"Halogenated ethers, alcohols, and alkanes activate TASK-3 tandem pore potassium channels likely through a common mechanism". Anita Luethy, James D. Boghosian, Rithu Srikantha, and Joseph F. Cotten. *Molecular Pharmacology.*

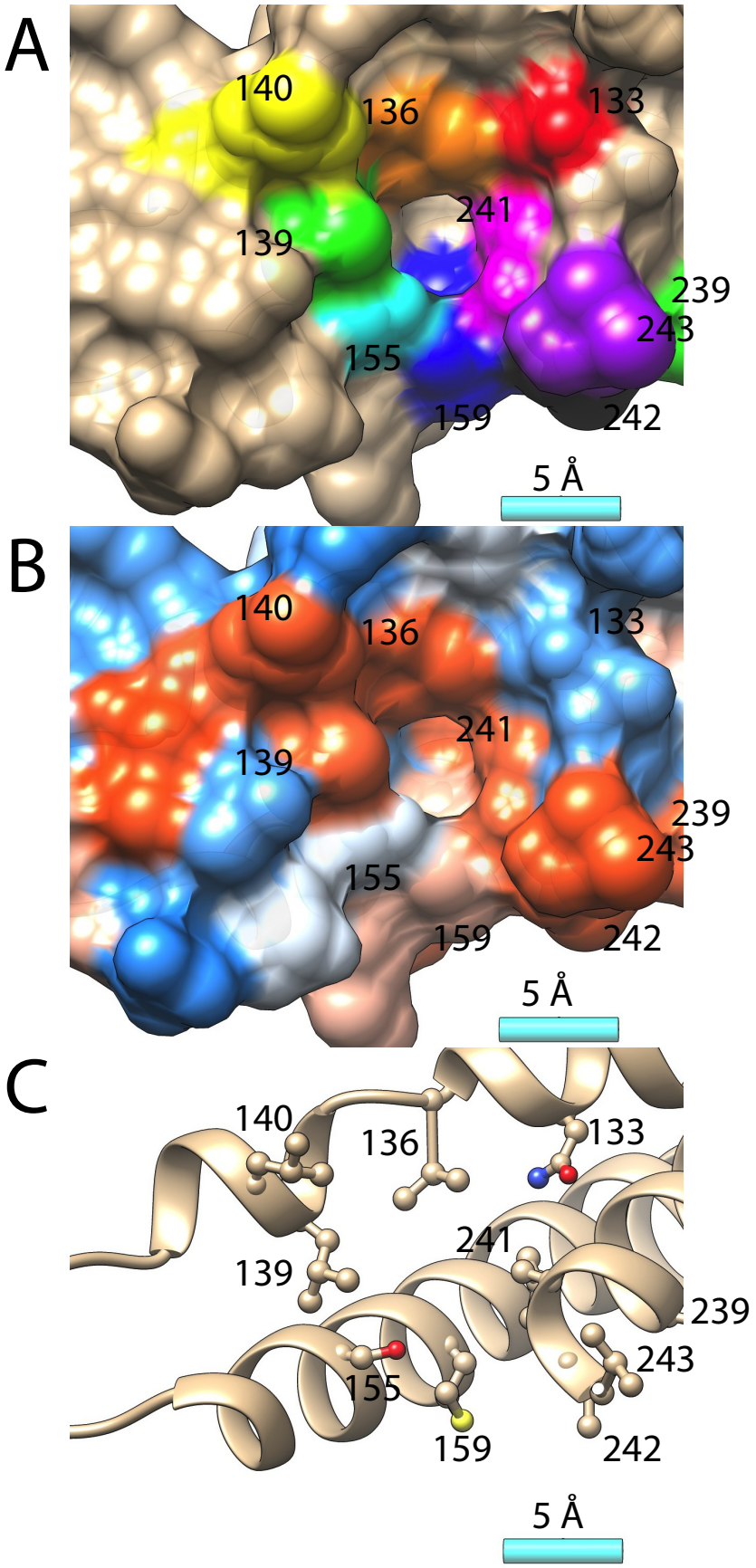

Figure S1. Surface rendering of the putative anesthetic binding site in the TASK-3 homology model. A, close up of the putative anesthetic binding site. Contribution of relevant amino acids to the surface are demarcated by color: Met-133 (red), Val-136 (orange), Leu-139 (green), Leu-140 (yellow), Met-159 (blue), Leu-239 (green), Leu-241 (magenta), Leu-242 (black), and Val-243 (purple). B, identical structure as in A, but with molecular surfaces colored by Kyte-Doolittle amino acid hydrophobicity (dodger blue for the most hydrophilic, to white, to orange-red for most hydrophobic). C, ribbon diagram of identical structure as in A & B, but with surface rendering removed and residue side chains represented by ball and stick.

Supplementary Figure S1

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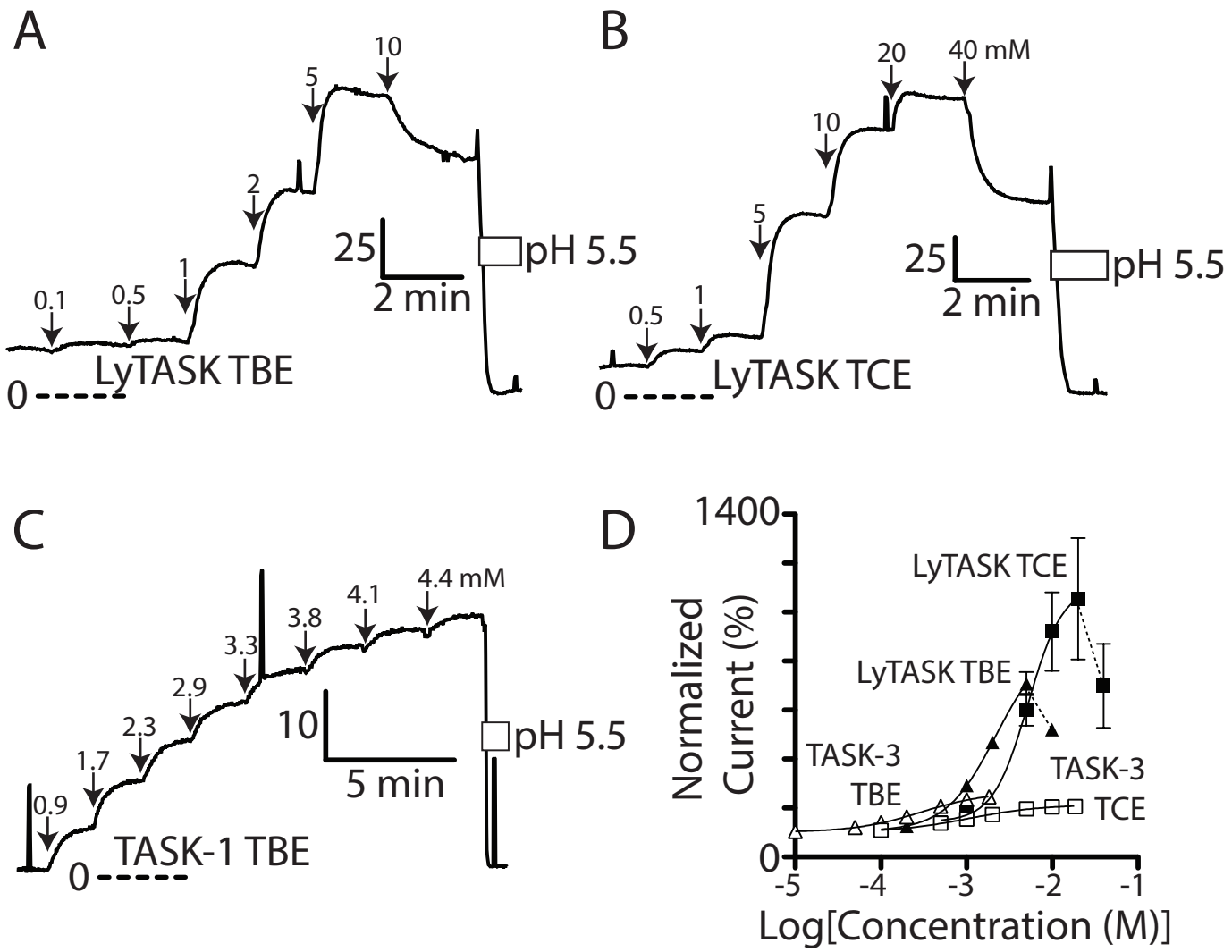

Figure S2. LyTASK and TASK-1 channel function is activated by halogenated ethanols. A, Ussing chamber potassium current records using Fischer rat thyroid cell monolayers transiently expressing LyTASK (A and B) and TASK-1 (C). Data were collected as described in Fig. 3 with n = 3 to 8 ± SD for each. D, LyTASK and TASK-3 concentration-response for TCE and TBE. Data points for LyTASK were fitted as in Fig. 3, but with the highest concentration point excluded. LyTASK TCE and TBE data, respectively, for Imax (in %; 95% confidence): 1179(849 to 1509) and 912(754 to 1070). EC50 (in mM): 5.2(3.1 to 8.9) and 2.3(1.6 to 3.2). HillSlope: 1.6(0.5 to 2.7), 1.4(1.1 to 1.7).

Supplementary Figure S2

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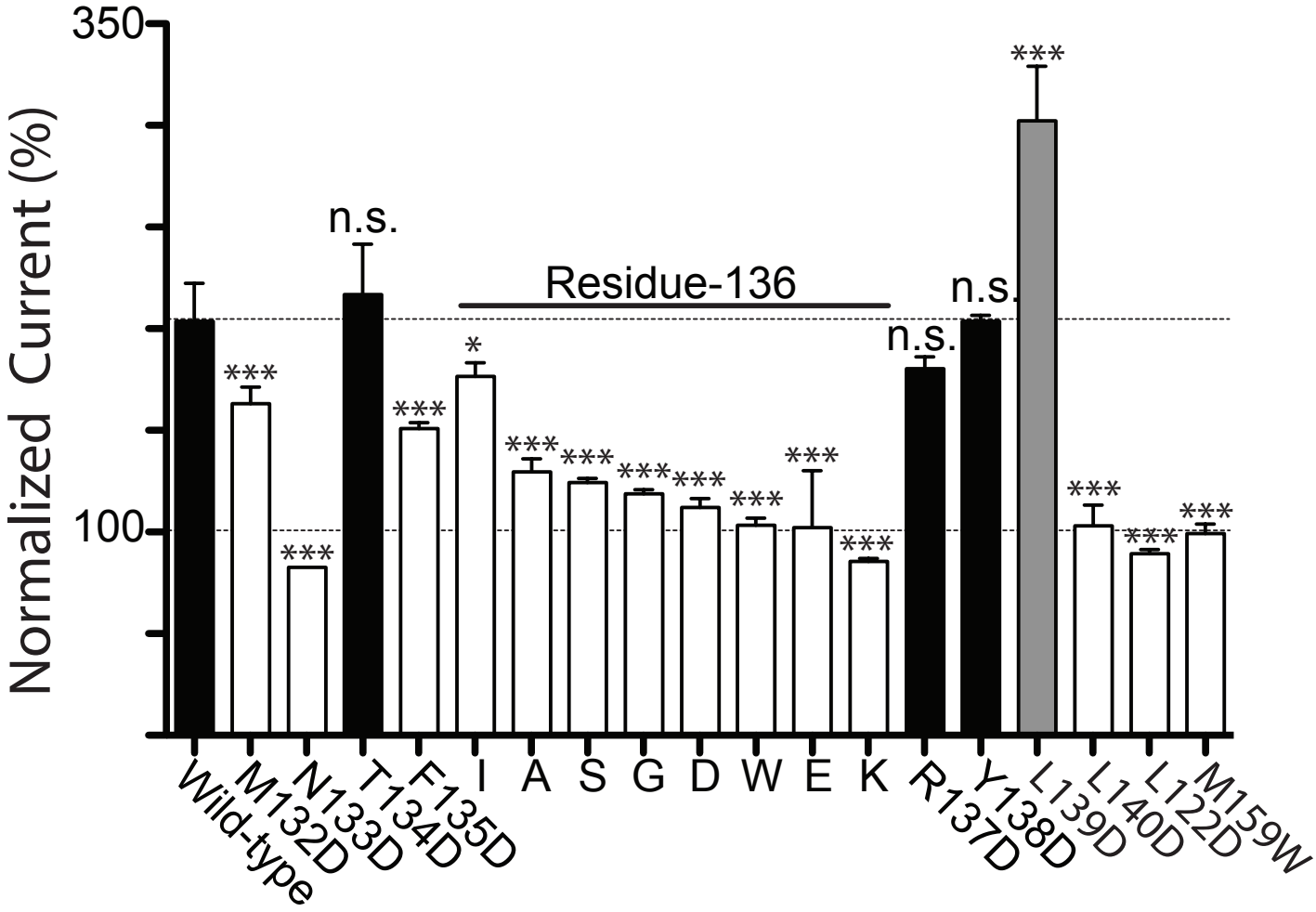

Figure S3. Comparison of 10 mM 2,2,2-trichloroethanol activation of TASK-3 at residues-132 to -140. Data were derived from that presented in Fig. 5. n = 3 to 8 ± SD for each. Asterisks (***, **, and *) indicate significance ($P < 0.001$, 0.01, and 0.05) relative to wild-type TASK-3 as determined by a one-way ANOVA and a post-hoc Bonferroni multiple comparison test.

Supplementary Figure S3

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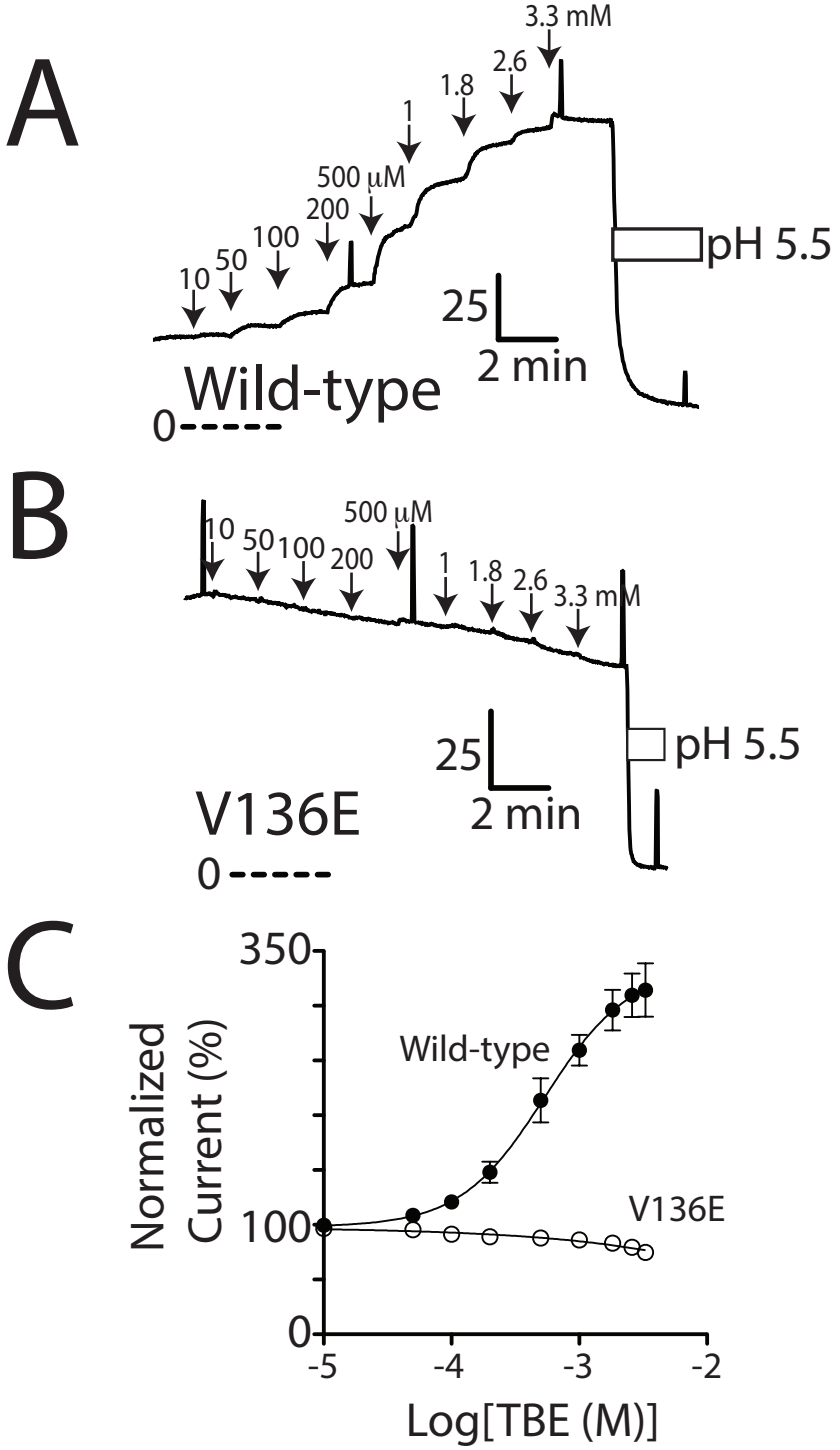

Figure S4. 2,2,2-tribromoethanol (TBE) activates "yeast optimized" wild-type TASK-3, but not "yeast optimized" V136E rTASK-3. TASK3 was optimized by random mutagenesis for yeast expression and contains the following missense mutations: T17I, D27E, D32G, K79E, F202L, and F246Y. Data were collected as described in Fig. 3. Ussing chamber potassium current records using Fischer rat thyroid cell monolayers expressing "yeast optimized" wild-type TASK-3 (A) and "yeast optimized" V136E TASK-3 (B) exposed to increasing concentrations of TBE. C, compiled TBE concentration-response data (n = 3 ± SD for each). Data points for "yeast optimized" wild-type rTASK-3 were fitted as in Fig. 3. Imax (in %; 95% confidence) = 333(308 to 359). EC50 (in µM): 539(414 to 712). HillSlope: 1.4(1.0 to 1.7).

Supplementary Figure S4

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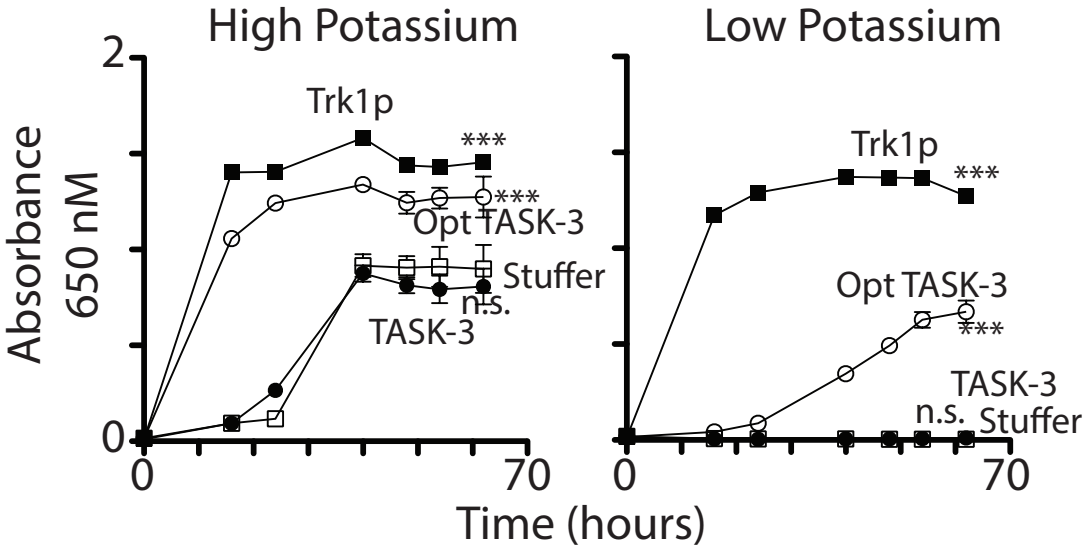

Figure S5. Trk1p and "yeast optimized" TASK-3 promote trk1Δtrk2Δ *Saccharomyces cerevisiae* **growth in low potassium media.** Growth measurements of the potassium-sensitive strain trk1Δtrk2Δ *Saccharomyces cerevisiae* after transformation with cDNA encoding Trk1p, "yeast optimized" TASK-3 (Opt TASK-3), wild-type TASK-3, or non-functional Kir 2.1 "stuffer". Yeast were cultured in 96 well format at 30°C in HIGH potassium (100 mM KCl; left side of figure) or LOW potassium (no added KCl; right side). Growth was quantified by repeated A650 nM measurements. "Yeast optimized" TASK-3 contains the following missense mutations: T17I, D27E, D32G, K79E, F202L, and F246Y. Statistical significance at the 62 hour data point was determined using a one-way ANOVA and a post-hoc Bonferroni multiple comparison test. n =4 to 6 ± SD for each. n.s., indicates no significant difference; and ***, indicates $P < 0.0001$ relative to yeast transformed with the non-functional Kir 2.1 "stuffer" channel.

Supplementary Figure S5

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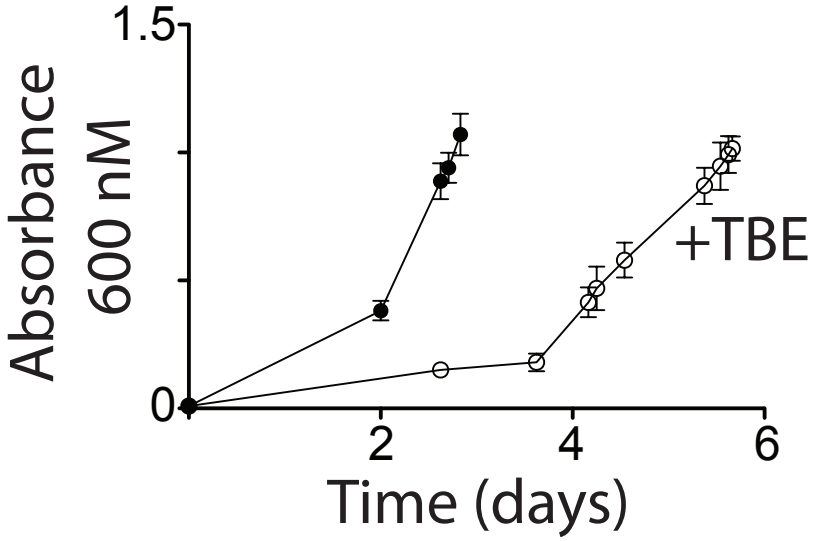

Figure S6. Rate of yeast growth in the presence of 2,2,2-tribromoethanol (TBE). Potassium-sensitive trk1Δtrk2Δ *Saccharomyces cerevisiae* yeast transformed with "yeast optimized" wild-type TASK-3 or "yeast optimized" V136E TASK-3 were mixed at an initial ratio of 1:1 and cultured in low potassium media in the presence (open circles) or absence (black circles) of 1 mM TBE. Growth was assessed by repeated A600 measurements. n = 4±SD for each.

Supplementary Figure S6

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Ethanol	190
2,2,2-Trifluoroethanol	23.8
2,2,2-Trichloroethanol	0.74
2,2,2-Tribromoethanol	0.30
Chloral Hydrate	1.6
α-Chloralose	0.53
Halothane	0.23
Isoflurane	0.29

Table S1. Anesthetic concentration (in mM) required for loss of righting reflex in Xenopus laevis tadpoles. Values were obtained from Krasowski and Harrison, 2000 and Firestone, 1986.