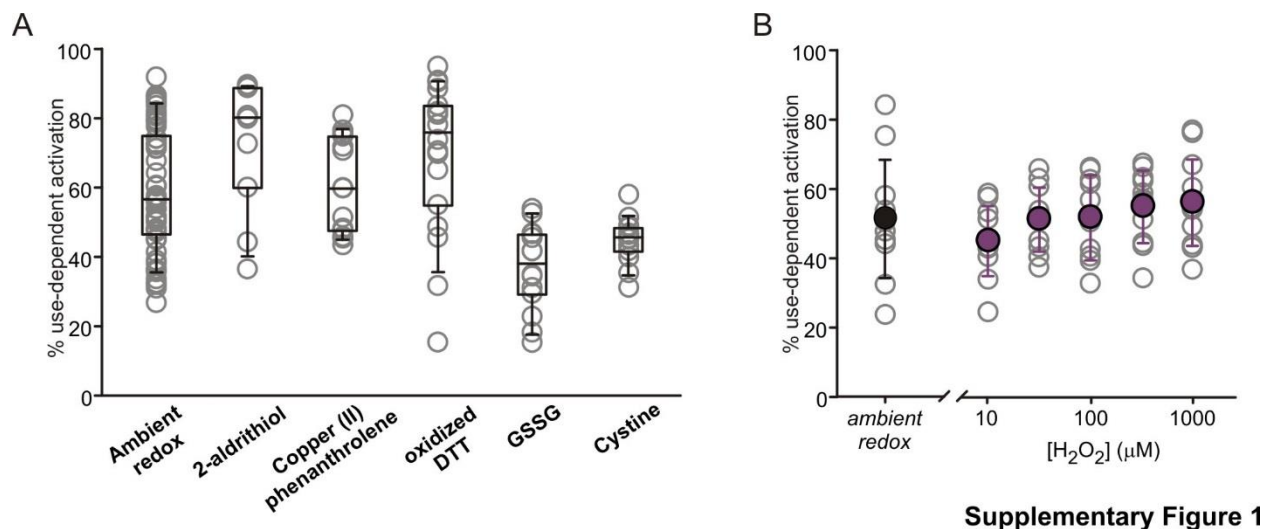


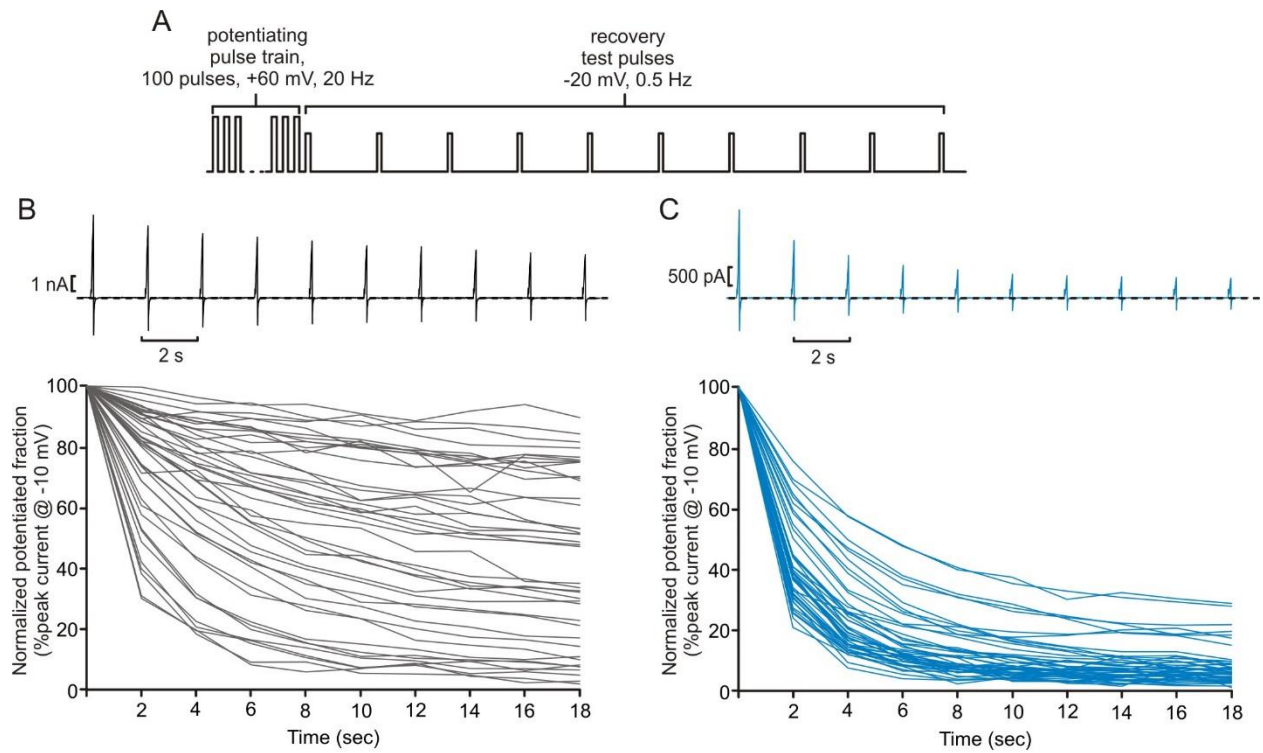
Extracellular redox sensitivity of Kv1.2 potassium channels

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SUPPLEMENTAL INFORMATION

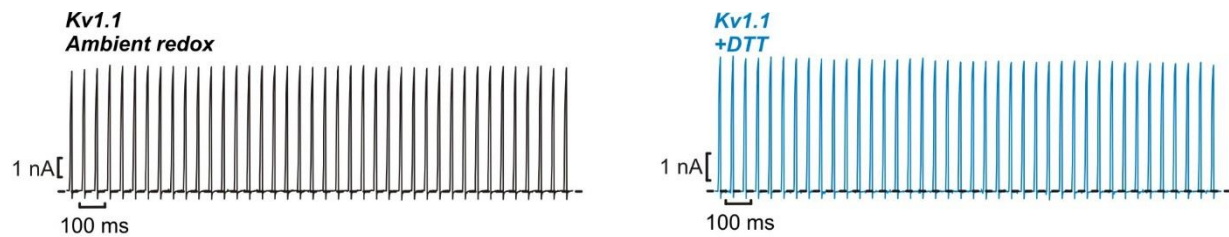


Supplemental Figure 1. Oxidizing agents do not affect use-dependent activation. (A) Cells expressing Kv1.2 were incubated with various oxidizing agents, 2-aldrithiol (100 or 500 μM, N = 10), copper(II) phenanthroline (2 μM/100 μM or 150 μM/500 μM, N = 14), oxidized DTT (0.5 or 1 mM, N = 18), oxidized glutathione (GSSG) (1 mM, N = 14) and cystine (500 μM, N = 14), or in ambient redox (N = 57) and % use-dependence was calculated as described in Figure 1. (B) Use-dependent activation was also quantified in cells over a range of H₂O₂ concentrations (10 μM – 1 mM) with no effect observed.



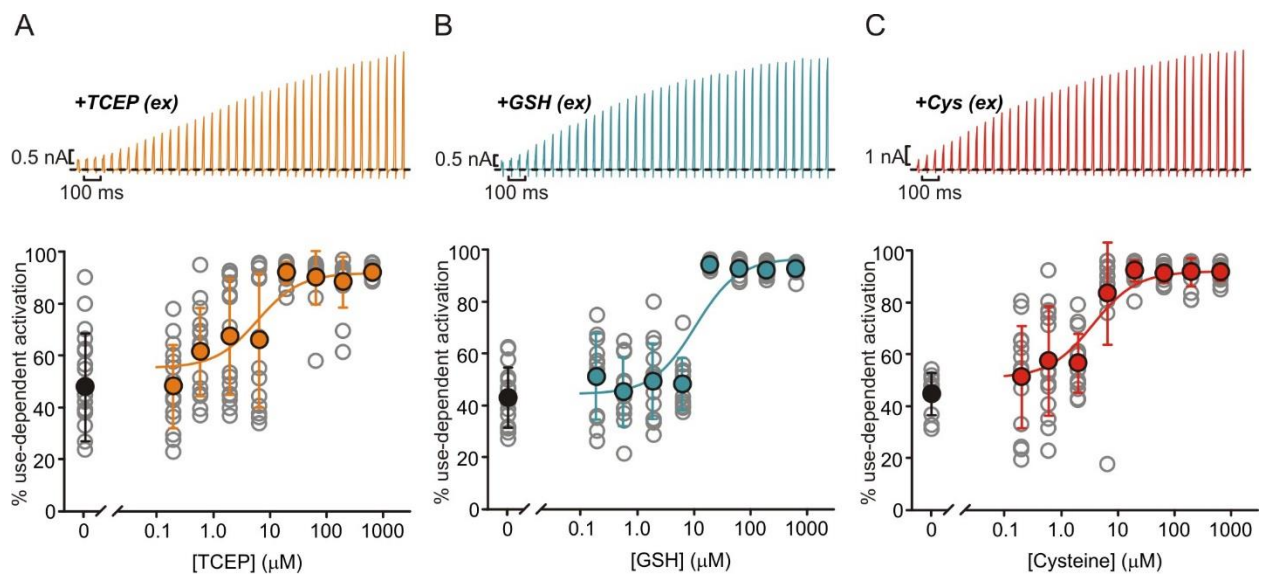
Supplementary Figure 2

Supplemental Figure 2. Quantification of recovery of inhibited gating mode in ambient redox and reducing conditions. (A) Detailed protocol for assessing recovery of inhibition. Cells were pulsed repetitively to +60 mV to populate the potentiated gating mode, then pulsed every 2 s to -10 mV to assess recovery to the inhibited gating mode. (B,C) Exemplar traces illustrate a sequence of recovery pulses in ambient redox (B) or 666 μ M DTT (C). The time course of recovery is illustrated for all individual cells collected to illustrate the variability in ambient redox vs. DTT conditions.



Supplementary Figure 3

Supplemental Figure 3. Redox insensitive currents of Kv1.1 channels. Exemplar currents recorded from Itk- mouse fibroblasts expressing Kv1.1 are presented in ambient redox conditions (black) or DTT (blue).



Supplementary Figure 4

Supplemental Figure 4. Dose-dependent shift in use-dependence with membrane impermeant reducing agents. (A-C) Concentration-response of use-dependent activation was measured with increasing concentrations of extracellularly applied (A) TCEP (EC₅₀ of 3.4 μ M and a Hill coefficient of 1.1), (B) reduced glutathione (GSH, EC₅₀ of 2.1 μ M and a Hill coefficient of 1.2), or (C) cysteine (EC₅₀ of 2.8 μ M and a Hill coefficient of 1.1). N = 10-20 for each condition.