

## Supplementary Figures

Supplementary Figure 1.  $\rho 1$ T6' mutants: GABA concentration-response curves. A. T6'Y (n= 7) B. T6'F (n= 7-8). C. T6'V (n= 4-5). D. T6'M (n= 4). Symbols and error bars represent means  $\pm$  SEM.

Supplementary Figure 2. Linear correlation between ethanol and hexanol effects on  $\rho 1$  T6' mutants and different variables. Amino acid volume and effect for ethanol (panel A, p = 0.045) and hexanol (panel B, p = 0.18). Hydrophobicity and effect for ethanol (panel C, p = 0.40) and hexanol (panel D, p = 0.29). GABA EC<sub>50</sub> and effect for ethanol (panel E, p = 0.17) and hexanol (panel F, p = 0.04).

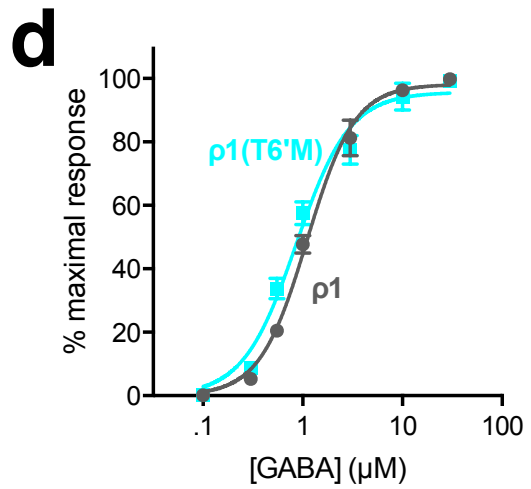
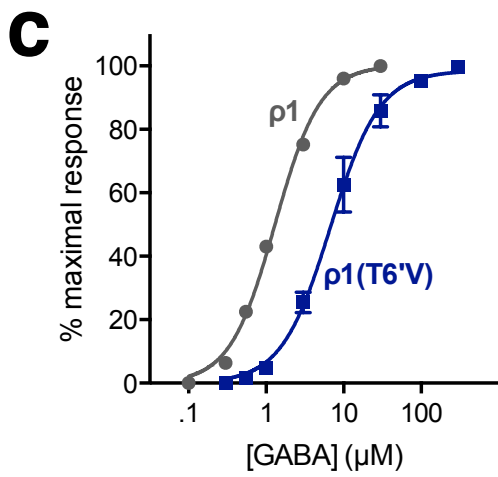
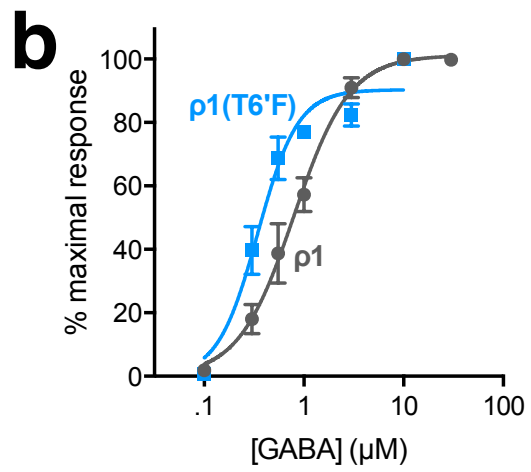
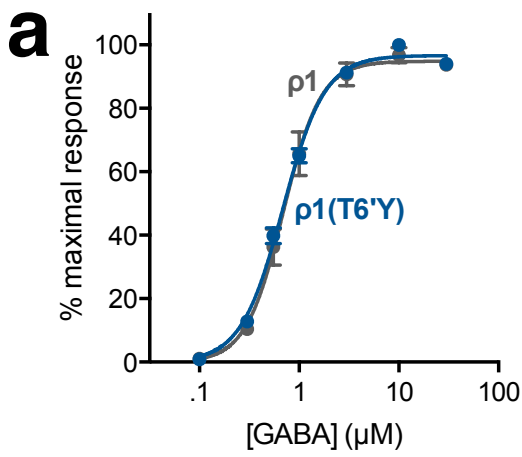
Supplementary Figure 3.  $\rho 1$ I5' mutants: GABA concentration-response curves. A. I5'A (n= 7-9). B. I5'V (n= 6). C. I5'F (n= 4). D. I5'W (n= 3-4). Symbols and error bars represent means  $\pm$  SEM.

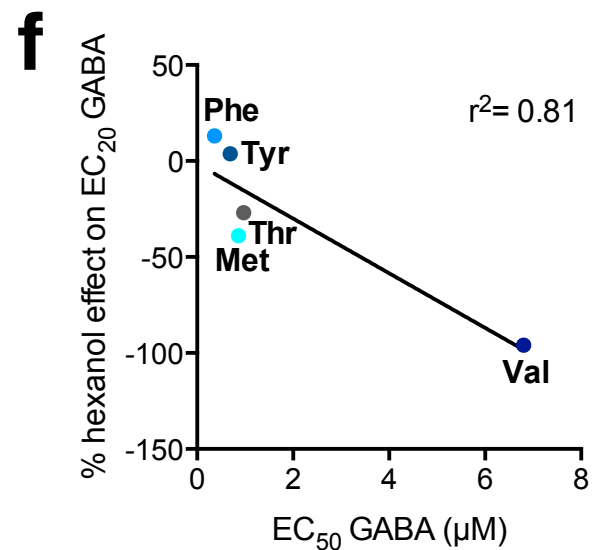
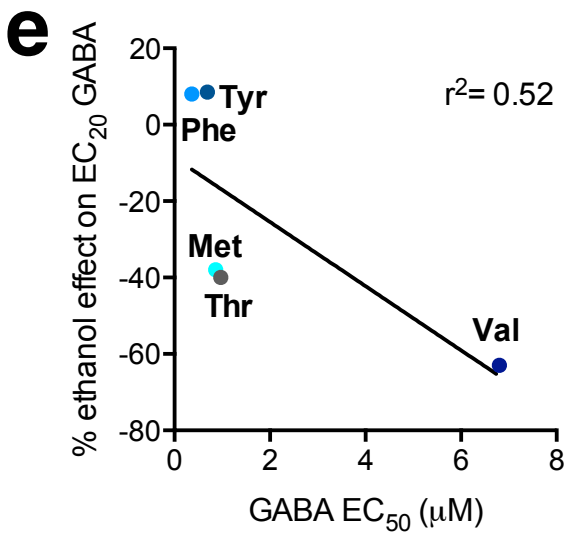
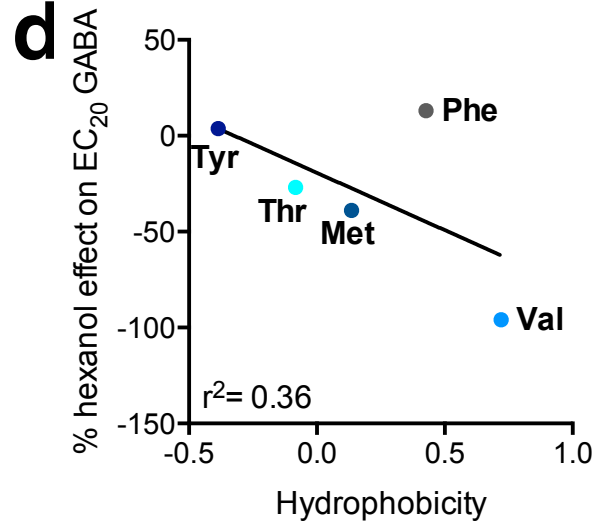
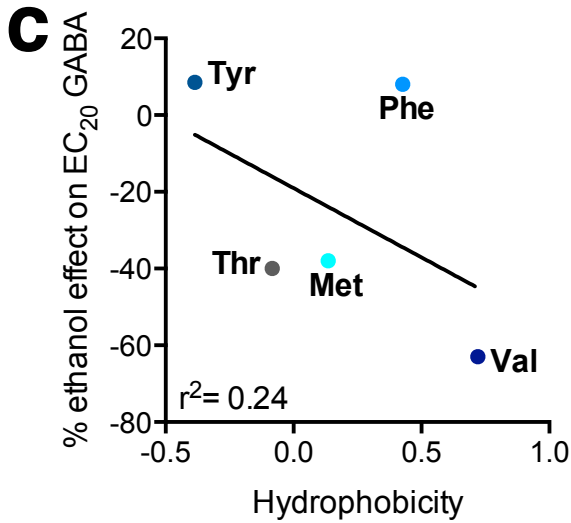
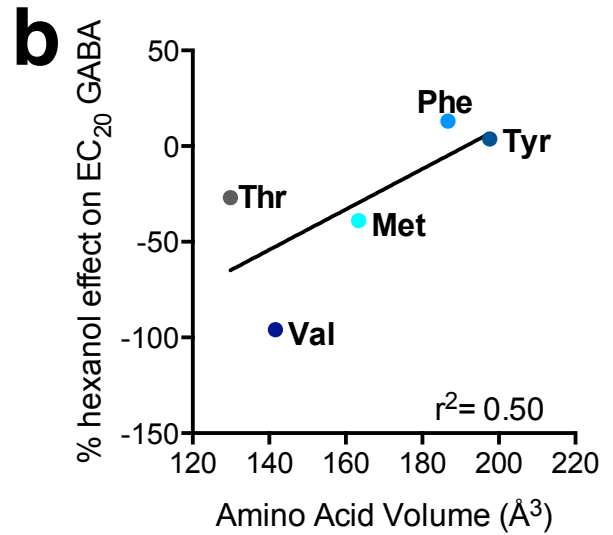
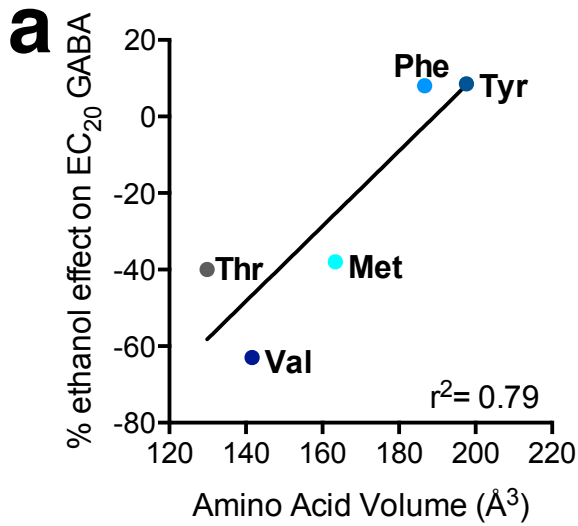
Supplementary Figure 4. Linear correlation between ethanol and hexanol effects on  $\rho 1$  I5' mutants and different variables. Amino acid volume and effect for ethanol (panel A, p = 0.22) and hexanol (panel B, p = 0.30). Hydrophobicity and effect for ethanol (panel C, p = 0.25) and hexanol (panel D, p = 0.02). GABA EC<sub>50</sub> and effect for ethanol (panel E, p = 0.30) and hexanol (panel F, p = 0.95).

Supplementary Figure 5. Neighboring  $\rho 1$  mutants in TM2 and TM1: GABA concentration-response curves. A. L9'F (n= 4-5). B. L3'F (n= 5). C. P2'G (n= 3-4). D. L277F (n= 4-6). Symbols and error bars represent means  $\pm$  SEM.

Supplementary Figure 6.  $\rho 1$ T6'Y and/or I15'S mutants: GABA concentration-response curves. Symbols represent means and bars indicate SEM (n= 6-13).

Supplementary Figure 1





Supplementary Figure 3

