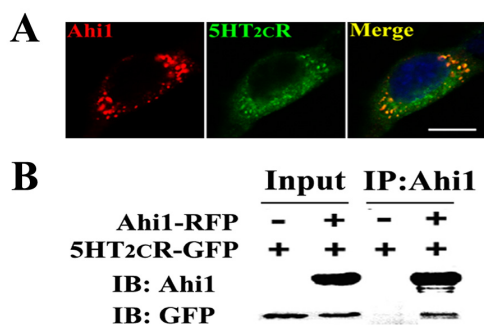
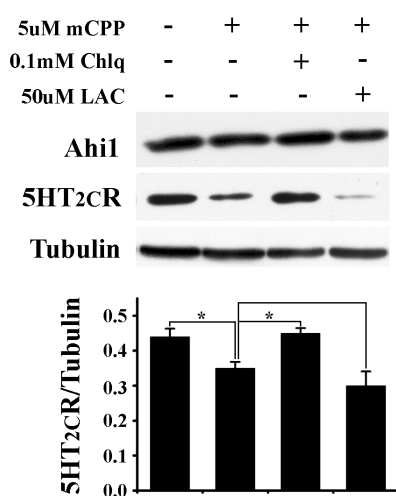


SUPPLEMENTAL DATA

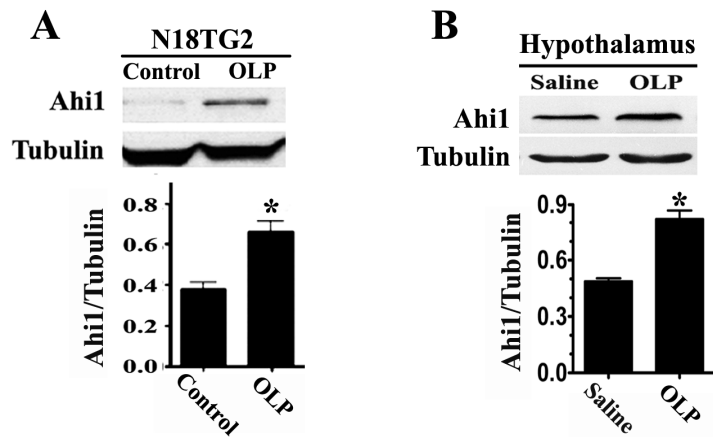


Supplemental Figure 1 DsRed-Ahi1 and GFP-5-HT_{2C}R were transfected into HEK293 cells, and immunofluorescence and co-IP were performed on these cells. (A) Immunofluorescence showed clear overlap of Ahi1 and 5-HT_{2C}R in HEK293 cells. (B) Co-IP of 5-HT_{2C}R with Ahi1 in transfected HEK293 cells. Scale bar: 10 μ m.

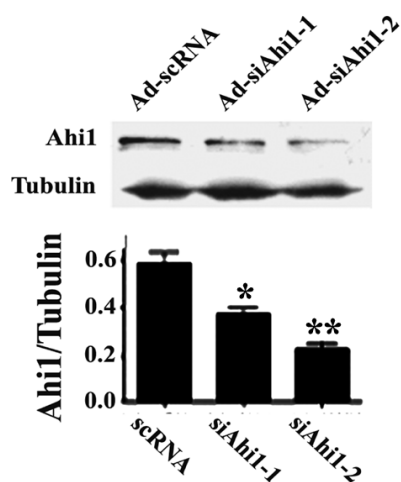
Abbreviation: IP, immunoprecipitation; IB, immunoblotting.



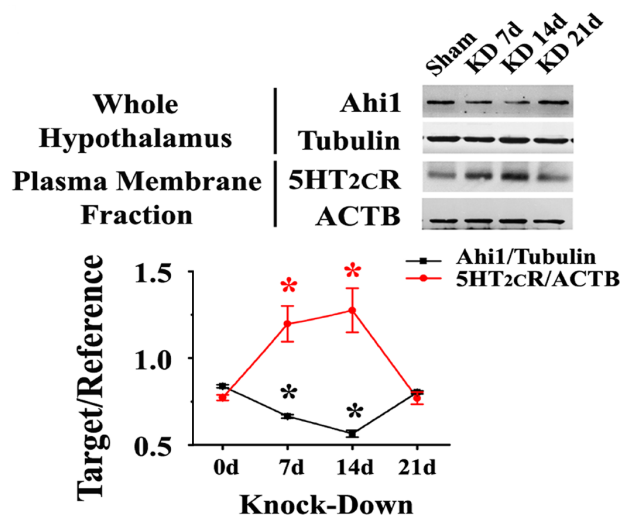
Supplemental Figure 2 The degradation of 5-HT_{2C}R mediated by Ahi1 was through lysosomal pathway rather than proteasome pathway. When we treated cells co-transfected with Ahi1 and 5-HT_{2C}R by chloroquine (Chlq, a lysosomal inhibitor), the degradation of 5-HT_{2C}R was reversed; whereas, the treatment of lactacystin (LAC, a proteasome inhibitor) did not reverse the degradation of 5-HT_{2C}R. Abbreviation: mCPP, *meta*-chlorophenylpiperazine.



Supplemental Figure 3 Olanzapine increased the expression of Ahi1. (A) Ahi1 levels were measured in N18TG2 cells treated by OLP (Sigma) for 72 hours. OLP induced a significant increase of Ahi1 protein. (B) Mice were injected (i.p.) with OLP (Sigma, 2 mg/kg) for 4 weeks and the hypothalamic Ahi1 was measured by western blot. OLP treatment significantly increased the expression of Ahi1 in hypothalamus. Data are expressed as mean \pm SEM. *Statistically different from control group. Abbreviation: OLP, olanzapine.



Supplemental Figure 4 Ahi1-specific siRNA effectively inhibited the expression of endogenous Ahi1 in N18TG2 cells. Western blot showed the concentration of 10^7 plaque-forming units of Ad-siAhi1-2 was effective in knocking down the expression of Ahi1. Data are expressed as mean \pm SEM. *Statistically different from control group. Abbreviation: Ad-siAhi1, adenoviral vector with Ahi1-specific siRNA; Ad-scrRNA, adenoviral vector with scramble-siRNA.



Supplemental Figure 5 5-HT_{2C}R located on the neuronal membrane under the condition of artificial knockdown by RNA interference. As shown by western blot, the membrane distribution of 5-HT_{2C}R significantly increased when the expression of Ahi1 was down-regulated, after which a regression followed that was due to Ahi1 expression recovery. Data are expressed as mean \pm SEM. *Statistically different from control group. Abbreviation: KD, knockdown; ACTB, actin-beta.