Pyrimidinyl biphenylureas act as allosteric modulators to activate cannabinoid receptor 1 and initiate β-arrestin-dependent responses

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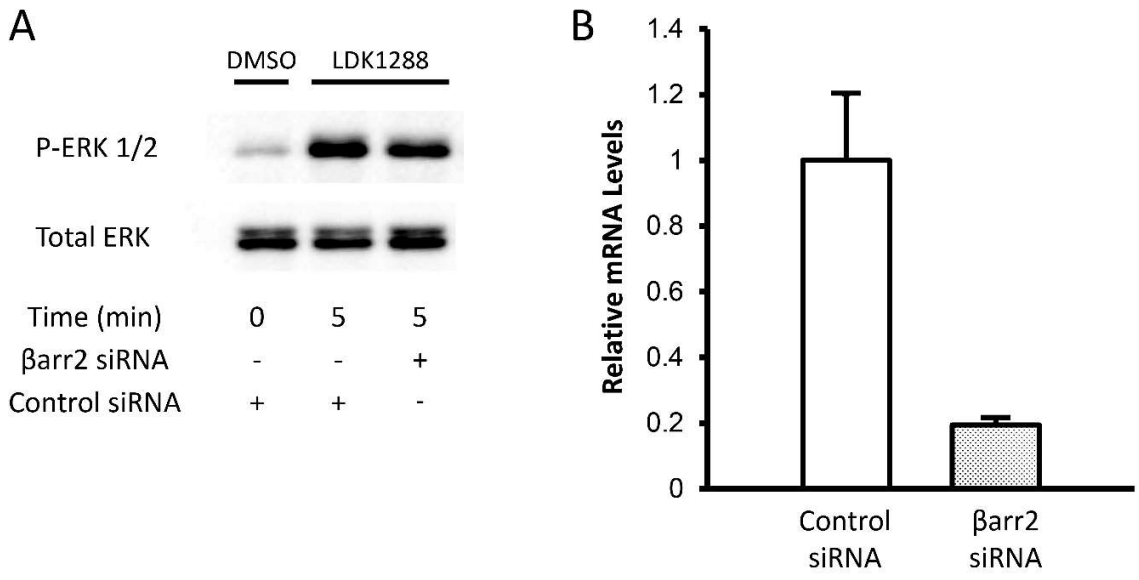
Molecular Pharmacology

Supplemental Figure 1.



Supplemental Figure 1. β-arrestin 2 knockdown.
(A) CB1 expressing HEK293 cells were treated with β-arrestin 2 siRNA or control siRNA, and phosphorylation levels of ERK1/2 were determined after vehicle (DMSO) or LDK1288 treatment for 5 minutes. (B) Knockdown efficiency by β-arrestin 2 siRNA was confirmed by measuring mRNA levels after β-arrestin 2 siRNA or control siRNA treatment. CB1 expressing HEK293 cells were transfected with β-arrestin 2 siRNA and harvested in TRIzol Reagent (Invitrogen), and total RNA extraction was performed following the manufacturer's instruction. Extracted RNA was reverse transcribed by High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) and quantitative real-time PCR was performed using Applied Biosystems 7500 Fast Real-Time PCR System. Primers used in the reaction are as follows: human GAPDH forward, 5'- AGCCACATCGCTCAGACAC -3', and human GAPDH reverse, 5'-AATGAAGGGGTCATTGATGG-3', human β-arrestin 2 forward, 5'-AGGGTCTTCAAGAAGTCGA -3', and human β-arrestin 2 reverse, 5'-CTCGAGACACCACCAGCTTCACC -3'. mRNA levels were analyzed by ΔΔCt method and expressed as mean ± S.E. (n=3). The siRNA to β-arrestin 2 change is given relative to control siRNA set at 1.0.