Supplemental Fig 1. Millipore GPCR Profiler™ Functional Screen. While a number of selective positive allosteric modulators have been recently reported for class C GPCRs, identification of selective modulators of Class A GPCRs, such as the muscarinic acetylcholine receptors, has proven more challenging, most likely due to the highly conserved orthosteric ligand binding site contained within the transmembrane regions. Because of the potential liability for non-selective activity at other class A GPCRs, VU0152099 was evaluated in Millipore's GPCR Profiler™ screen for activity at 16 GPCR targets using a Fluorimetric Imaging Plate Reader ($FLIPR^{Tetra}$), (Molecular Device Corp). This cell-based assay relies on endogenous expression of the promiscuous G protein, $G\alpha_{15/16}$, to couple recombinant receptors to calcium mobilization, and uses a two addition protocol to assess agonist, antagonist, and allosteric potentiator activity. VU0152099 exhibited no agonist activity at any of the targets tested, and was found to possess antagonist activity only at the $5HT_{2B}$ receptor (as indicated by an ≈16 fold rightward shift in the agonist CRC in the presence of VU0152099). Potentiator activity was assessed by performing agonist CRCs in the absence (vehicle, ▲) or presence (VU0152099, ■) of 10μM test compound. **A.** At the human M_4 mAChR, 10μM VU0152099 elicits a robust ≈80 fold leftward shift in the carbachol CRC (EC_{50} values: CCh alone ≈ 800nM, CCh +10μM VU0152099 ≈ 10nM). **B-P.** Similar CRC curves were generated for additional GPCRs: Muscarinic M_1 (**B.**), Adenosine A_{2B} (**C.**), α_{1A}-, $_{2A}$-, $_{2B}$-Adrenergic (**D-F.**), Dopamine D_1 ,D_{2L}, D_5 (**G-I**), Histamine H_1, H_2, H_3 (**J-L**), and Serotonin $5HT_{1A,\ 2A,\ 2B,\ 2C}$ (**M-P**), with no substantial leftward shift observed at any other target tested. All data represent the mean of duplicate determinations.