Supplementary Information

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A Discrete Alcohol Pocket Involved in GIRK Channel Activation

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Supplementary Item & Number	Title
Supplementary Figure S1	Dual modulation of GIRK2 by 1-PeOH
Supplementary Figure S2	Assay to detect surface expression of mutant channels.
Supplementary Figure S3	Mutation in GIRK4 pore-helix converts 1-BuOH from an inhibitor to activator.
Supplementary Figure S4	Site for regulating sensitivity to alcohol-dependent inhibition.
Supplementary Figure S5	Mutations L342 and Y349 in the βL-βM loop of GIRK2 reduce both alcohol and/or m2R-activated currents.

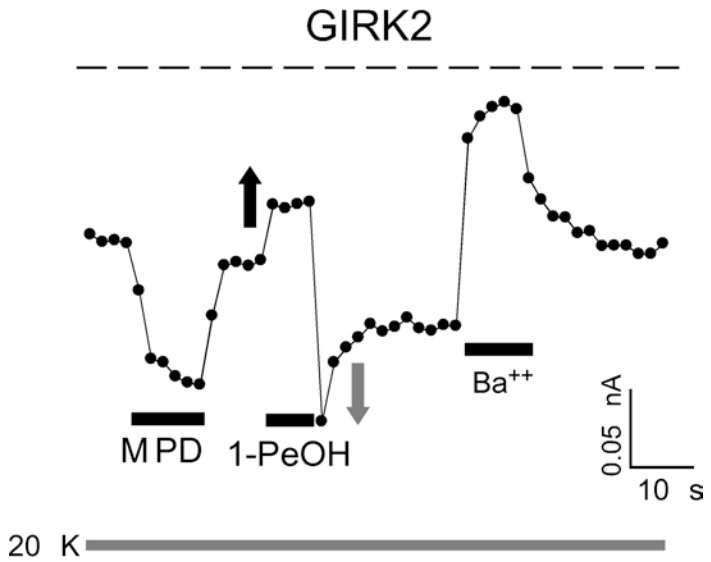

Figure S1. Dual modulation of GIRK2 by 1-PeOH. Example of inward K^+ current for GIRK2 in response to 25 mM MPD, 25 mM 1-PeOH or 1 mM Ba^{++}. Note that 1-PeOH inhibits (black arrow) the basal current and reveals a potentiated current (grey arrow) immediately following washout of 1-PeOH. If the rate of deactivation is slower than that for unblocking, then the potentiated current reflects activated GIRK2 channels that have unblocked. Holding potential was –100 mV.

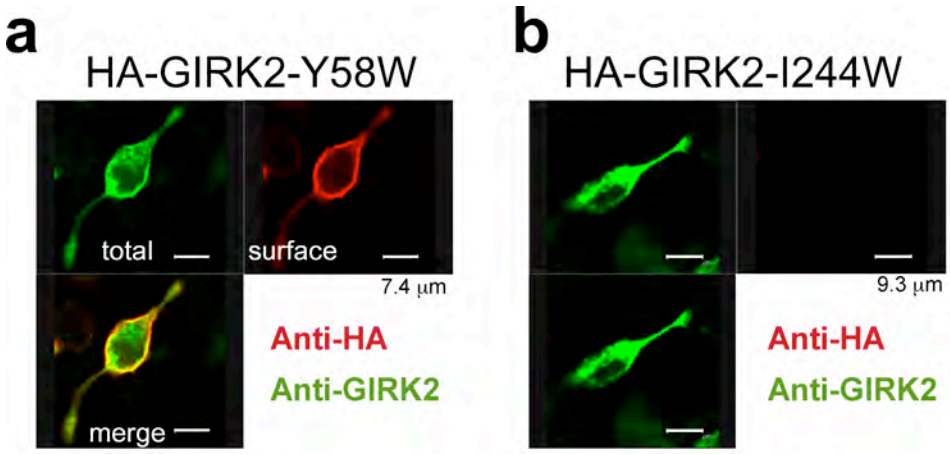

Figure S2. **Assay to detect surface expression of mutant channels**. HA-tagged channels were transfected in HEK cells and immunostained with anti-HA antibodies (unpermeabilized), followed by permeabilization and immunostaining with anti-GIRK2 antibodies. **a**) HA-GIRK2-Y58W expressed on the surface of unpermeabilized cells (red) as well as in the cytoplasm (green). **b**) GIRK2-I244W was only detected in permeabilized cells (green), indicating no surface expression.

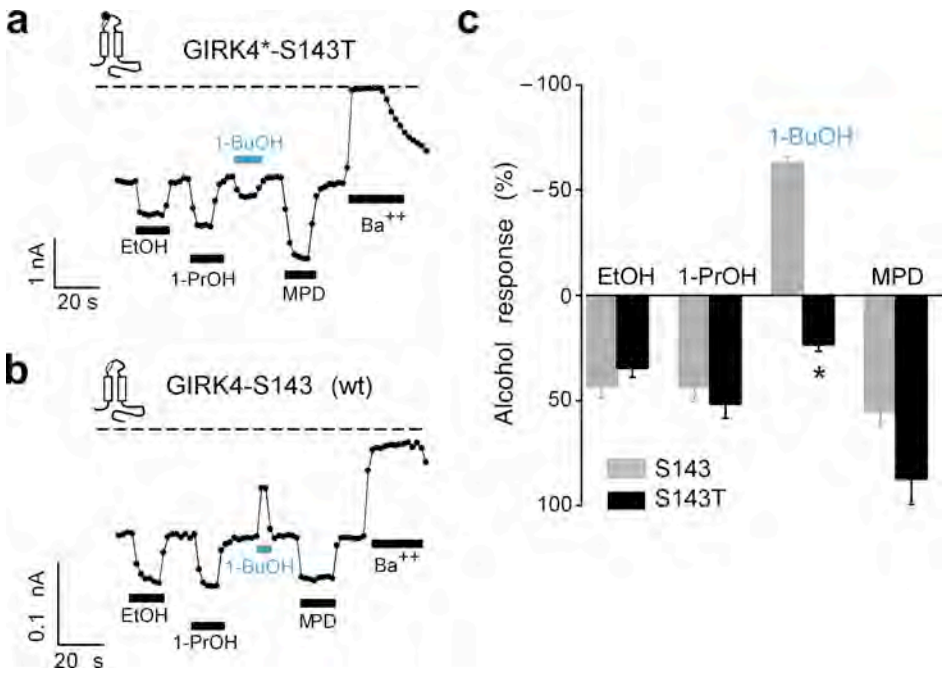

Figure S3. **Mutation in GIRK4 pore-helix converts 1-BuOH from an inhibitor to activator. a-b**) Examples of currents for GIRK4* (S143T) (**a**) and GIRK4 wild-type (wt) (**b**) channels measured at –100 mV in response to 50 mM EtOH, 50 mM 1-PrOH, 50 mM 1-BuOH or 50 mM MPD or 1 mM Ba^{++} in 20K solution. The K+ basal currents (Ba^{++} sensitive) were significantly larger for GIRK4*-S143T (–174 ± 25 $pApF^{-1}$, n = 7) than for wild-type GIRK4-S143 (–9.57 ± 2.21 $pApF^{-1}$, n = 7). **c**) Bar graph shows mean percentage alcohol response normalized to Ba^{++}-sensitive basal current (n = 6-7).

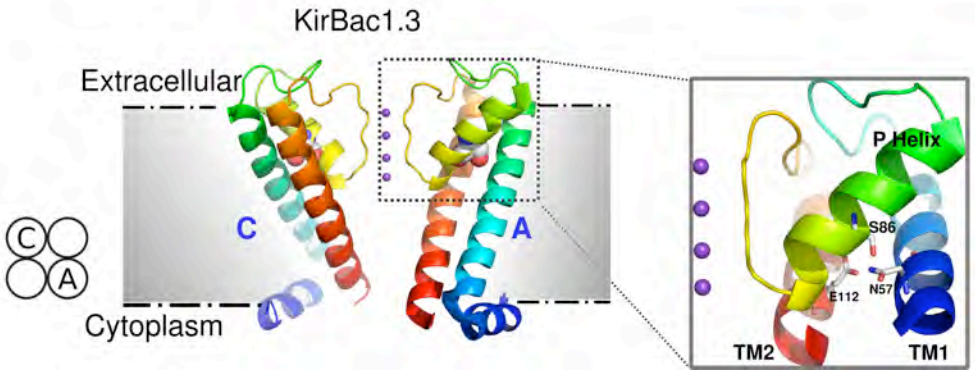

Figure S4. Site for regulating sensitivity to alcohol-dependent inhibition. S86, which is the amino acid homologous to S148 in GIRK2, is located in the pore-helix of KirBac1.3. Two opposing subunits (labeled A and C) are shown from the putative closed state of the Kirbac1.3-GIRK1 structure (PDB: 2QKS) and with four K^+ in the selectivity filter. Inset, zoom shows S86 from the pore-helix interacts with amino acids N57 and E112 from M1 and M2 transmembrane domains, respectively. There is no appreciable space to accommodate alcohol, suggesting this site influences an inhibition site located elsewhere in the channel.

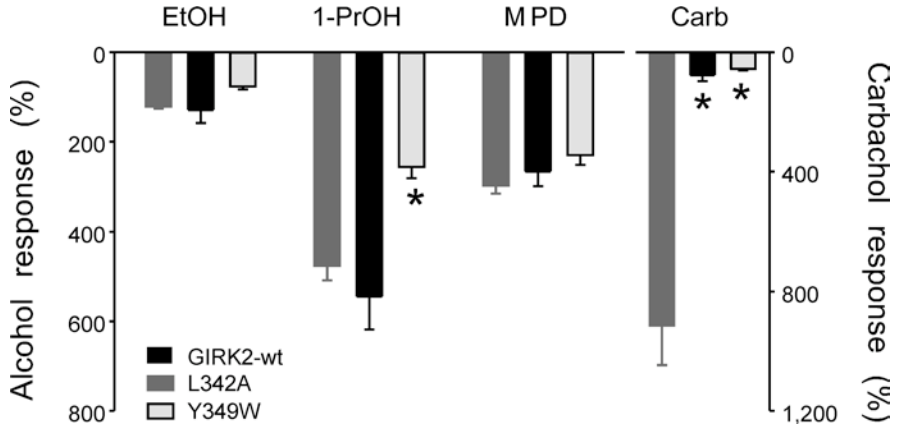

Figure S5. **Mutations L342 and Y349 in the βL-βM loop of GIRK2 reduce both alcohol and/or m2R-activated currents.** Bar graphs show the mean response (%) for GIRK2 (dark grey, n = 34), GIRK2-L342A (black, n = 10) and GIRK2-Y349W (light grey, n = 10) in response to 100 mM 1-PrOH, 100 mM MPD, 100 mM EtOH, or 5 μM carbachol, normalized to K^+ basal current (Ba^{++} sensitive). *$P < 0.05$ vs. wild-type. Substitutions at A, F, Y or W at L342 and A, T, L, F at Y349 produced little or no Ba^{++} sensitive basal currents (< –1 $pApF^{-1}$).