Oxidation driven reversal of PIP_2-dependent gating in GIRK2 channels

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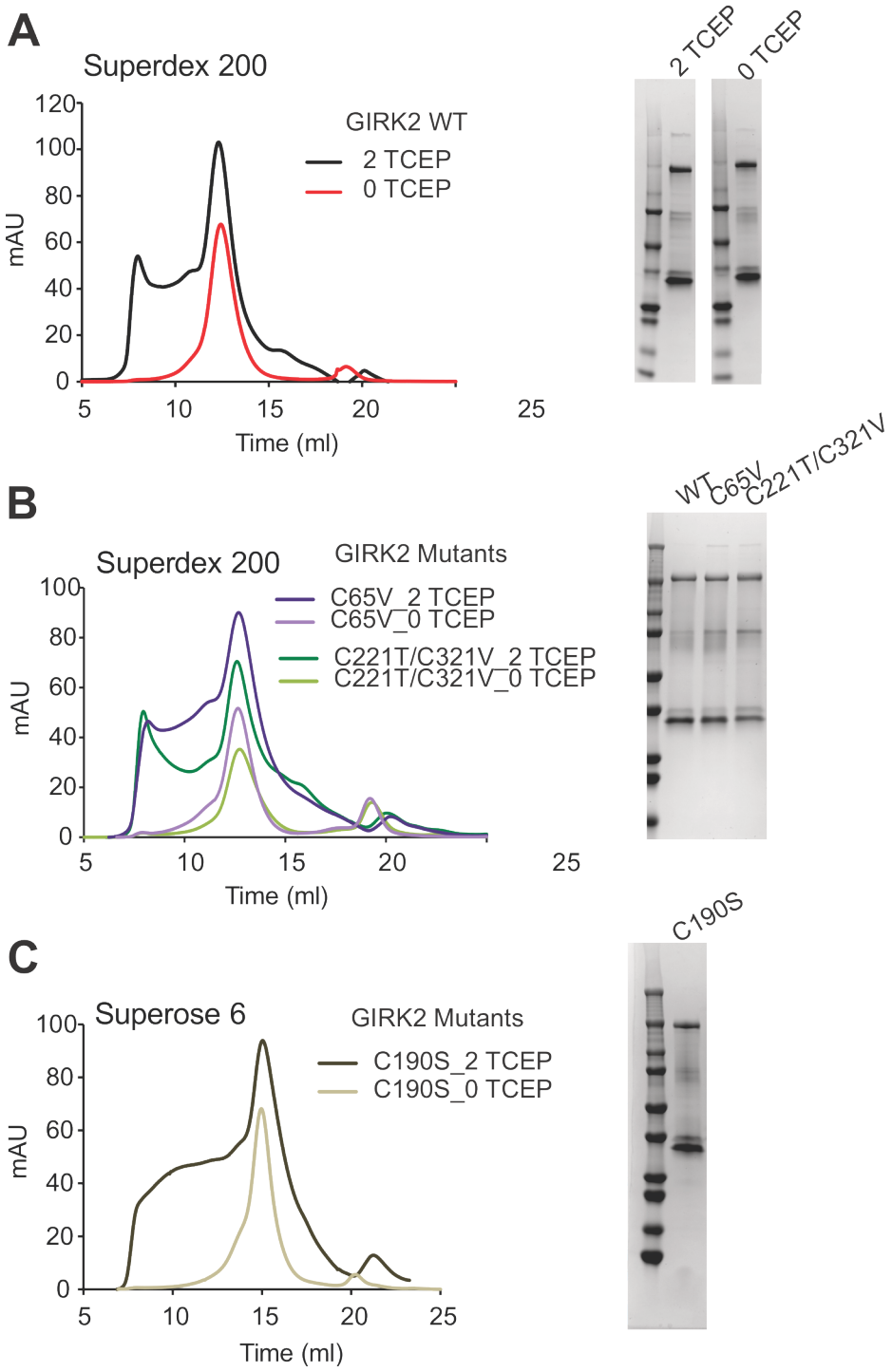
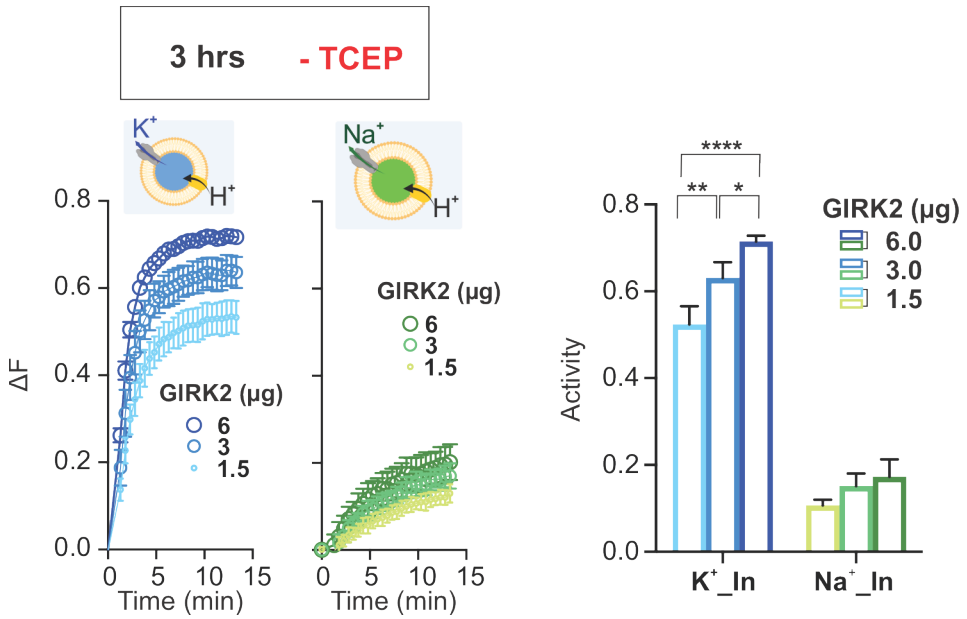
Supplementary information

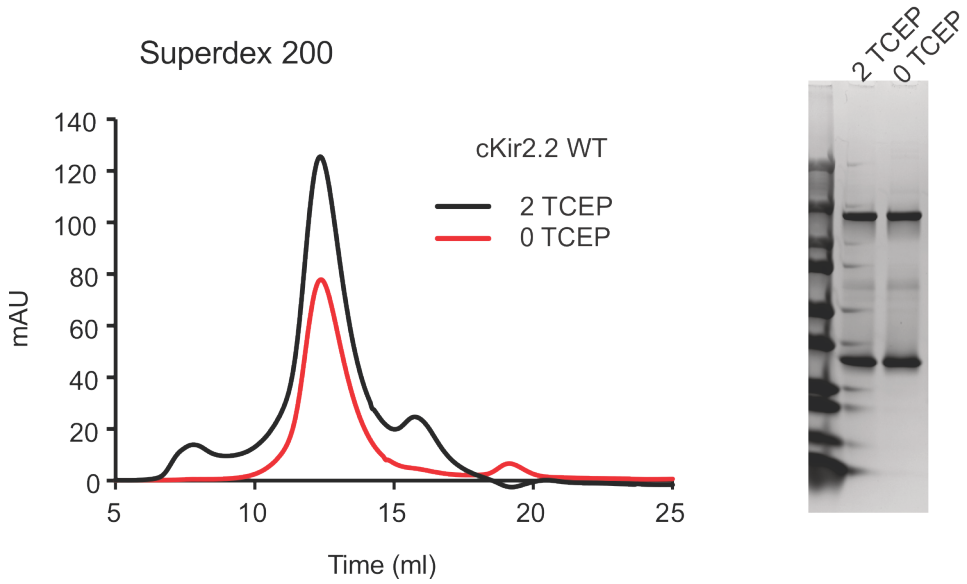


Supple Fig. S1. Purified GIRK2 proteins in reducing and oxidizing condition
The elution profiles of GIRK2 wild type protein (**A**), GIRK2 mutants (C65V and C221T/C321V) (**B**), and C190S mutant (**C**) off the size exclusion columns equilibrated in reducing (with 2 mM TCEP; black) or in oxidizing (without TCEP; red) condition. Coomassie stained 1D SDS-PAGE image of the each sample. The type of the used size exclusion column is noted above the profiles.

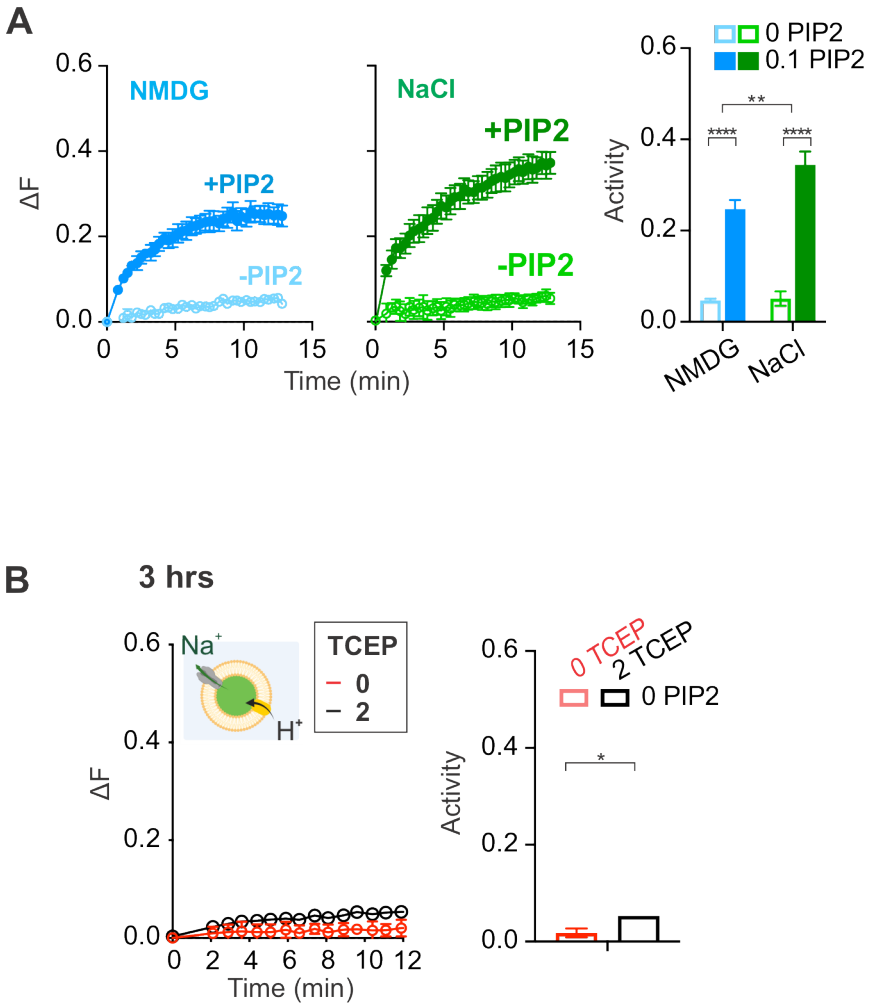


Supple Fig. S2 K^+ specific basal activity elevated in oxidized GIRK2 proteins

Differential ACMA signal decay (ΔF) of GIRK2 proteins in the oxidizing condition, purified and incubated for 3 hours without TCEP to test basal channel activity with increasing amount of proteins and with the internal salt KCl (Left) or NaCl (Middle). Comparison of K^+ vs Na^+ specific basal activities (Right).



Supple Fig. S3. Purified cKir2.2 protein in reducing and oxidizing condition
A. The elution profiles of cKir2.2 off the size exclusion column equilibrated in reducing (+2 mM TCEP; black) or oxidizing (0 mM TCEP; red) condition, and the Coomassie stained 1D SDS-PAGE profiles of the protein samples.



Supple Fig. S4. cKir2.2 channel activities in the reducing and oxidizing condition
A. Differential ACMA signal decay (ΔF) by cKir2.2 purified with 2 mM TCEP in reducing condition, and activities shown under different combinations of agonists with external NMDG (blue) or NaCl (green) and with 0 (open) or 0.1 (filled) % PIP2. **B**. **Left:** Differential ACMA signal decay (ΔF) by cKir2.2 purified without TCEP in oxidizing condition, incubated for 3 hours either with (black) or without (red) TCEP. The buffer composition for the ACMA assay with internal NaCl and external NMDG is shown in the schematic diagram. **Right**: Activity in oxidizing condition is in red and in reducing condition in black. Paired student t-test was applied with n = 3.