Supplemental Material

Development of a High-Throughput Screening-Compatible Assay for Discovery of GPR3 Inverse Agonists Using a cAMP Biosensor

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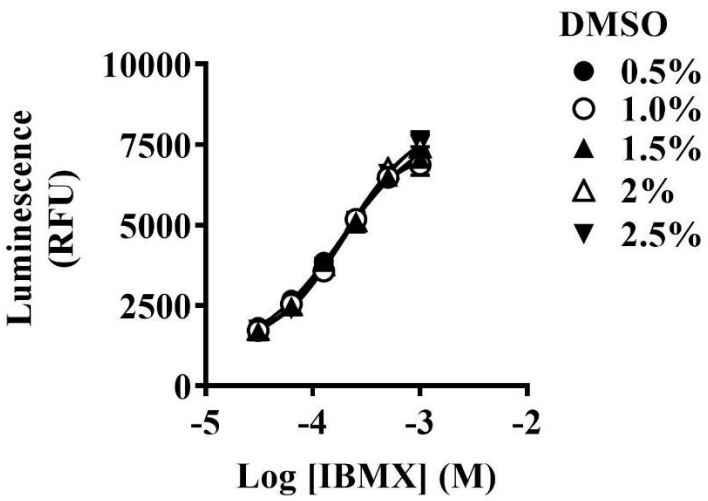
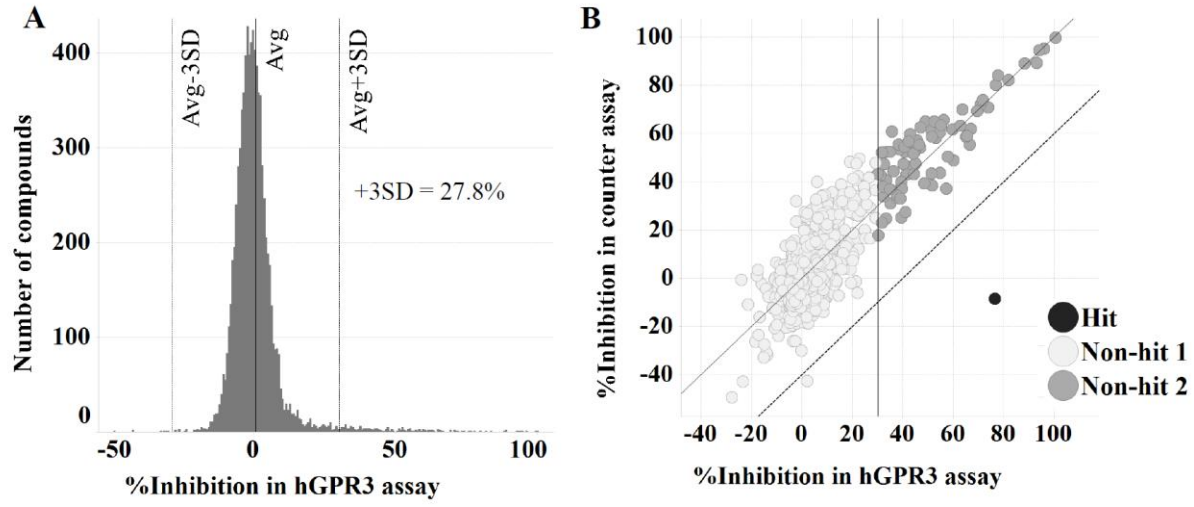
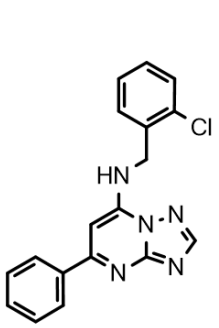
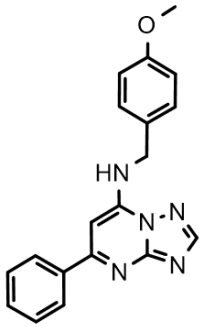


Supplemental Figure. S1. DMSO tolerance of the hGPR3 GloSensor assay.
T-REx-293/hGPR3-GS22F cells were cultured overnight in the presence of tetracycline and then cryopreserved. The hGPR3 GloSensor assay was preliminarily conducted using the cryopreserved cells immediately after thawing without cell culture for recovery in the presence of different concentrations of DMSO. DMSO had no significant effect on the GPR3 GloSensor assay in the range of the concentrations tested. Data are the means ± S.D. from one experiment performed in quadruplicate.



Supplemental Figure. S2. Results of the pilot screening. Compounds (4218) were screened at the final concentration of 10 μM using the hGPR3 GloSensor assay and the counter assay. Histogram of the %inhibition of all compounds in the hGPR3 assay **(A)**. The mean + 3S.D. was determined to be 27.8%. Comparison of compound activities in the pilot screening between the GPR3 GloSensor assay and the counter assay **(B)**. The x-axis and the y-axis represent %inhibition in hGPR3 assay and %inhibition in counter assay, respectively. The vertical line indicates 30% inhibition in hGPR3 assay. The slanted line is defined by %inhibition in hGPR3 assay = %inhibition in counter assay (y = x), and the dotted slanted line is defined by %inhibition in hGPR3 assay = 40% + %inhibition in counter assay (y = 40 + x). Closed circle represents a hit compound showing more than 30% inhibition in hGPR3 assay without significant activity in the counter assay (Hit). Light gray circles represent compounds with less than 30% inhibition in hGPR3 assay, suggesting less active compounds (Non-hit 1). Dark gray circles represent compounds showing more than 30% inhibition in hGPR3 assay with similar inhibition in the counter assay, suggesting nonspecific compounds (Non-hit 2).





	Compound #28	**Compound #46**
hGPR3 EC_{50}	0.55 μM	4.4 μM
hMC4R EC_{50}	No significant effect at 30 μM	No significant effect at 30 μM

Supplemental Figure. S3. Chemical structures of representative hit compounds with triazolopyrimidine scaffold. The compound numbers correspond to the same numbers as in Fig. 6. The potencies of these compounds toward GPR3 were consistent with those in the previous report[25].