SUPPLEMENTAL FIGURE LEGENDS:

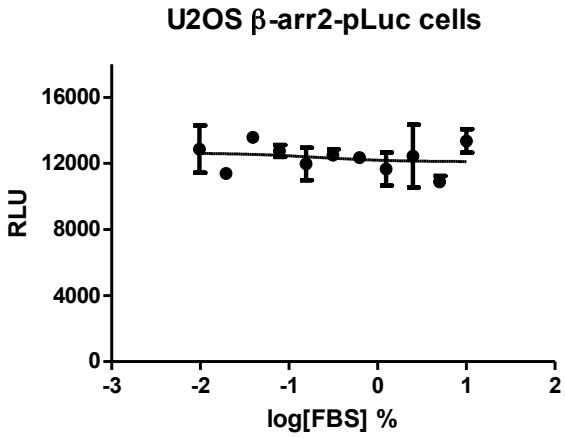

Figure S1. Absence of FBS response in U2OS cells only expressing β-arrestin-2-pLuc. Cells were seeded in a 384-well plate at 20,000 cells/well and cultured for overnight. Transfection was done next day with 0.03 µg β-arrestin-2-pLuc/well using Lipofactamine2000 according to manufacturer's instruction. Cells were cultured for another 24 hours. The culture media were then replaced with 20 µl DMEM with no FBS, and 5 µl of a serial dilution of FBS were added to cells with final FBS concentrations from 20 to 0.01%. After 90 min incubation, luciferase detection reagent was added and luminescent signals were determined.

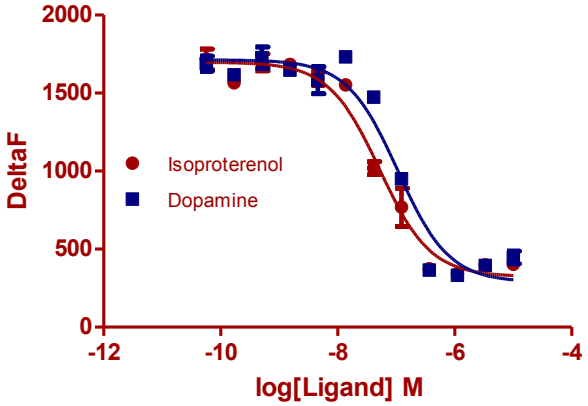

Figure S2. Exogenous expression of ERK2-TEV and β-arrestin-2-pLuc in U2OS cells did not alter endogenous GPCR signaling. HTRF cAMP assays were performed using cisbio cAMP dynamic 2 kit. The cells were seeded in a 384-well plate at 8,000 cells/well and cultured for overnight, the cells were then treated with a serial dilution of isoproterenol or dopamine. The assays were performed according to the manufacturer's instruction. Fluorescence (665/620 nM) was recorded by Tecan Pro 200.

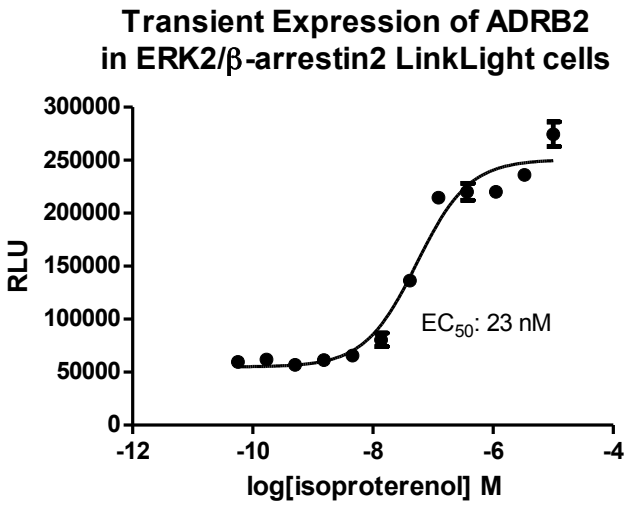

Figure S3. Activation of exogenous ADRB2 expressing in ERK/β-arrestin U2OS cells. Cells were seeded in a 384-well plate at 10,000 cells/well and cultured for 24 hr. The cells were transfected with an ADRB2 expression plasmid with 0.03 μg DNA/well using the lipofectin method. After 24 hour transfection, the medium was replaced with a FBS-free medium. Isoproterenol was added to cells for 90 min incubation. The luminescent signals were determined after addition of the luciferase detection reagent. Transient over-expression of ADRB2 in cells is known to have increased basal level activity due to small portion of receptor having constitutively active conformation.[1, 2]

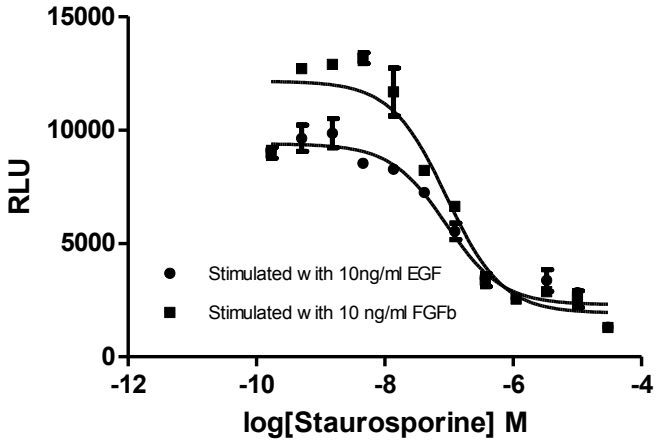

Figure S4. Inhibition of EGF and FGF-basic induced ERK/β-arrestin interaction by staurosporine. Cells were incubated with staurosporine in a serial dilutions for 15 min in the FBS-free medium followed by addition of 10 ng/ml (final concentration) of EGF or FGF-basic. The luminescent signals were determined after addition of the luciferase detection reagent.

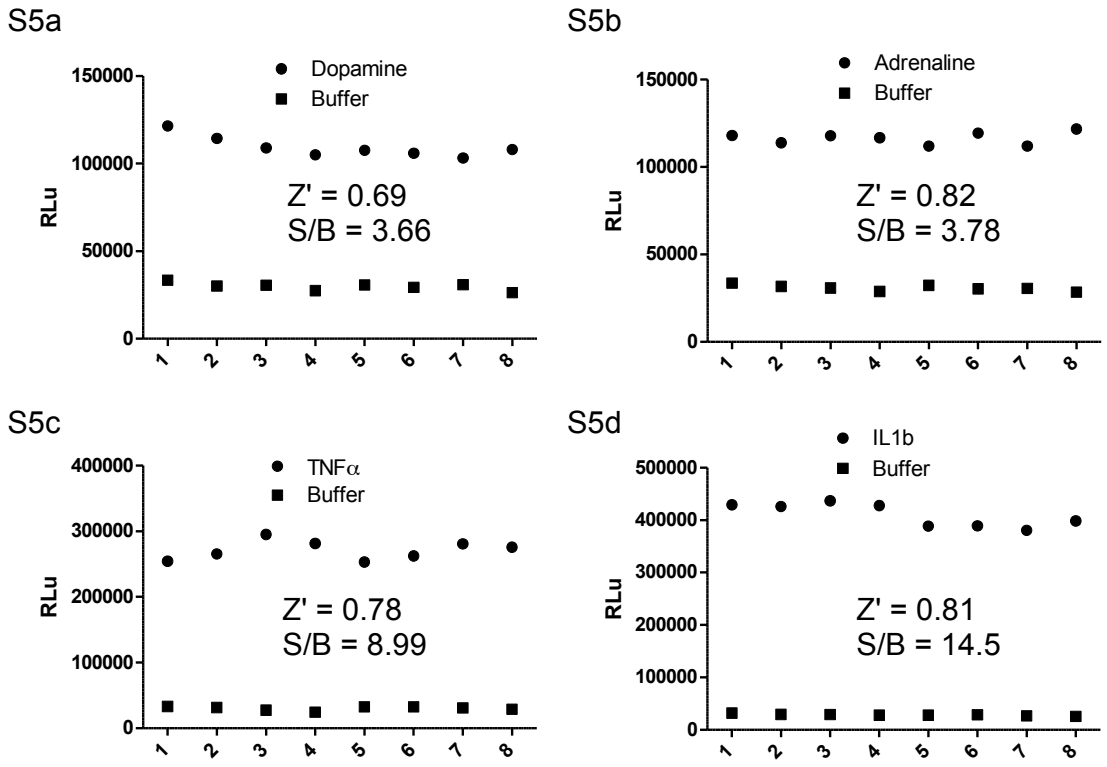

Figure S5. Z' factors and S/B ratios of ERK/β-arrestin LinkLight Assay. Measuring the S/B and Z' factor of the ERK/β-arrestin assay**.** Cells were incubated with either assay buffer (square dots) or 10 µM dopamine (S5a, circle dots), 10 µM adrenaline (S5b, circle dots), 0.1 µg/ml TNFα (S5c, circle dots), and 0.1 µg/ml IL1α (S5d, circle dots) for 90 min at 37°C. The luminescent signals were determined after addition of the luciferase detection reagent. Each condition was repeated 7 times. The statistical parameters of S/B ratio and *Z′* are calculated based on equations defined in the literature.[3]

1. Chidiac, P., et al. Inverse agonist activity of beta-adrenergic antagonists. *Mol Pharmacol* **1994,** *45* (3), 490-9.
2. de Vries, B., et al. Beta-agonist-induced constitutive beta(2)-adrenergic receptor activity in bovine tracheal smooth muscle. *Br J Pharmacol* **2000,** *131* (5), 915-20.
3. Zhang, J. H., et al. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen* **1999,** *4* (2), 67-73.