Supplemental Data

Supplement Table 1. Normalized values of Gα_s expression and R_{max} for LHR and β_2-AR[a].

Gα_s	W[b]	CTX[c]	W/C[d]	R_{max}[e]	R_{max}[f]	R_{max}[g]	R_{max}[e]	R_{max}[h]	R_{max}[i]
	Gα_s	Gα_s	Gα_s	LHR	LHR	LHR	AR	AR	AR
WT	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
L394+A	0.72	0.71	1.01	0.58	0.80	0.81	0.22	0.30	0.31
Δ394	1.15	0.45	2.53	0.56	0.49	1.24	0.02	0.02	0.04
Δ393-4	0.92	1.23	0.75	0.03	0.03	0.02	0.00	0.00	0.00
Δ392-4	0.37	0.37	1.00	0.01	0.04	0.04	0.00	0.00	0.00
Δ391-4	0.83	0.37	2.25	0.01	0.01	0.01	0.00	0.00	0.00
L394A	1.14	1.29	0.89	0.93	0.81	0.72	0.18	0.16	0.14
L394F	0.16	1.28	0.12	2.59	16.67	2.02	0.81	5.22	0.63
L394D	0.75	1.20	0.62	2.42	3.22	2.01	0.32	0.42	0.26
L394K	0.93	0.35	2.64	0.74	0.80	2.11	0.12	0.12	0.33
L393A	1.69	0.45	3.76	0.02	0.01	0.04	0.00	0.00	0.00
L393I	1.28	1.24	1.03	1.97	1.54	1.58	0.73	0.57	0.59
L393D	0.78	0.34	2.32	0.01	0.01	0.02	0.00	0.00	0.00
L393K	0.73	0.48	1.51	0.00	0.00	0.00	0.00	0.00	0.00
E392A	0.58	0.13	4.52	0.44	0.75	3.40	0.22	0.37	1.69
E392R	1.02	0.43	2.37	0.27	0.26	0.62	0.24	0.23	0.55
E392Q	0.50	0.61	0.81	0.67	1.35	1.09	0.43	0.87	0.71
E392V	0.73	0.14	5.25	0.79	1.08	5.66	0.06	0.09	0.46
E392L	0.13	0.23	0.59	0.78	5.84	3.47	0.15	1.08	0.64
E392S	0.24	0.73	0.33	0.84	3.47	1.14	0.36	1.49	0.49
Y391A	0.34	0.48	0.70	0.22	0.65	1.88	0.17	0.51	0.36
Y391F	0.61	0.13	4.84	1.08	1.77	8.55	0.20	0.33	1.61
Q390A	1.34	0.47	2.84	0.88	0.66	1.88	0.88	0.66	1.87
R389A	0.64	0.31	2.03	0.93	1.45	2.94	0.68	1.06	2.17
R389P	1.00	0.36	2.79	0.01	0.01	0.03	0.00	0.00	0.00

[a] For clarity of presentation, mean values rounded to two decimal positions are shown without the SEMs, and all values are normalized to that of wild-type Gα_s.*[N.B. In some cases there may appear to be small discrepancies between the numbers given and their ratio. This arises because three decimal positions were used to determine the ratios; then the number was rounded to two decimal positions.]*

[b] Normalization of Western blots (W) to wild-type (WT) Gα_s. A representative blot is shown in Fig. 4.

[c] Normalization of CTX-stimulated cAMP to WT Gα_s (Fig. 3A).

[d] Ratio of the normalized Western results to the normalized CTX data.

[e] For each receptor, basal cAMP was subtracted from the respective R_{max} for each Gα_s mutant, and this difference was normalized to that of wild-type Gα_s minus basal cAM as shown below. $[(R_{max} - \text{basal})_{mutant}/(R_{max} - \text{basal})_{WT}]$ The data were taken from Table 1 and Figs. 3B, 5, and 6.

[f] The values of $[(R_{max} – \text{basal cAMP})_{mutant}/(R_{max} – \text{basal cAMP})_{WT}]_{LHR}$ were normalized to the expression (normalized) as monitored by Western blots. The results reported in Table 1 and Figs. 3B and 5 were used for these calculations.

[g] The values of $[(R_{max} – \text{basal cAMP})_{mutant}/(R_{max} – \text{basal cAMP})_{WT}]_{\beta2\text{-}AR}$ were normalized to the expression (normalized) as monitored by CTX stimulation of cAMP. The data shown in Table 1 and Figs. 3B and 6 were used for these determinations.

[h] The values of $[(R_{max} – \text{basal cAMP})_{mutant}/(R_{max} – \text{basal cAMP})_{WT}]_{\beta2\text{-}AR}$ were normalized to the expression (normalized) as monitored by Western blots. The results reported in Table 1 and Figs. 3B and 6 were used for these calculations.

[i] The values of $[(R_{max} – \text{basal cAMP})_{mutant}/(R_{max} – \text{basal cAMP})_{WT}]_{\beta2\text{-}AR}$ were normalized to the expression (normalized) as monitored by CTX stimulation of cAMP. The results reported in Table 1 and Figs. 3B and 6 were used for these calculations.

Supplement Table 2. Summary of predicted interactions between Gα_s and LHR and β_2-AR[a].

Gα_s	LHR	Mode of interaction	β_2-AR	Mode of interaction
L394	K563 (helix 6)	Salt bridge[b]	K270 plus A271 (helix 6)	Salt bridge plus van der Waals[c]
L393	I468(helix 3)	van der Waals	I135 (helix 3) L275 (helix 6)	van der Waals
E392	R464 (helix 3, DRY motif)	Salt bridge	R131 (helix 3, DRY motif)	Salt bridge
Y391	Y470 (IL2)	van der Waals	I135 (helix 3)	van der Waals

[a] These predicted interactions are from the models presented in Figs. 7 and 8.

[b] The salt bridge involves the α-carboxylate group of L394 (Gα_s) and the ε-amino group of K563 (LHR).

[c] The salt bridge involves the α-carboxylate group of L394 (Gα_s) and the ε-amino group of K270; in addition, there are van der Waals interactions between the side chain of L394 and those of K270 and A271.

Discussion of results in Supplement Tables 1 and 2

A comparison of the expression levels as judged by Western blots and CTX stimulation of cAMP (Supplemental Table 1) shows there is excellent agreement with several of the Gα_s mutants: L394+A, Δ392-394, L394A, and L393I. For others the agreement is within 2-2.5-fold (and generally less): Δ393-394, Δ391-394, L394D, L393K, E392R, E392Q, E392L, Y391A, and R389A. Expression levels for the other mutants differ more than 2.5-fold. As discussed in the text, the levels estimated by CTX stimulation of cAMP are preferred to the data from Western blots as these values reflect to a greater extent functional Gα_s. Interestingly, a qualitative assessment of Gα_s functionality with ligand stimulation of both LHR and β_2-AR is similar using just the normalized values, $[(R_{max} – \text{basal cAMP})_{mutant}/(R_{max} – \text{basal cAMP})_{WT}]$, or the same corrected for expression by either Western blots or CTX stimulation of cAMP.

In Supplemental Table 2 the predicted interactions of the Gα_s CT with LHR and with β_2-AR involve the same four CT amino acid residues of the G protein. There are similarities in many of the contacts with the two receptors, but there are also significant differences. For example, a salt bridge occurs between the α-carboxylate of Gα_s L394 and the ε-amino group of a Lys in helix 6 of the receptors: K563 of LHR and K270 of β_2-AR. In addition, the side chains of β_2-AR K270 and A271 form hydrophobic interactions with the side chain of Gα_s L394. Gα_s L393 interacts with an Ile in helix 3 of the receptors: I468 in LHR and I135 in β_2-AR. Additional hydrophobic interactions in β_2-AR include V222 (helix 5) and V275 (helix 6). A salt bridge is predicted between Gα_s E392 and the Arg of the DRY motif (helix 3) of both receptors; Gα_s Y391 interacts with Y470 in IL2 of LHR and with I135 in helix 3 of β_2-AR. The additional interactions between Gα_s and β_2-AR, compared with LHR, are consistent with the differences observed in the potencies associated with the mutants of Gα_s.