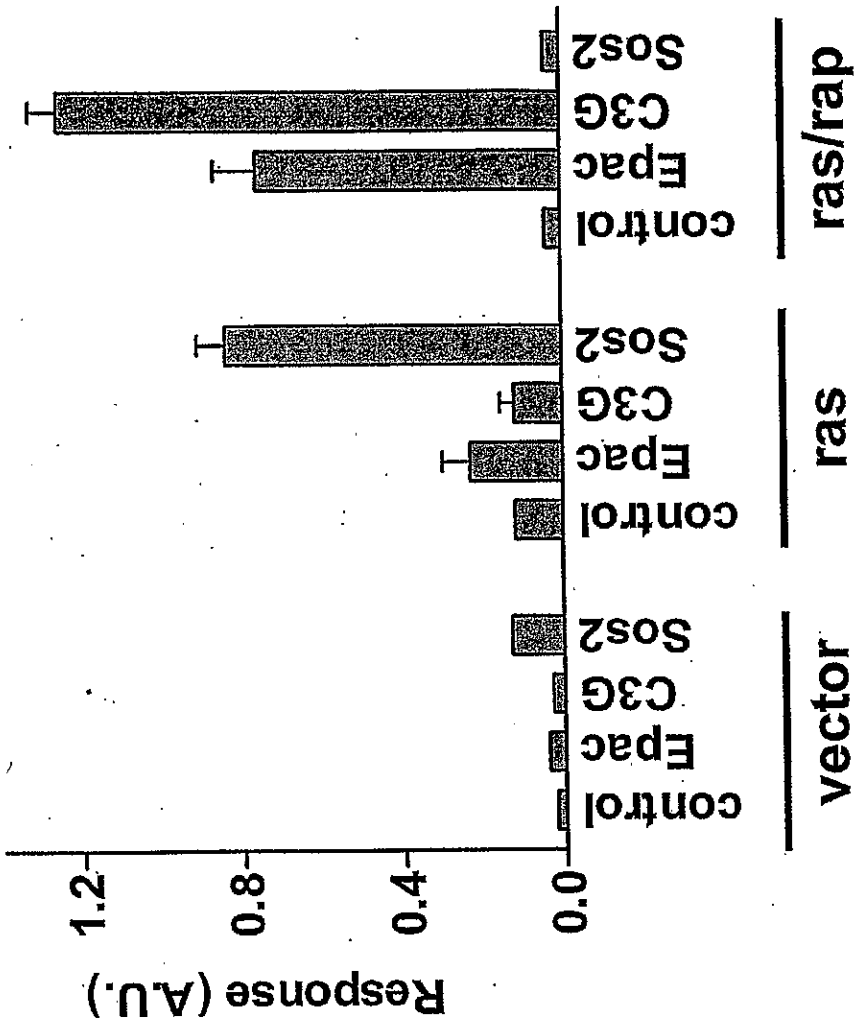


Figure 3. Non-transforming G-protein coupled receptors activate cellular proliferation in a ras/rap dependent manner

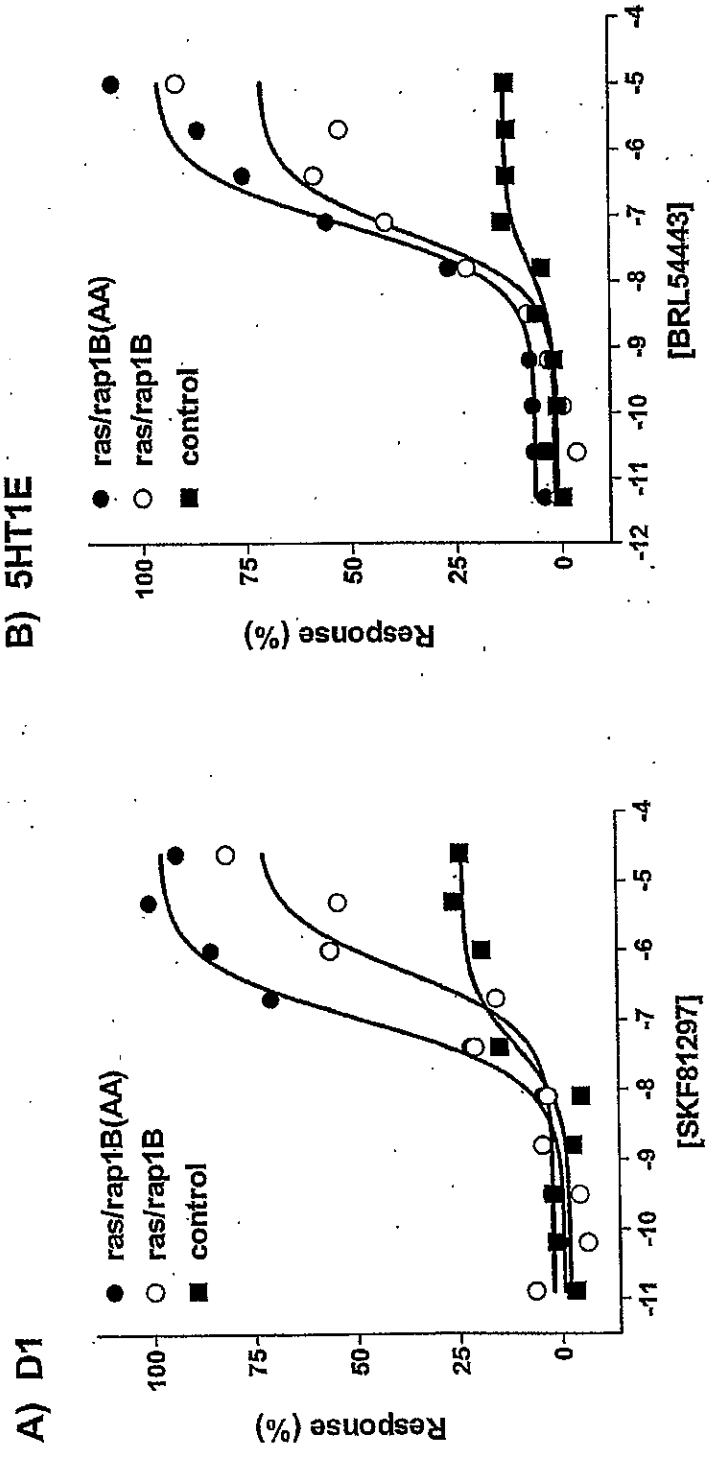


Figure 4. Pertussis toxin sensitivity of ras/rap dependent proliferative responses to G-protein coupled receptors.

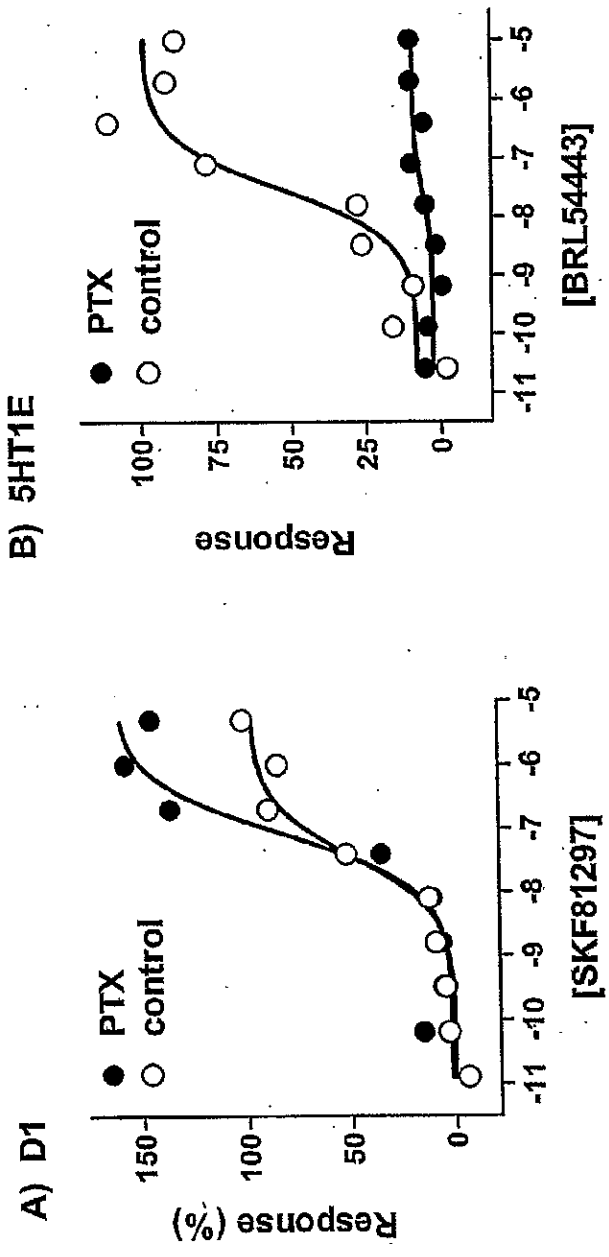


Figure 4C: Gi-coupled receptors enabled by Ras/rap are blocked by PTX

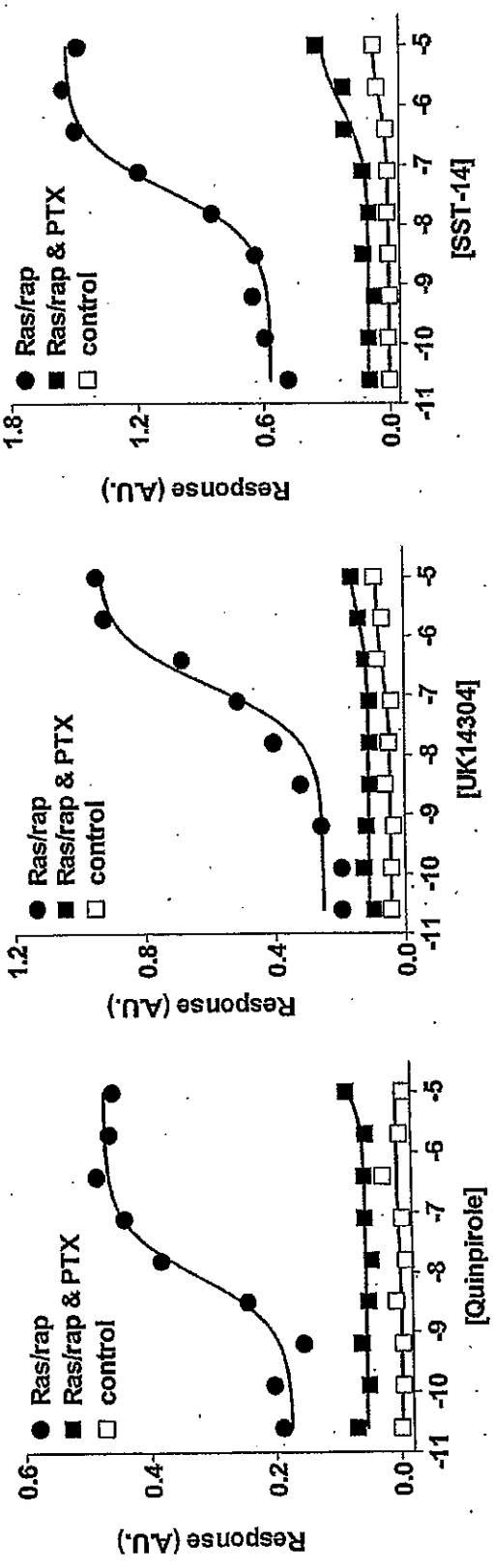


Figure 5. Adenylyl cyclase augments ras-rap dependant responses to D1 and D2

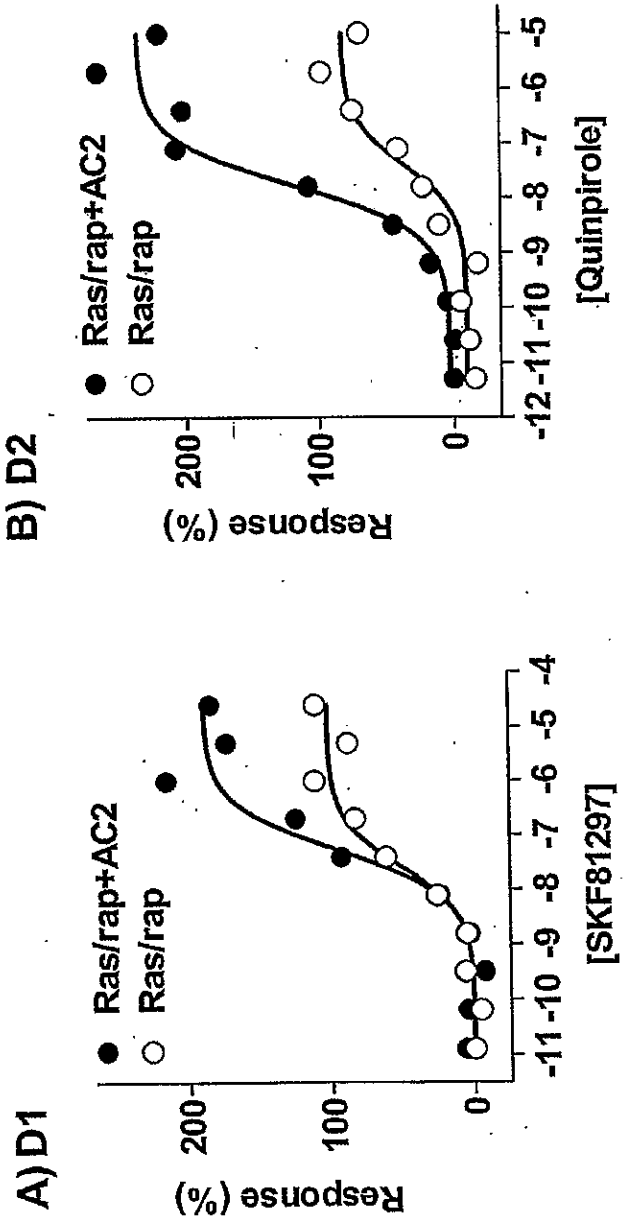


Figure 5C & 5D: AC2 augments enabling effect of Ras/rap: Gi-linked receptors

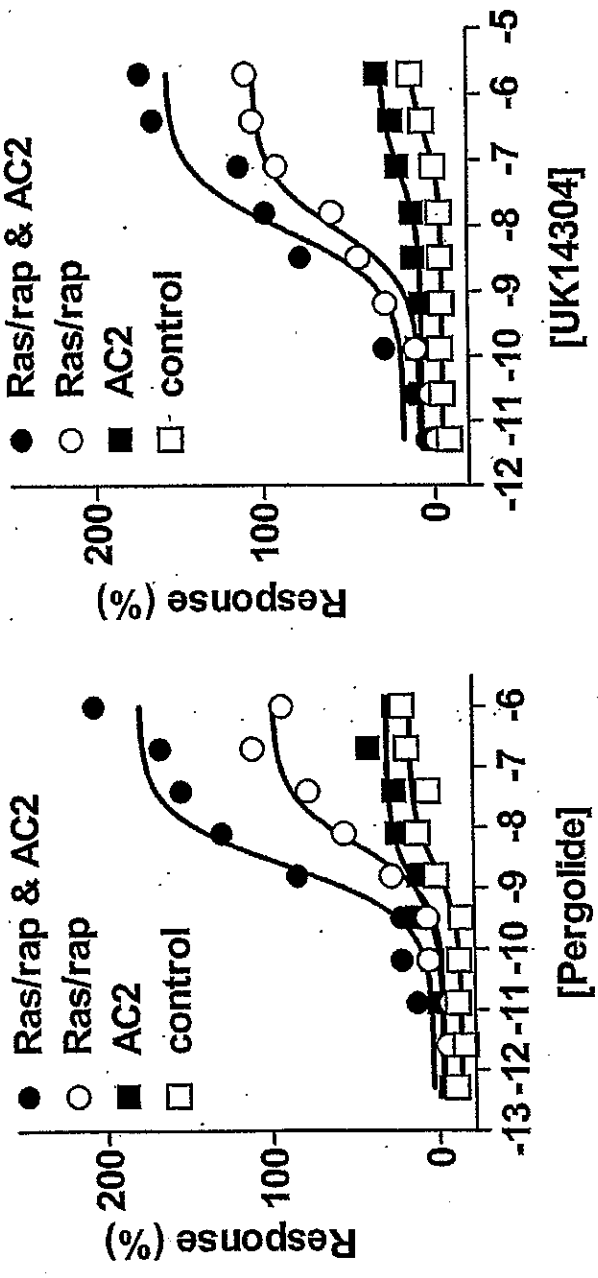


Figure 5E: AC2 augments enabling effect of Ras/rap: Gs-linked receptors

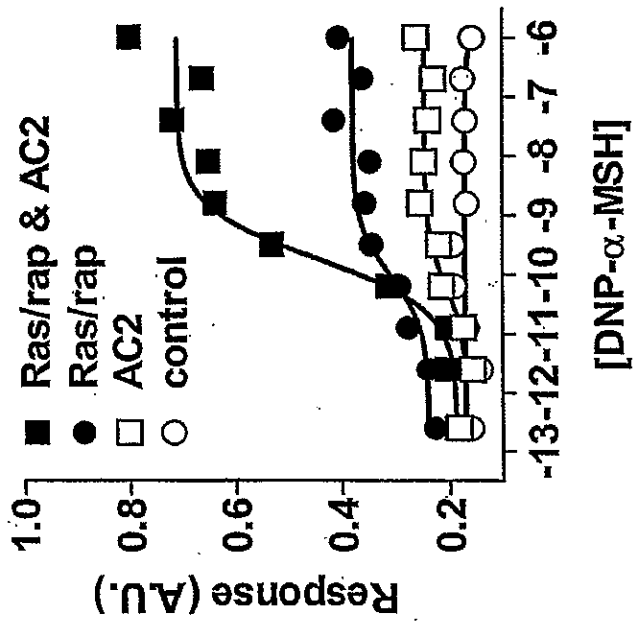


Figure 5F: Suppression of beta-gamma signaling by BarkCT

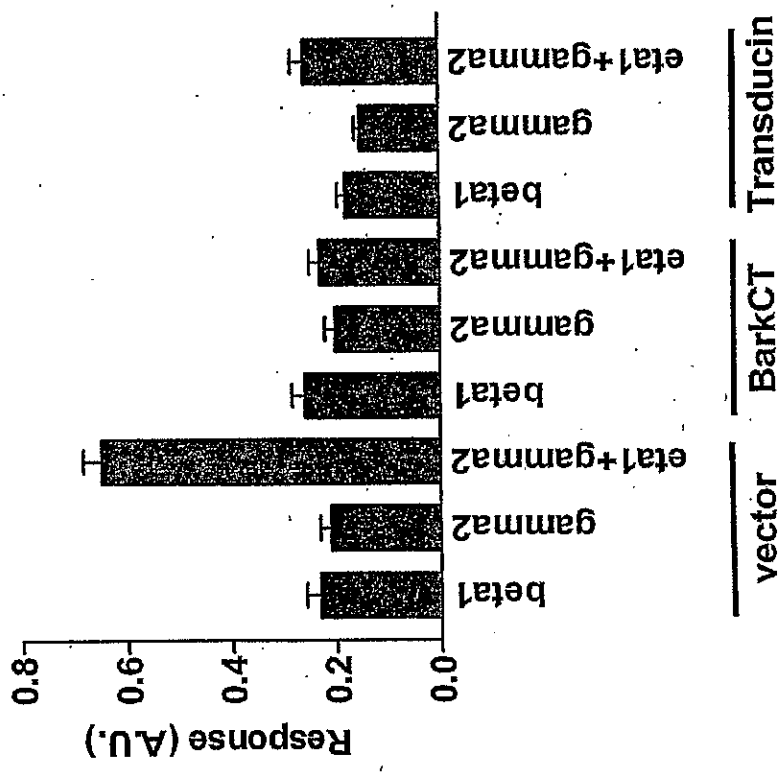


Figure 5G: Synergy of helper genes

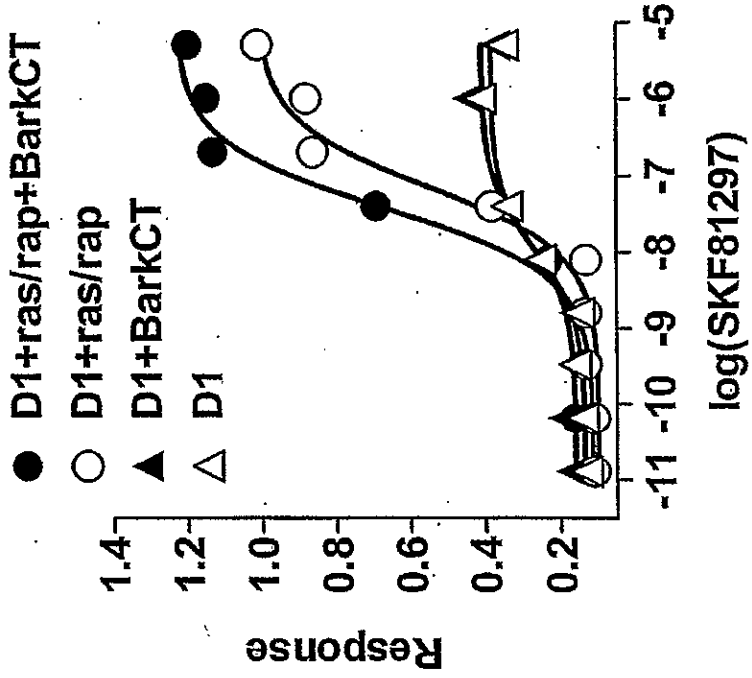


Figure 5H: Synergy of helper genes

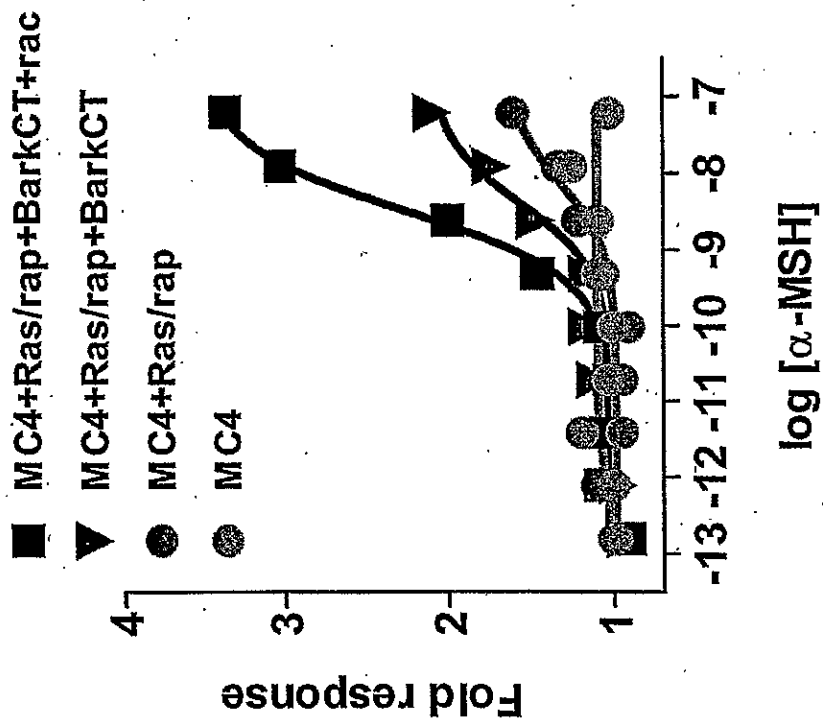


Figure 6A. Gs-coupled receptors enabled with Ras/rapAC2 for cell proliferation assay

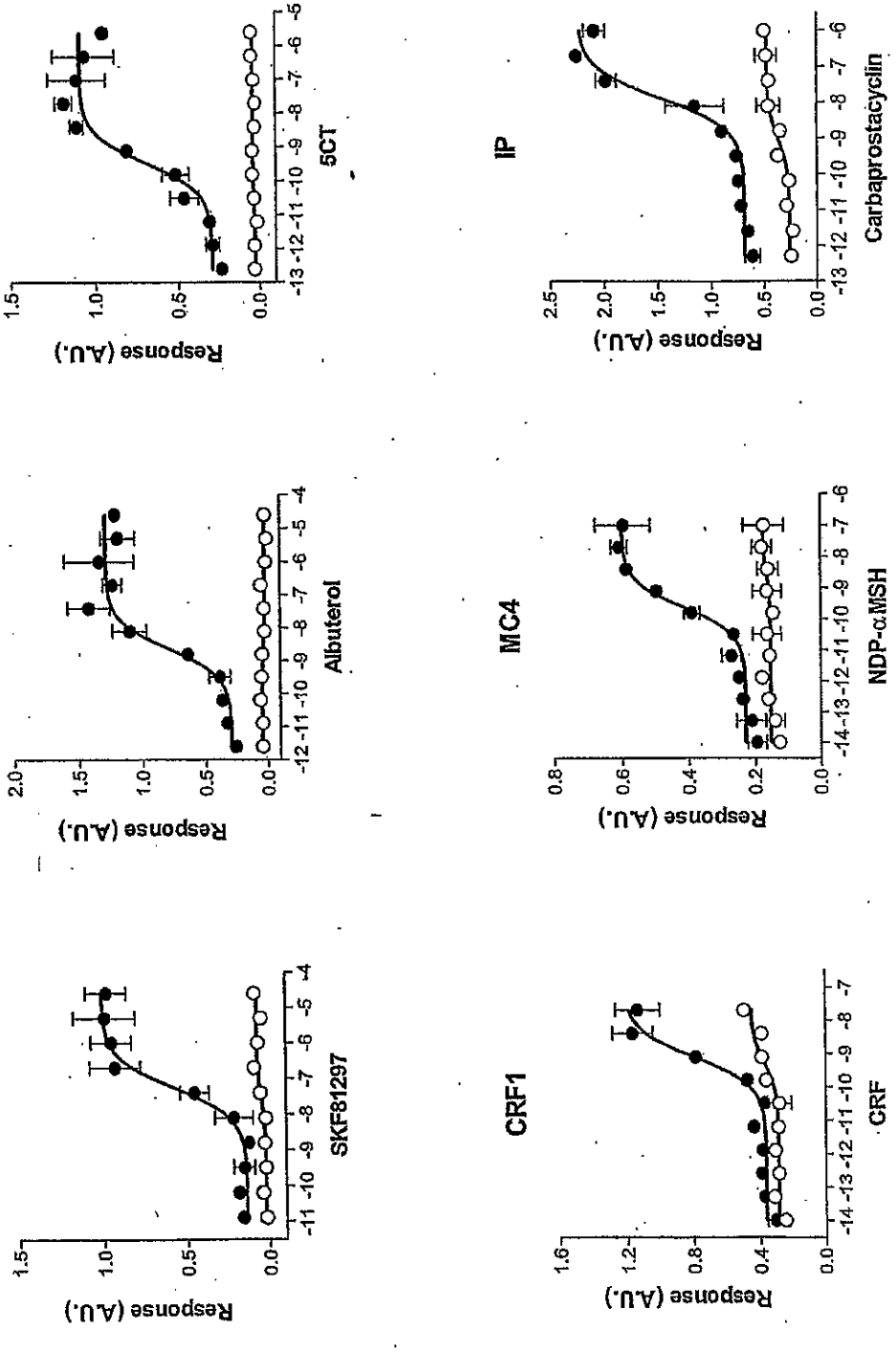


Figure 6B. Gi-coupled receptors enabled with Ras/RapAC2 for cell proliferation assay

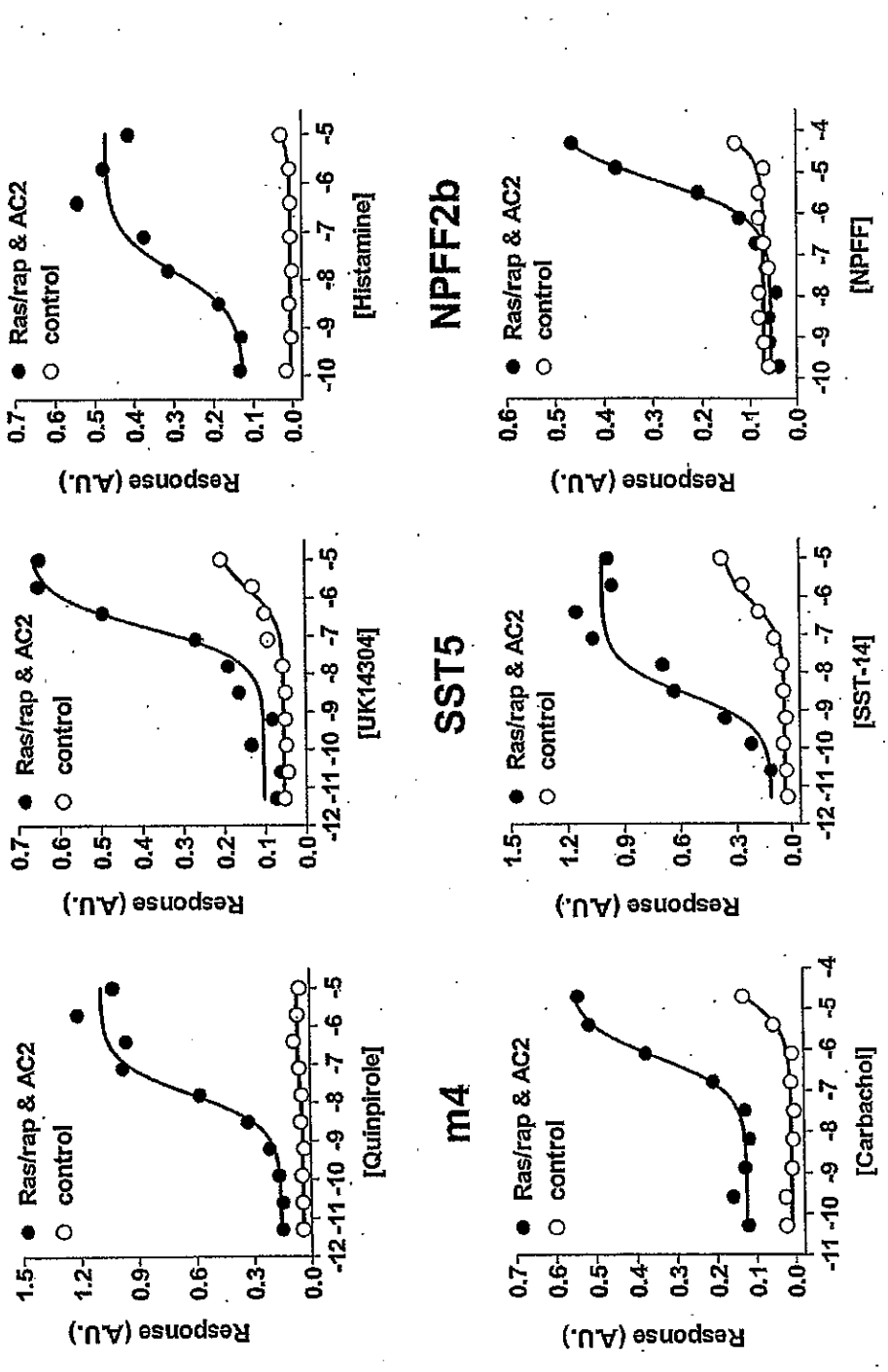
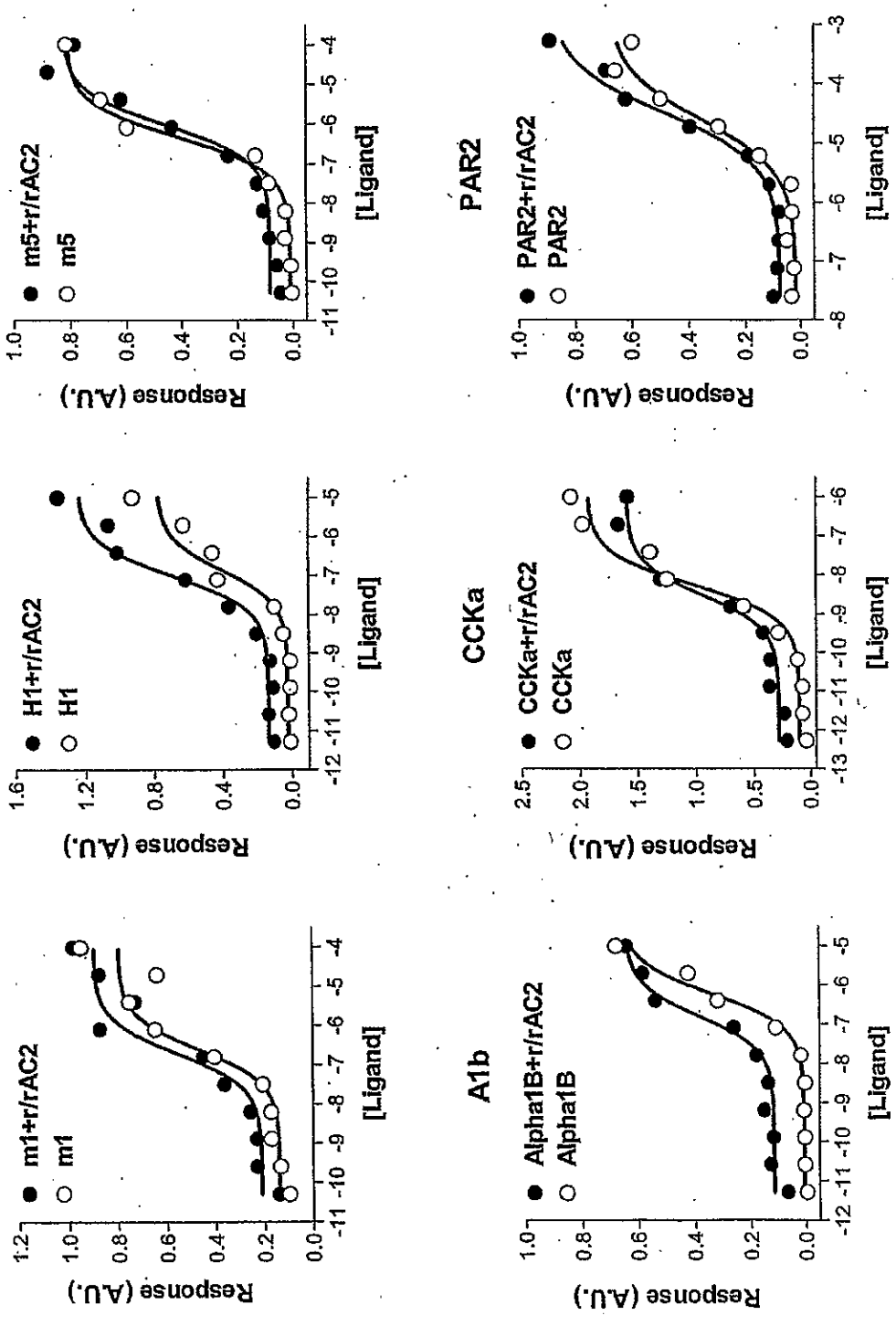


Figure 6C: Group of Gq receptors enabled +/- Ras/RapAC2 for cell proliferation assay



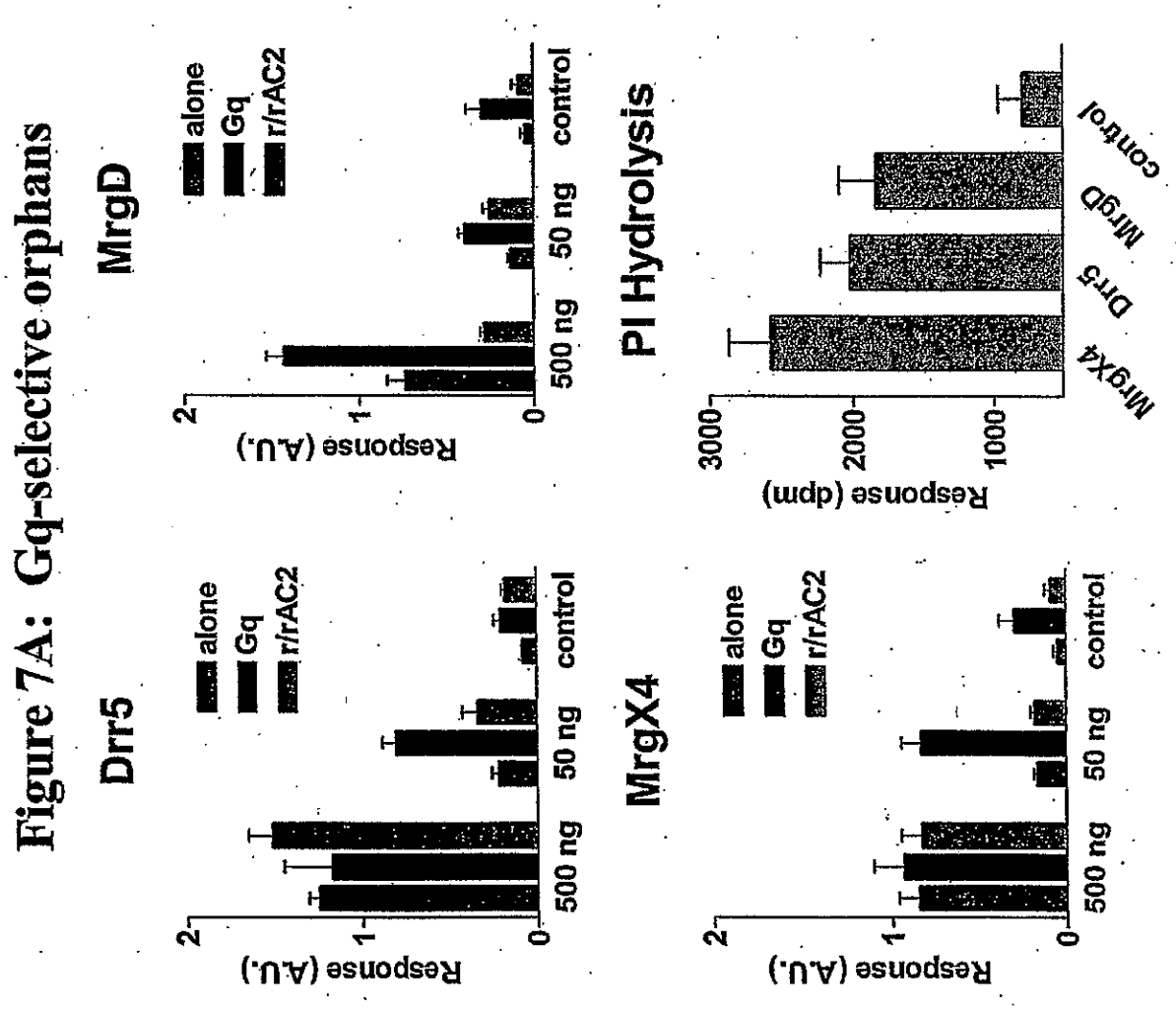

Figure 7A: Gq-selective orphans
Drr5
MrgD
MrgX4
PI Hydrolysis
alone
Gq
r/rAC2
Response (A.U.)
500 ng
50 ng
control
Response (dpm)
MrgX4
Drr5
MrgD
control

Figure 7B: Gs-selective orphans

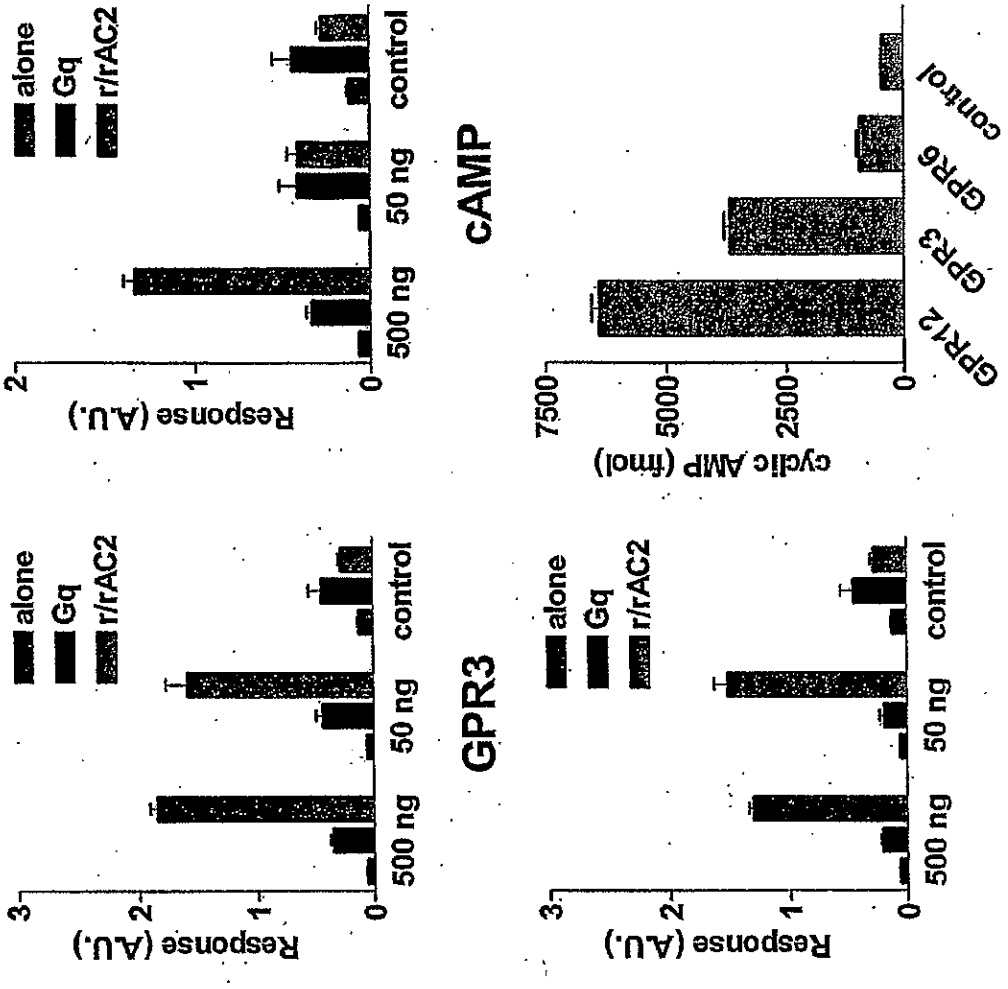
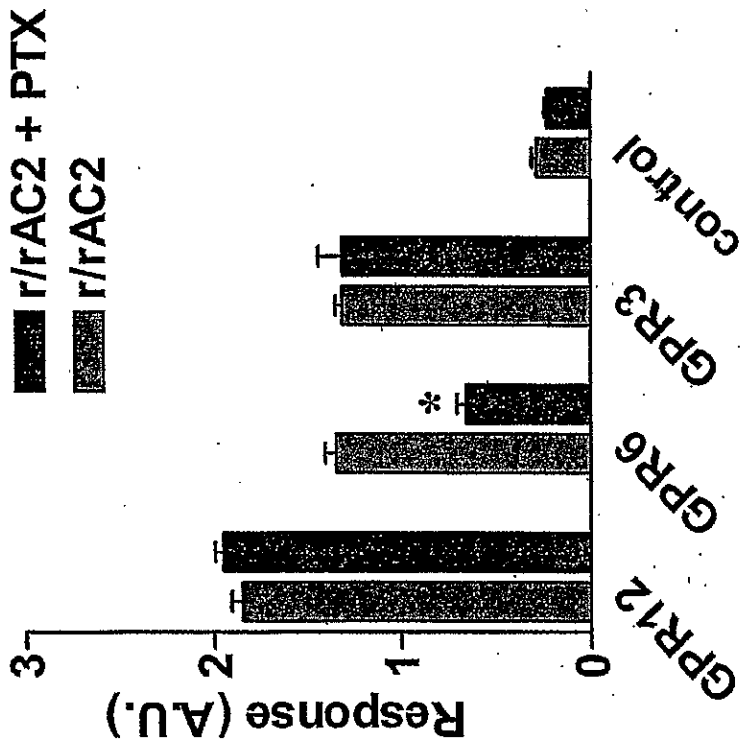


Figure 7C: Orphan specificity for the Gi-pathway



*significant repression

Figure 7D: Orphan specificity for the Gs-pathway

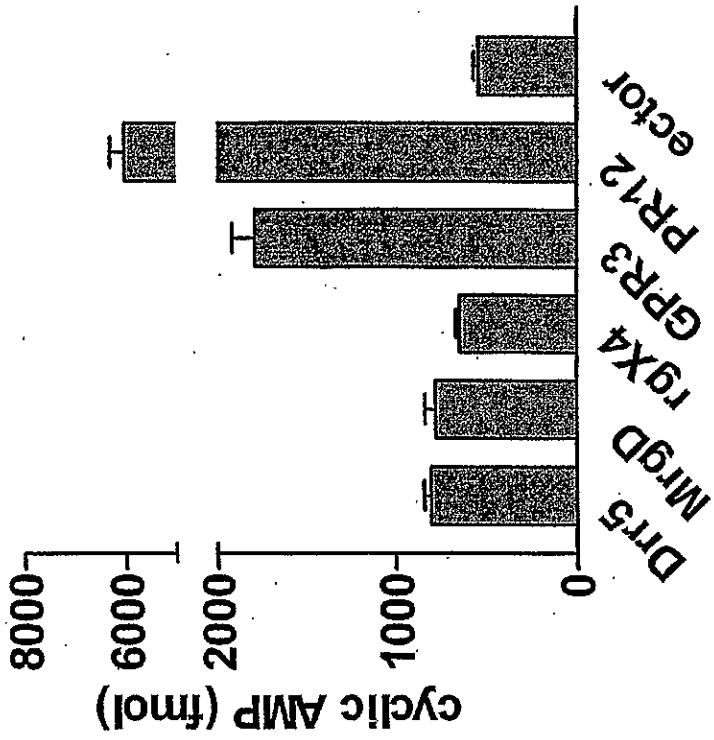


Figure 8. HTS screening using helper genes: proof of concept

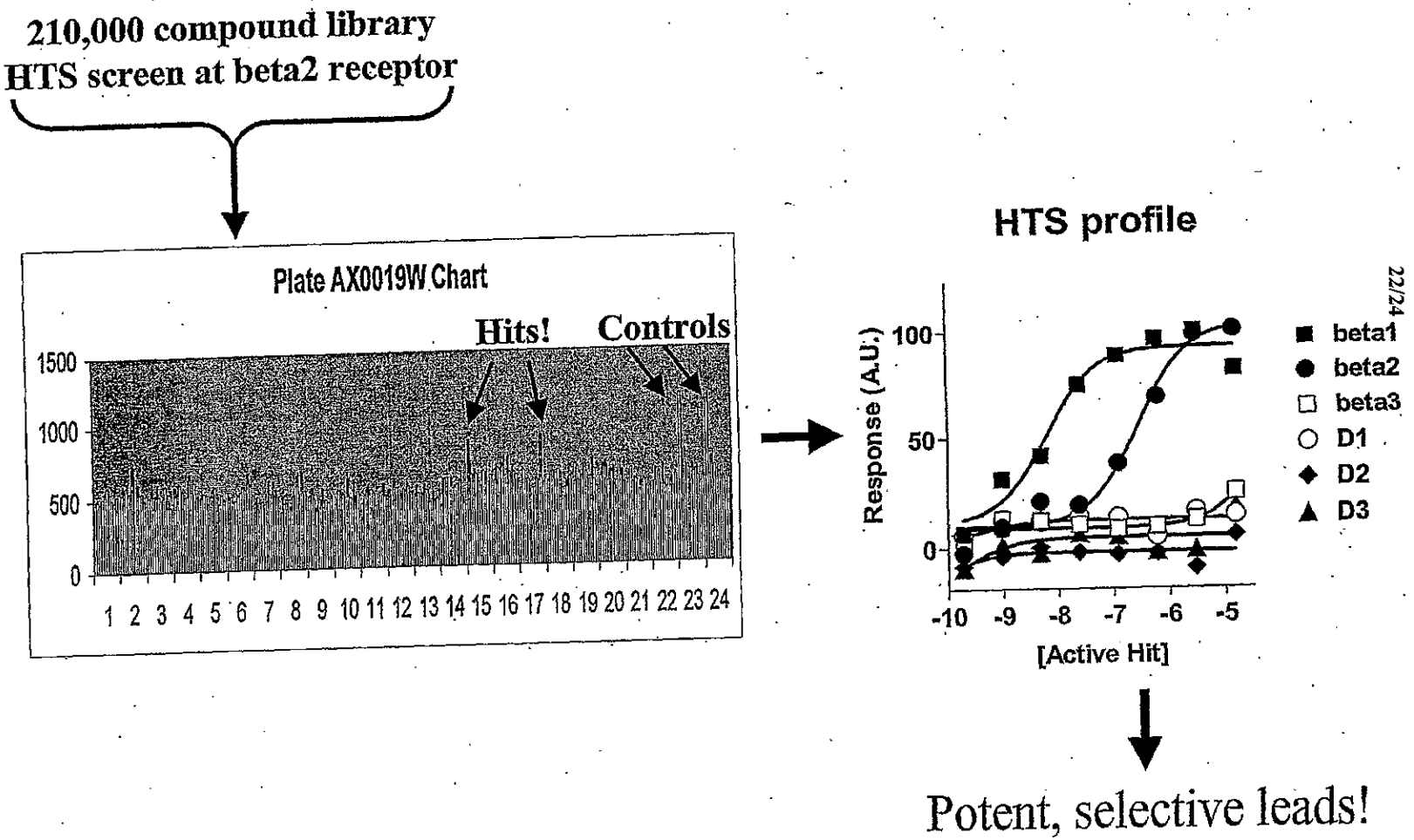


Figure 9. Homogeneous detection of GPCR signaling using ras/rap chimeras

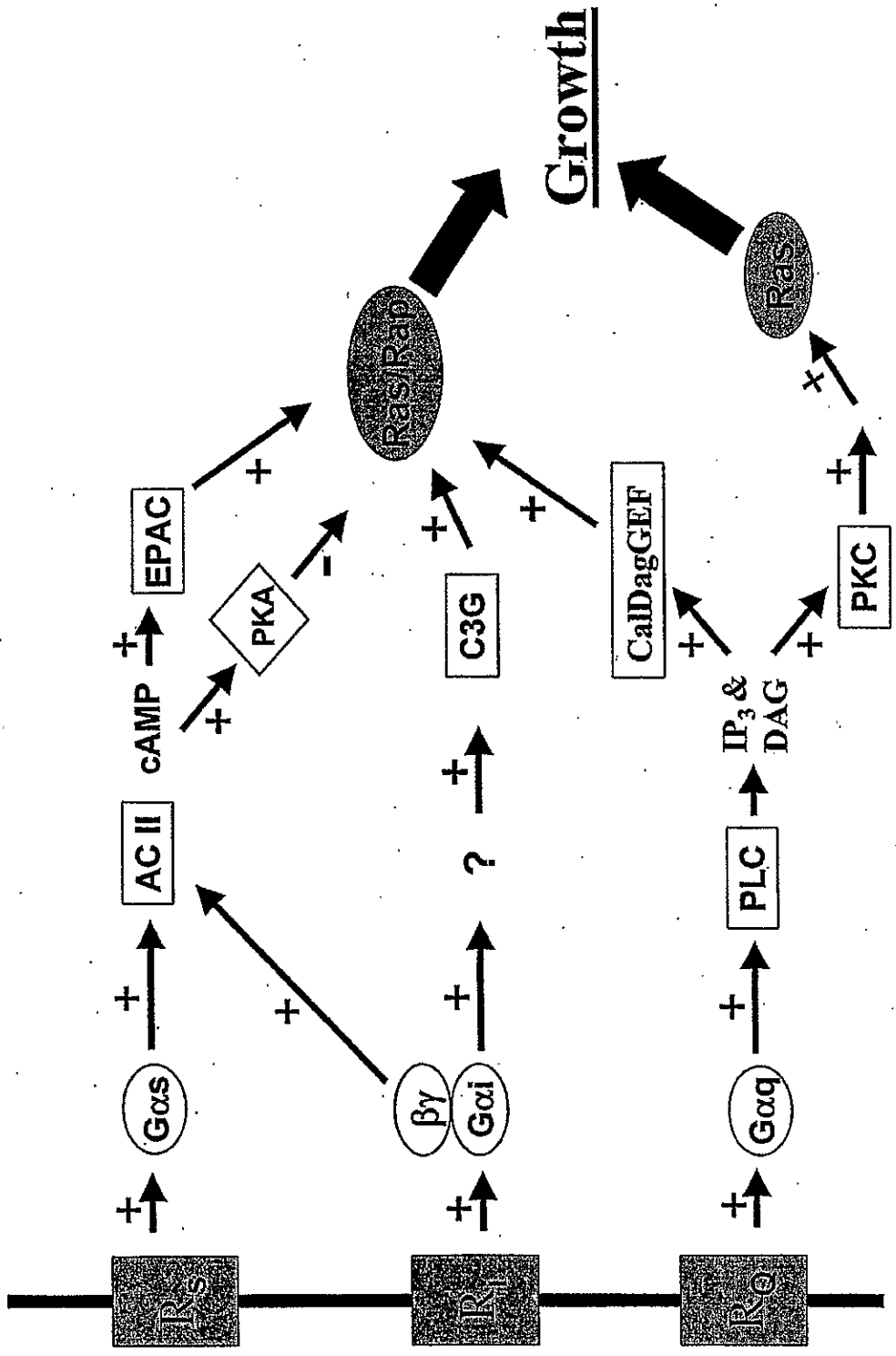


Figure 10: R-SAT vs. 2nd messenger assays

Gs receptors:

Receptor	R-SAT	cAMP stim	ratio
5HT7a	10.1	9.4	5
Beta2	8.8	8.1	5
CRF1	9.0	9.1	1
D1	7.3	7.6	0.5
IP	7.4	7.0	3
MC4	9.8	9.1	6

Gi receptors:

Receptor	R-SAT	cAMP inhib	ratio
M4	6.3	6.4	1
5HT1E	7.4	7.6	1
D2	8.2	7.7	3
alpha2C	6.9	7.5	0.3
SSTR5	8.0	8.7	0.2
NPFF2b	5.2	nd	-

Gq receptors:

Receptor	R-SAT	R-SAT & helpers	PI hydr	Ratio h/no h	Ratio PI/RSAT
Alpha1B	6.2	6.8	6.9	1.1	1.1
CCKa	8.3	8.5	8.2	1	1.0
H1	6.9	7.0	7.4	1	1.1
m1	6.6	6.7	5.9	1	0.9
m5	6.3	6.0	5.9	1	0.9
PAR2	4.6	4.5	5.0	1	1.1