**Supplementary Information for**

**Direct single-molecule detection of CoA-SH and ATP by the membrane proteins TMEM120A and TMEM120B**

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Figure 1. Representative chromatogram of gel filtration purification of TMEM120A (A) and TMEM120B (B). The fractions were analyzed by SDS-PAGE (C).
Figure 2. Current trace recording of TMEM120A/TMEM120B embedded into lipid bilayer membrane ((-trans) 100 mM NaCl - (-cis) 500 mM NaCl, 10 mM HEPES, pH 7.5.).
Figure 3. Current trace of TMEM120A and TMEM120B under different electrolytes conditions including 100 mM NaCl - 500 mM NaCl (A), 100 mM KCl - 500 mM KCl (B), 50 mM MgCl$_2$ - 250 mM MgCl$_2$ (C), 50 mM CaCl$_2$ - 250 mM CaCl$_2$ (D) ((-trans) - (-cis), voltage: +20 mV).
Figure 4. Current trace of TMEM120A and TMEM120B under different electrolytes conditions including 500 mM KCl - 500 mM KCl (A), 500 mM NaCl - 500 mM KCl (B), 250 mM MgCl$_2$ - 500 mM KCl (C) and 250 mM CaCl$_2$ - 500 mM KCl (D) ((-trans) - (-cis), voltage: +20 mV).
Figure 5. TMEM120B present higher voltage-gating probability than TMEM120A in the presence of Ca\(^{2+}\) and Mg\(^{2+}\). A. Ion condition: (-trans) 250 mM CaCl\(_2\) – (-cis) 500 mM KCl, 10 mM HEPES, pH 7.5. B. Ion condition: (-trans) 250 mM MgCl\(_2\) – (-cis) 500 mM KCl, 10 mM HEPES, pH 7.5. C. Voltage-gating probability of TMEM120A and TMEM120B under two different electrolyte conditions, with a voltage range from -100 mV to 100 mV.
Figure 6. Represent signal and possible forms of interaction between COA-SH and TMEM120A. A. One-step block signal with relatively low blockage rate. B. Two-step block signal with relatively high blockage rate and two-step of dissociation. C. Two-step block signal with one step dissociation (+20 mV, (-trans) 100 mM NaCl - (-cis) 500 mM NaCl, 10 mM HEPES, pH 7.5.).
Figure 7. Represent signal and possible forms of interaction between COA-SH and TMEM120B. 

A. One-step block signal with relatively low blockage.

B. One-step block signal with relatively high blockage rate.

C. Block signal with two steps during block process (+20 mV, (-trans) 100 mM NaCl - (-cis) 500 mM NaCl, 10 mM HEPES, pH 7.5.).
Figure 8. Power spectrum analysis of TMEM120A/TMEM120B control (black) and TMEM120A/TMEM120B with 1 mM ATP in the -cis side (blue/yellow). The electrolyte buffer is (-trans) 250 mM MgCl₂ - (-cis) 500 mM KCl. The voltage was +20 mV.
Figure 9. Current trace of COA-SH sensing by TMEM120A (blue) and TMEM120B(yellow) under the electrolyte condition of \((-\text{trans}) 250 \text{ mM MgCl}_2\) - \((-\text{cis}) 500 \text{ mM KCl}\). The voltage was +20 mV.