Online supplementary data: dose-response curves in mM

The following graphs are the same data presented in Figures 1-4 and 6, with effect plotted against anesthetic concentration in mM rather than %. The conversion uses the data of Franks [ref], who reviewed published data in the literature to conclude that, on average, 1% halothane equates to 0.24 mM in aqueous solution and 1% isoflurane is 0.28mM.

Figure S1. Re-plot of Figure 1B. Quantitative effect of halothane and isoflurane across a range of concentrations tested on depressing the glomus cell $[Ca^{2+}]i$ response to hypoxia. Each point is mean ± SD, with a total of 44 recordings for halothane and 52 for isoflurane (different cells for each agent; a maximum of for concentrations in any one cell for any single agent). The exact n values for each data point are (lowest to highest concentrations): 7, 7, 13, 5, 7, 5 (halothane); 3, 11, 5, 8, 6, 9, 5, 5 (isoflurane). This is plotted as a conventional 'dose-response' relationship, where 'response' is the agent's ability to diminish the $[Ca^{2+}]i$ response to hypoxia (100% being complete abolition of the response). This plot makes clearer how isoflurane appears to have a lower intrinsic activity for this effect.

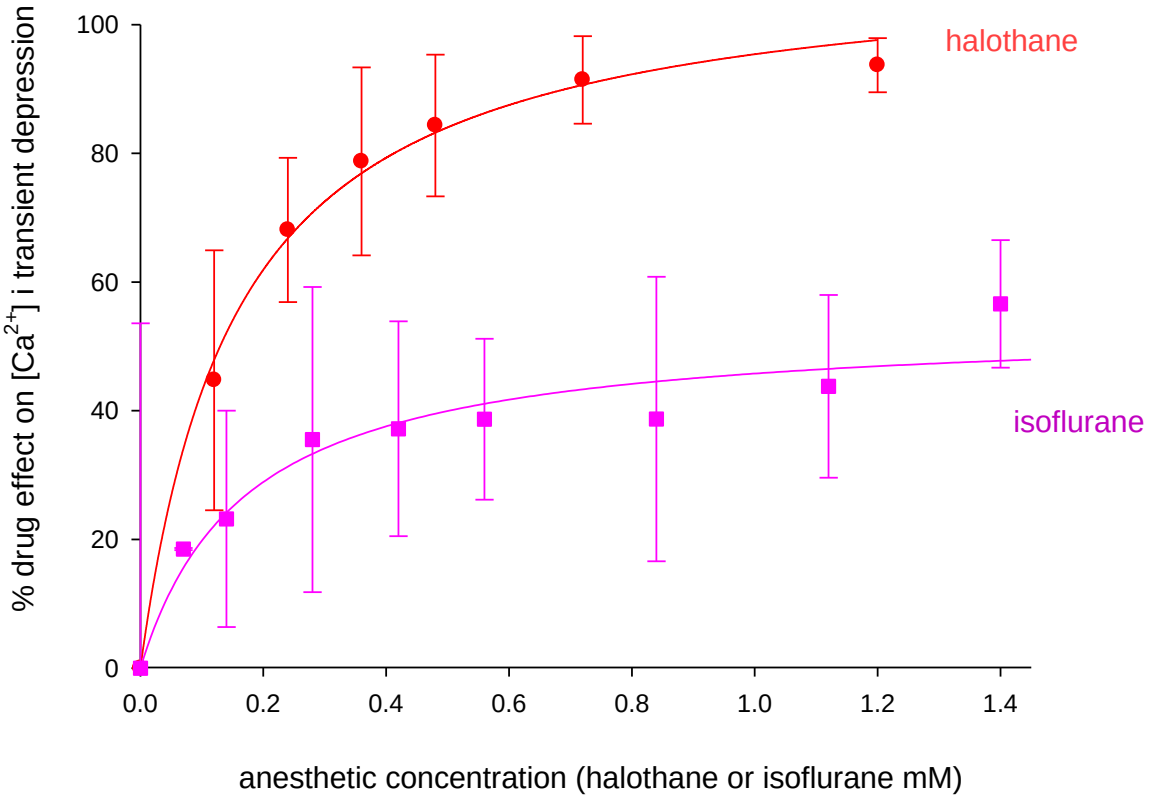

Figure S2. Re-plot of Figure 2. The effect anesthetic combinations on depression of the hypoxic $[Ca^{2+}]_i$ transient. Halothane 0.36 mM depressed the hypoxic $[Ca^{2+}]_i$ transient by 78.7 ± 14.6% (see Figure 1B), which is shown by the single red symbol. At this constant background level of 0.36 mM halothane, progressively adding isofurane to the mix (0.28, 0.42 and 0.84) in separate experiments does not increase the depressive effect of halothane on hypoxic $[Ca^{2+}]_i$ transient to >80%, but instead diminishes it. For clarity, also shown (purple) is the best fit line using Equation 1 transposed from Fig. 1B for the effects of isoflurane alone to illustrate the fact that as more isoflurane is added to the mix, the effect tends towards that for isoflurane alone. Also shown (green line) is the estimated plot from Equation 2, assuming the agents compete at a single receptor. Each symbol is mean ± SD; n = 25 total for the mix. Exact n values for the mix (lowest to highest concentrations) are: 8, 8, 9. There were two conditions per cell in a paired design (halothane and mix).

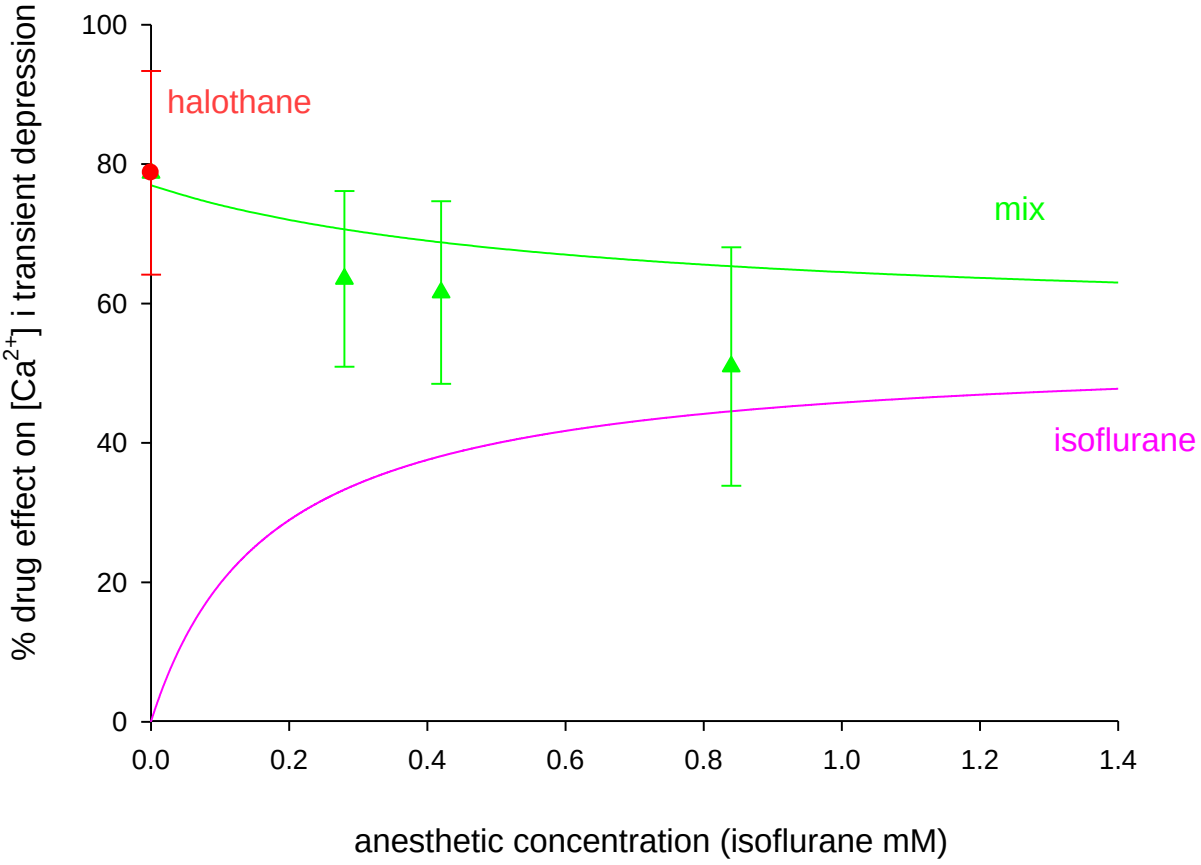

Figure S3. Re-plot of Figure 3B. Quantitative analysis of nPopen activities for halothane (red) and isoflurane (purple) and mix of the following concentrations of agent (green: 0.36 mM halothane + 0.42 mM isoflurane; 0.6 mM halothane + 0.7 mM isoflurane; 0.96 mM halothane + 1.12 mM isoflurane). The red and purple lines (for halothane and isoflurane respectively) are the result of applying Equation 1 to the data, from which individual agent Kd and Bmax were estimated. The green line is the result of applying Equation 2 using these Kd and Bmax values. Values are mean ± SD (unidirectional bars for clarity), with a total of 176 separate patches in separate cells performed. The exact n values for each point (lowest to highest concentrations) are: 5, 13, 17, 6, 15, 13 (halothane); 5, 5, 13, 16, 14, 15, 10 (isoflurane); 9, 10, 10 (mix).

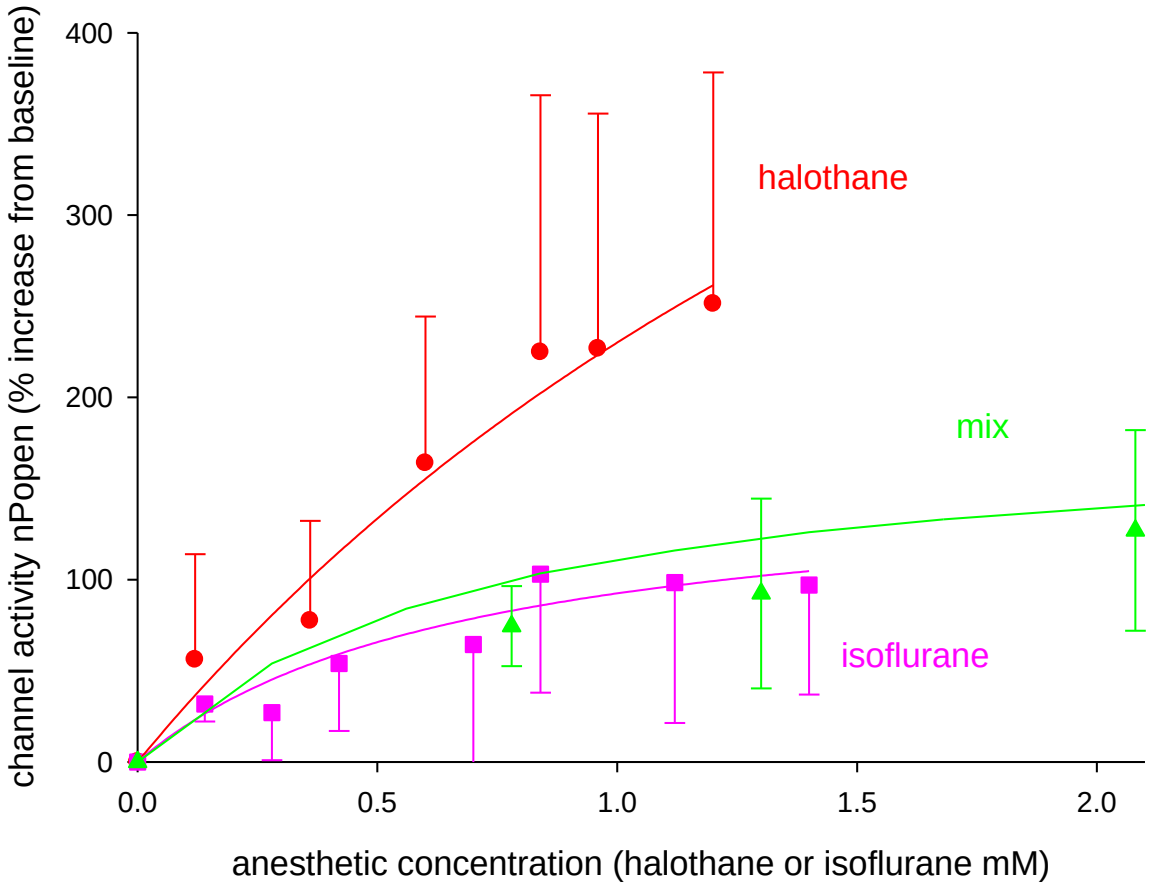

Figure S4. Re-plot of Figure 5B. Results of quantitative analysis of TASK-1 channel activation expressed in HEK cells (nPopen values, mean ± SD; n = 81 separate recordings in separate cells). The exact n values are (lowest to highest concentrations): 6, 21, 7, 5, 8 (halothane); 5, 5, 7, 6, 7, 4 (isoflurane). Halothane (red) is a stronger activator than isoflurane (purple) with both agents reaching apparent saturation, and isoflurane (as in Figure 1) appearing to exhibit partial agonist behaviour. The red (halothane) and purple (isoflurane) lines are the result of applying Equation 1 to the data, and estimating individual Kd and Bmax values.

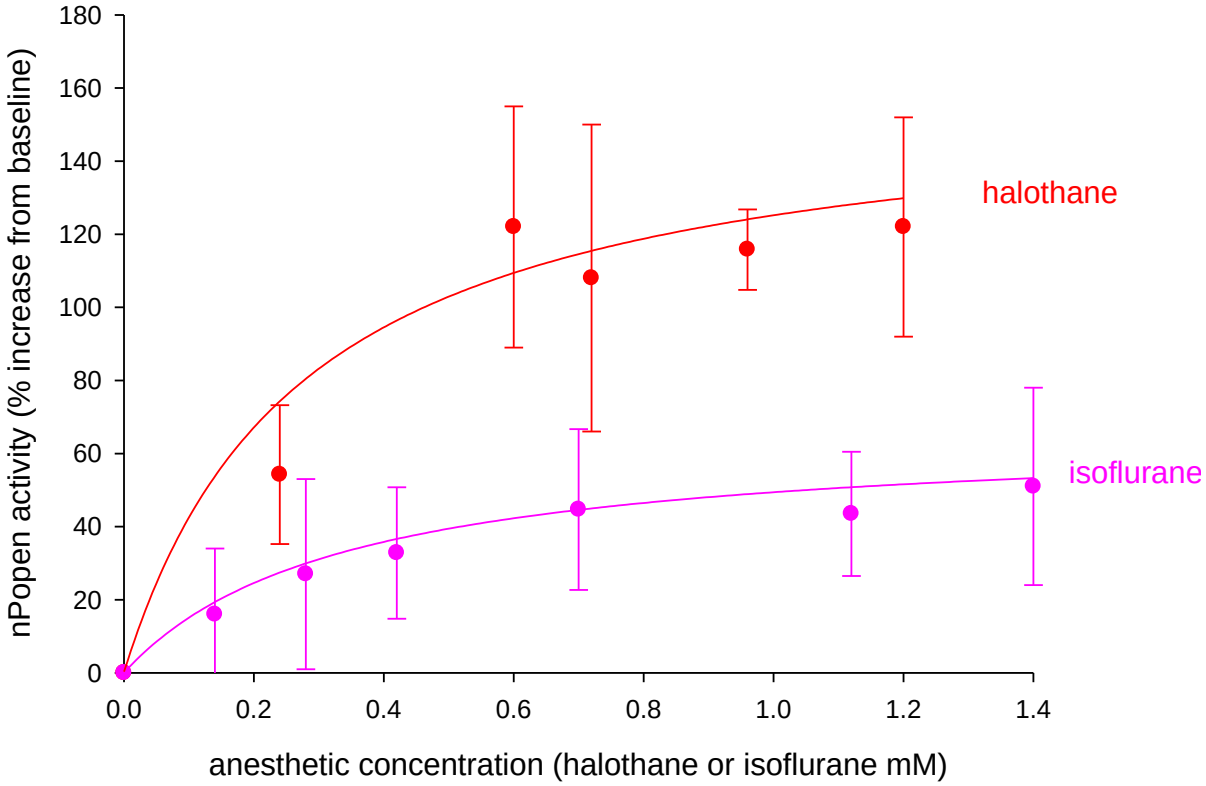

Figure S5. Re-plot of Figure 6 (the effect of anesthetic combinations on TASK1 open probabilities expressed in HEK293 cells) in two ways: the anesthetic concentrations are presented as mM and therefore, the total aqueous concentration is presented on the x-axis. Compare with Figure 2. Halothane 0.6 mM (single red symbol) activates TASK1 channels to just under 120% (see also Figure 5B). At this constant background level of 0.6 mM halothane (red symbol), progressively increasing concentrations of isoflurane were added to the mix (concentrations of 0.42, 0.7, 1.12, 1.4 mM). The effect was not additive, but infra-additive. Also for clarity is shown (purple) is the plotted line from Equation 1 for ligand binding copied from Fig. 5B for isoflurane, to show that as isoflurane is added to the mix, the result tends towards that for isoflurane alone, and not away from it. The green line is the line plotted from Equation 2, using the estimated values of Bmax and Kd for halothane and isoflurane. Each symbol is mean ± SD of n = 30 paired recordings in 30 separate cells for the mix line. The exact values are (lowest to highest concentrations): 9, 8, 5, 8.