Fig. Supplementary 1 : **Poly-lysine inhibition of TREK-1**

A) Effect of pL (30 μg/ml) and heparin (20 μg/ml) (hpn) on TREK-1 recorded in an inside-out patch (physiological K^+ gradient). The membrane voltage was depolarized from –80 mV to 0 mV (as indicated). B) Effect of pL (30 μg/ml), heparin (20 μg/ml), PIP_2 (5 μM), pH_i 5.5 and following cytosolic acidosis (pH_i 5.5) after PIP_2 (5 μM) washout (open lock indicated by a star) on TREK-1 channel activity (top histogram). Same experiments have been performed on control EYFP-transfected COS cells (bottom histogram). C) Current traces recorded during a depolarization to 140 mV from a holding potential of –80 mV for control EYFP (top) and TREK-1-transfected COS cell (bottom). Control traces are shown in red and currents recorded in the presence of intracellular pL (30 μg/ml) are shown in black. In these experiments, currents were recorded in the inside-out patch configuration in a symmetrical K^+ gradient lacking divalent cations. The basal TREK-1 current, in the absence of stretch, pH_i or lipid stimulation, is composed of two separate components : i) a time-dependent component that slowly activates for a depolarization higher than 40 mV and that slowly deactivates upon repolarization to –80 mV (I_{st}); 2) a K^+-selective leak component recorded at all voltages that shows a very mild outward rectification and lacks current kinetics (I_{to}). D) Normalized current traces of TREK-1 in control and after pL (30 μg/ml) treatment (same experiment as C). E) The time-dependent component I_{st} was subtracted from the leak component measured at the beginning of the pulse (I_{to}). In the presence of intracellular pL, the time-dependent component (I_{st}-I_{to}) is completely inhibited (filled circles). In EYFP-transfected COS cells, the time-and voltage-dependent current component is absent (gray circles). F) I-V curve of the leak component (I_{to}) in control and in the presence of pL (30

μg/ml)(filled squares). In EYFP-transfected COS cells, the leak current component is absent (gray squares).

Fig. Supplementary 2 : **EYFP-ΔC119TREK-1 is not expressed at the plasma membrane**

A) Expression of the fusion protein EYFP-ΔC119 TREK-1 in an ethanol permeabilized and WGA/DAPI stained COS cell. The plasma membrane is shown in red (WGA), EYFP-ΔC119 TREK-1 in green (EYFP) and the nucleus in blue (DAPI). Images were collected with a 63x oil immersion lens. High magnification of the boxed region is illustrated in the lower panels. B) Confocal image of an EYFP-ΔC119 TREK-1 transfected ethanol permeabilized COS cell. The image was acquired with a 100x oil immersion objective and laser excitation for FITC. The image corresponds to a section of 0.1 μm.