## SUPPLEMENTARY INFORMATION

## Distinct active conformations of a ligand-gated ion channel

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Supplementary Figure 1. Chemicals used in this study.

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Supplementary Figure 2. Actions of 5-HT analog 3-(2-hydroxyethyl)indole (3-2-HEI). *A*, Current traces show the responses elicited by 30  $\mu$ M 5-HT (left) and 5 mM 3-2-HEI alone (right) in HEK 293 cells expressing the 5-HT<sub>3A</sub> (left) and 5-HT<sub>3AB</sub> (right) receptors at a holding potential of -60 mV. The drug application protocol was similar to that described in Fig. 3A. *B*, Superimposed traces compare the responses activated by 5-HT and 3-2-HEI. Similar responses were obtained from 8-10 cells.



Supplementary Figure 3. Kinetics of 5-HI-suppressed constitutive activity in HEK 293 cells expressing the 5-HT<sub>3AB</sub> receptor. *A*, Whole-cell current traces show the detection of constitutive receptor activity by 5 and 20 mM 5-HI. *B*, Representative responses illustrate the kinetics for onset ( $\tau_{Block}$ ) and offset ( $\tau_{Unblock}$ ) of the inhibition of constitutive activity by 5 mM 5-HI. The onset and offset and of inhibition could be described by a mono-exponential function. The bar graphs show summaries of kinetics for  $\tau_{Block}$  and  $\tau_{Unblock}$  for 5, 10 and 20 mM 5-HI. *C*, Linear regression show the rate of inhibition ( $1/\tau_{Block}$ ) was dependent on concentration (slope = 5.9 X 10<sup>4</sup> M<sup>-1</sup> s<sup>-1</sup>; intercept = 4.5 s<sup>-1</sup>; r<sup>2</sup> > 0.99), whereas the concentration-dependence for unblock is not obvious (intercept = 4.8 s<sup>-1</sup>, n = 7-12 cells per point). The calculation of *Ki* for 5-HI depressing the constitutive activity was based on the method described by Gunthorpe and Lummis (1). Error bars are s.e.m.



Supplementary Figure 4. A schematic summary of the effects of 5-HT analogs.

## References

1. Gunthorpe, M. J. & Lummis, S. C. (1999) J Physiol 519, 713-722.