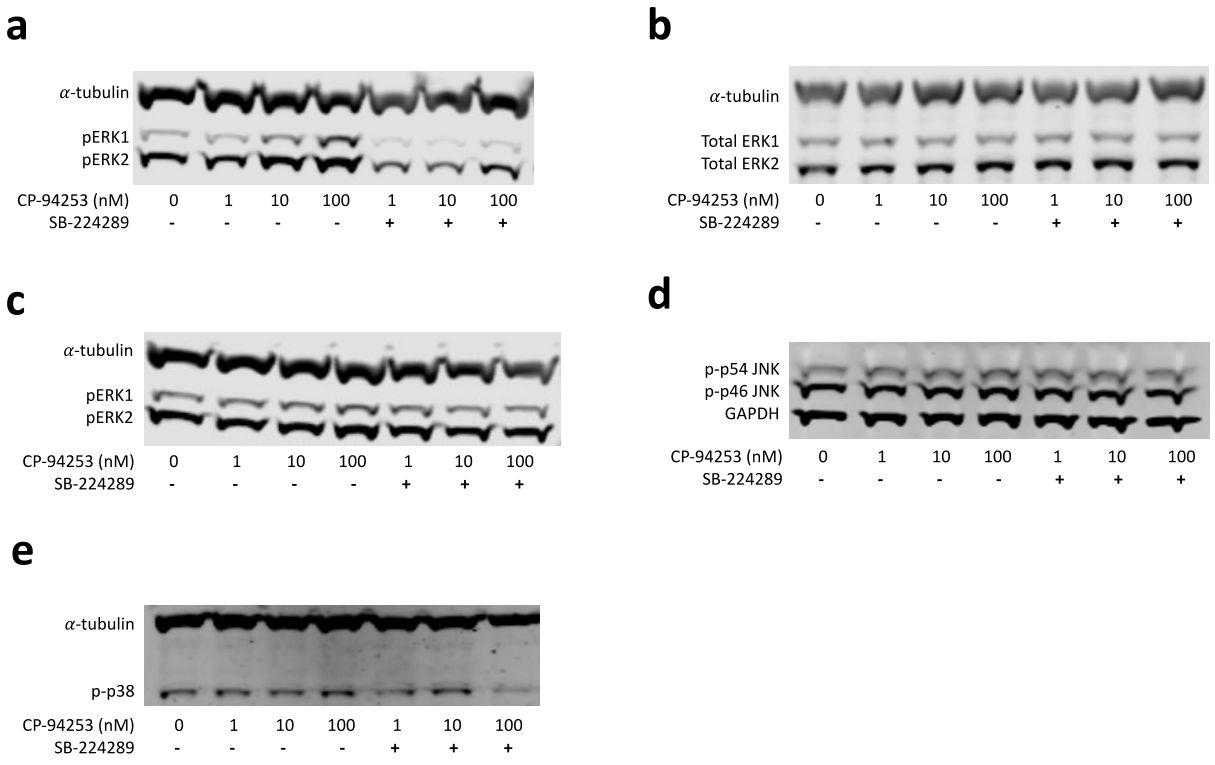
Supplemental Table 1. Primers used for qPCR in untransfected N2A and stably transfected N2A-1B cells.

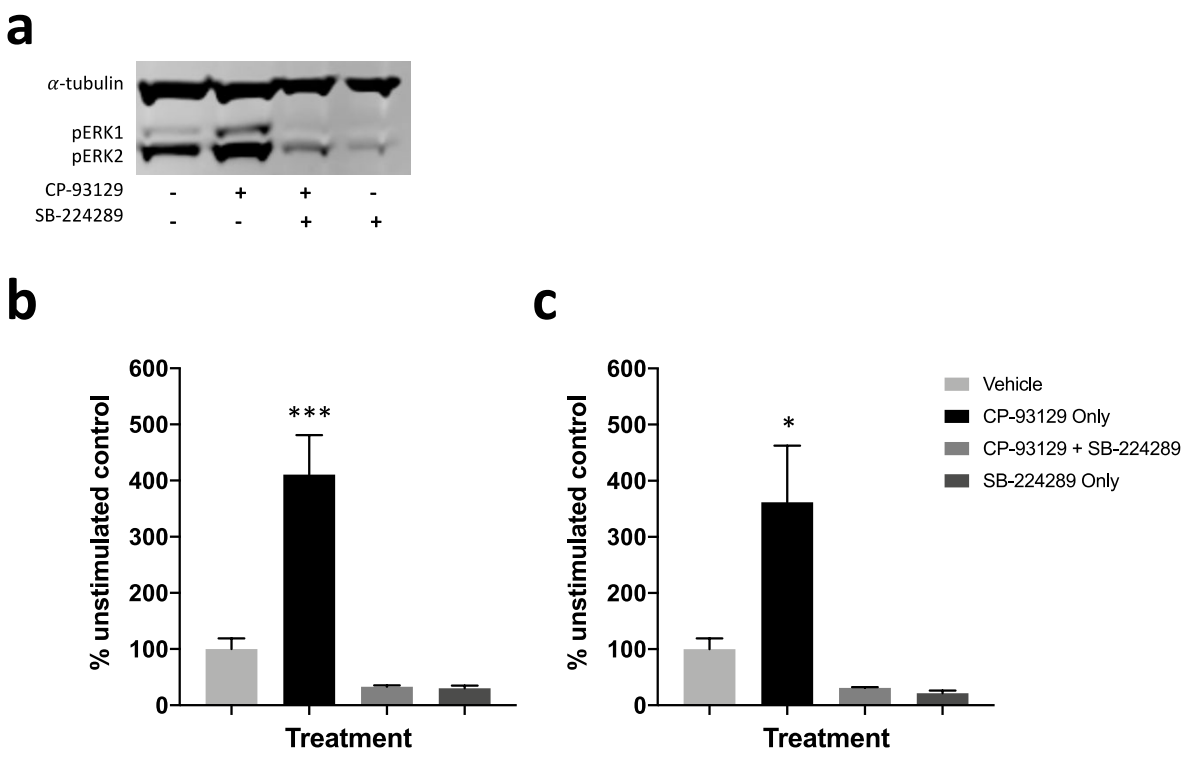
Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
5-HT_{1B}	CACCCTTCTTCTGGCGTCAA	GAGAGCGGGCTTCCACATAG
5-HT_{1A}	GCCAACTATCTCATCGGCTCC	TGGTACAGAGCAGCCATGGG
SERT	TCACGGTGCTTGGCTACATG	GGCAAAGAATGTGGATGCTGG
Pet-1	GTCGGAGATGGTCTTTTTAAGG	TGCCACAACTGGATCTGC
Tph2	CTACCCGACTCATGCTTGCC	CAGGAAGTCTCTTGGGCTCAG
Tph1	AAGAAATTGGCCTGGCTTC	GTTTGCACAGCCCAAACTC
AADC	CTAAGGCCAACCGTGAAAAG	ACCAGAGGCATACAGGGACA
GCH1	GCCTCACCAAACAGATTGC	CACGCCTCGCATTACCAT
VGLUT3	TTTGTCCCCTCATTGTTGGT	GCGCTGCTATGAGGAACAC
VMAT2	TGCTGAAGGACCCATACATTC	CACATGGTCTCCATCATCCA

Supplemental Table 2. Primers used for site-directed mutagenesis of the wild-type 5-HT_{1B} receptor.

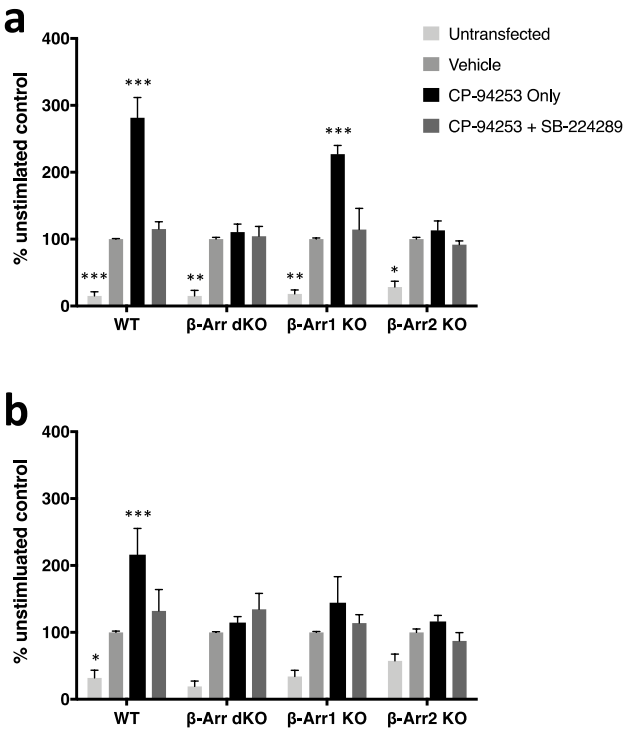
Mutant	**Forward primer (5' to 3')**	**Reverse primer (5' to 3')**
S256A	CAGTTGATAACAGACGCTCCAGGATCCACG	CGTGGATCCTGGAGCGTCTGTTATCAACTG
S291A	GTCAAAGTGCGAGTCGCAGACGCCCTGCTGG	CCAGCAGGGCGTCTGCGACTCGCACTTTGAC
S277A+S279A	AGGTGCCCAGTGAGGCCGGAGCTCCTGTGTACGTGAA	TTCACGTACACAGGAGCTCCGGCCTCACTGGGCACCT
S154A	CACTGATGCGGTGGACTATGCTGCTAAAAGAACTCCCAA	TTGGGAGTTCTTTTAGCAGCATAGTCCACCGCATCAGTG
T158A	TGGACTATTCTGCTAAAAGAGCTCCCAAAAGGGCGGCCATC	GATGGCCGCCCTTTTGGGAGCTCTTTTAGCAGAATAGTCCA
S154A+T158A	TGATGCGGTGGACTATGCTGCTAAAAGAGCTCCCAAAAGGGCGGC	GCCGCCCTTTTGGGAGCTCTTTTAGCAGCATAGTCCACCGCATCA



Supplemental Figure 1: CP-94253 increases levels of phospho-ERK1/2, but not phospho-SAPK/JNK or phospho-p38, in N2A-1B cells, but not in untransfected N2A cells. (a) Treatment with CP-94253 (1-100 nM) for ten minutes increased phosphorylation of ERK1 and ERK2 in N2A-1B cells compared to unstimulated N2A-1B control cells treated with vehicle (PBS), but this was blocked by pretreatment with the antagonist SB-224289. (b) No change was observed in total ERK in N2A-1B cells. (c) No change was observed in phospho-ERK1/2 with agonist treatment in untransfected wild-type N2A cells. Agonist treatment did not change levels of (d) phospho-p54 JNK, phospho-p46 JNK, or (e) phospho-p38.



Supplemental Figure 2: CP-93129 increases levels of phospho-ERK1/2 in N2A-1B cells. (a and b) Treatment with CP-94253 (100 nM) for ten minutes increased phosphorylation of ERK1 in N2A-1B cells compared to unstimulated N2A-1B control cells treated with vehicle (PBS) ($p = 0.0008$), but this was blocked by pretreatment with the antagonist SB-224289 ($p = 0.467$ for CP-93129 + SB-224289, $p = 0.437$ for SB-224289 only). (a and c) Treatment with CP-94253 (100 nM) for ten minutes also increased phosphorylation of ERK2 in N2A-1B cells compared to unstimulated N2A-1B control cells treated with vehicle (PBS) ($p = 0.017$), but this was blocked by pretreatment with the antagonist SB-224289 ($p = 0.673$ for CP-93129 + SB-224289, $p = 0.591$ for SB-224289 only). Data are expressed as the percent change in pERK signal compared to the no agonist control from each independent biological replicate. Error bars represent SEM and data are averages of 3 independent biological replicates for all groups (one-way ANOVA with Dunnett's post hoc tests; $***p < 0.001$, $*p < 0.05$).



Supplemental Figure 3. 5-HT_{1B}-mediated phosphorylation of ERK1/2 is dependent on β-arrestin signaling in mouse embryonic fibroblast (MEF) cells. Wild-type (WT), β-arrestin 1/2 double knockout (β-Arr dKO), β-arrestin 1 knockout (β-Arr1 KO), and β-arrestin 2 knockout (β-Arr2 KO) MEF cells were transiently transfected with HA-5-HT_{1B} plasmid. Untransfected control cells were treated with vehicle (PBS), and transfected cells were treated with vehicle, agonist alone (100 nM CP-94253 for 10 min), or agonist and antagonist (1 hour preincubation with 1 μM SB-224289, followed by 100 nM CP-94253 for 10 min). Levels of phospho-ERK1/2 were examined using western blot. (a) Phosphorylation of ERK1 in MEF cells differed significantly by treatment ($F_{3,64} = 62.73$, $p < 0.0001$) and by cell type ($F_{3,64} = 8.54$, $p < 0.0001$), with a significant interaction between treatment and cell type ($F_{9,64} = 7.363$, $p < 0.0001$). 5-HT_{1B} receptor activation significantly increased levels of phospho-ERK1 in WT ($p < 0.0001$) and β-Arr1 KO cells ($p < 0.0001$) but not in β-Arr dKO ($p = 0.954$) or β-Arr2 KO cells ($p = 0.922$). Expression of 5-HT_{1B} receptors alone activated pERK1 in all cell lines compared with untransfected controls (WT $p < 0.0001$; β-Arr dKO $p = 0.004$; β-Arr1 KO $p = 0.006$; β-Arr2 KO $p = 0.019$). (b) Phosphorylation of ERK2 did not significantly differ by cell type ($F_{3,64} = 1.618$, $p = 0.194$), nor was there a significant interaction between

treatment and cell type ($F_{9,64} = 1.436$, $p = 0.192$). However, there was a significant main effect for treatment ($F_{3,64} = 14.14$, $p < 0.0001$). 5-HT_{1B} receptor activation significantly increased levels of phospho-ERK2 in WT cells ($p = 0.0002$) but not in β-Arr dKO ($p = 0.963$), β-Arr1 KO ($p = 0.515$), or β-Arr2 KO cells ($p = 0.950$). Expression of 5-HT_{1B} receptors alone activated pERK2 in WT cells ($p = 0.037$). In all groups receiving preincubation with the 5-HT_{1B} antagonist SB-224289 prior to treatment with CP-94253, levels of ERK1/2 activation were similar to that of vehicle-treated cells ($p > 0.05$). Data are expressed as the percent change in pERK signal compared to the no agonist control from each independent biological replicate. Data are averages of 4 independent biological replicates for all groups (two-way ANOVA with Dunnett's post hoc tests; ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$).