Supplemental materials for *Biophysical Journal*-Biophysical Letters

Prestin modulates mechanics and electromechanical force of the plasma membrane

Cell Preparations and transfection

Human embryonic kidney (HEK) (also called HEK-293) cells (ATCC, CRL-1573) were seeded in Dulbecco's Modified Eagle Medium (DMEM, Mediatech, Herndon, VA) with 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA) at a density of ~100,000 cells/well on a 60 mm plate and incubated in a water-jacketed CO2 incubator (Nuaire, Plymouth, MN) at 37oC in 5% CO2 overnight. Isolated cells were selected for experiment if they displayed a uniform spindle-like shape.

For transfection, one dish of 20% confluent HEK cells were incubated overnight to grow to approximately 50% confluence. The medium in the wells was replaced with fresh DMEM+10% FBS. Then 94 μL of serum-free DMEM was mixed with 6 μL of FuGene 6 (Roche, Indianapolis, IN), incubated for 5 minutes at 37°C, followed by addition of plasmid DNA (2 μg) and incubated for an additional 30 minutes. The mixture was then added to the 60mm plate containing HEK cells. This plate was maintained in incubator overnight. Cells were seeded onto poly-d-lysine coated glass-bottom culture dishes (MatTek Corp., Ashland, MA.) containing 3 mL DMEM + 10% FBS 24 hours post-transfection, and maintained in incubator overnight for next day use.

To measure membrane current, non-linear capacitance (NLC) and electromechanical force (EMF), we changed the extracellular solution in the petri dish from the DMEM/FBS solution to a block solution consisting of 20mM TEA, 20mM CsCl, 2mM CoCl2, 1.47mM MgCl2, 100mM NaCl, 2mM CaCl2, and 10mM HEPES. For measurements of mechanical parameters, we used normal extracellular solution (NES) containing 142 mM NaCl, 5.37 mM KCl, 1.47 mM MgCl2, 2 mM CaCl2, and 10 mM HEPES. The pH of both block and NES solutions were adjusted to 7.2 and osmolarity of both solutions were adjusted to 290~300 mOsm.

Cloning of wild type (WT) prestin and mutagenesis to prestin A100W have been previously described (1). A100W prestin contains a residue substitution in the region of the sulfate transporter domain of prestin. Specifically, alanine at 100th position in the amino acid sequence was replaced by tryptophan.

Electrophysiology

For electrophysiological experiments, patch pipettes with resistances of 2~3 MΩ were pulled from borosilicate glass capillary tubing (World Precision Instruments, TW100-4, Sarasota, FL) using a CO_2 laser pipette puller (Sutter Instrument, P2000, Novato, CA). The patch pipette solution contained 140mM CsCl, 1mM EGTA, 2mM MgCl2, and 10mM HEPES, adjusted to a pH of 7.2 and an osmolarity of 290-300 mOsm/kg. Cells were visualized under 100X magnification oil immersion objectives (Carl Zeiss, Gottingen, Germany) with a halogen fluorescence light source. Single isolated cells displaying robust GFP fluorescence (488 nm) were selected for experiments. Whole-cell capacitive currents were recorded by pCLAMP 9.0 software (Axon Instruments, Axopatch 200 B, Sunnyvale, CA).

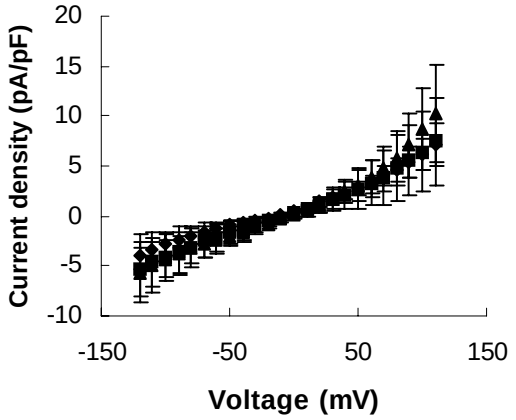

Figure 1 I-V curves of wild type prestin transfected (◆), A100W transfected (■) and normal HEK cells (▲) in block solution.

NLC and current-voltage (I-V) relationships were obtained by applying a sequence of 10 ms-duration voltage steps, in 10 mV increments, from –130 mV to 120 mV. The holding potential was set to –70 mV. The nonlinear capacitance can be described as the first derivative of a two state Boltzmann function (2):

$$C_m = \frac{Q_{\max}\alpha}{\exp[\alpha(V_m - V_{1/2})](1+\exp[-\alpha(V_m - V_{1/2})])^2} + C_{lin}$$

where C_{lin} is linear membrane capacitance, V_m is membrane potential, Q_{max} is maximum nonlinear charge moved through the membrane electrical field, $V_{1/2}$ is the voltage at half-maximum nonlinear charge transfer, α is the slope factor of the voltage dependence. Processing and fitting of data were performed with Matlab (MathWorks, Natick, MA) programs. I-V curves for WT prestin-transfected, A100W transfected and untransfected HEK cells are shown in Fig. 1. There was no significant difference between the different test groups indicating that prestin transfection did not affect cell conductance, nor cause cell membrane leakage.

Mechanical measurements

Once a tether was formed, it was pulled at a rate of 1μm /s for 30s until reaching a peak force F_{pk}. When the tether length reached 30μm, movement was halted and tether was maintained at this length for several minutes, allowing the tether force to relax to a non-zero equilibrium force F_{eq} (Fig. 2).

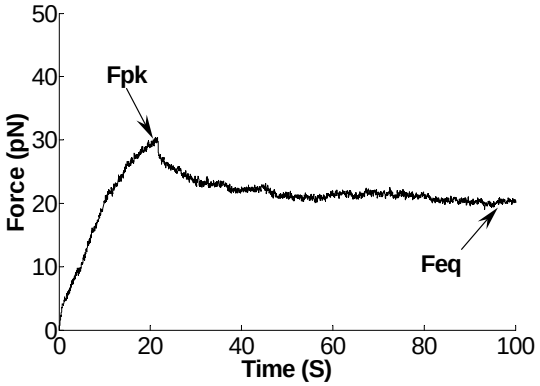

Figure 2 Typical tether force relaxation profile (from untrasfected HEK cell) when the tether length reached 30 μm.

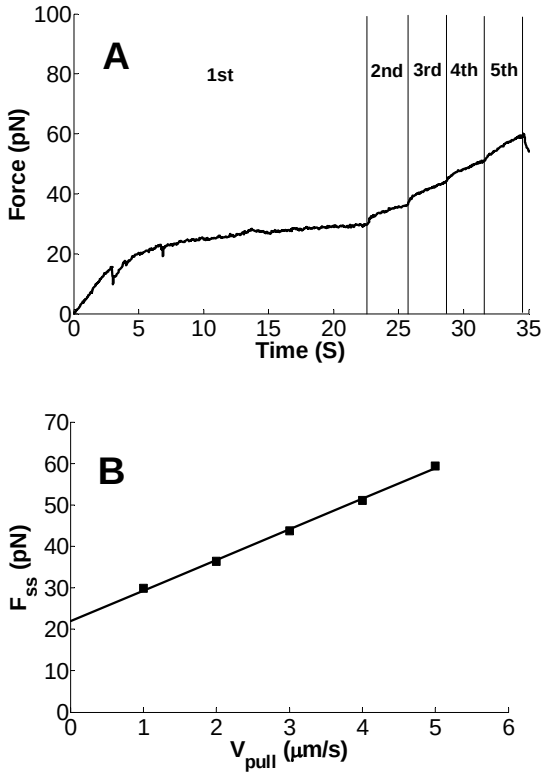

Figure 3 (A) Typical temporal force profile (from untransfected HEK cell) measured at different pulling rates. (B) Plot of steady-state forces vs. tether pulling rates. Eq.2 was used to fit the data: $F_{ss(0)}$ = 21.98 pN, η_{eff} =1.17 *pN/(μm/s).*

To measure the membrane effective viscosity (η_{eff}), the tether was pulled at subsequently increasing rates. Specifically, the cell was moved away from the trapped bead at 1μm /s for 30s, then the speed of the piezoelectric stage was increased every 3s by 1μm /s until it reached 5μm /s. Fig. 3 (A) shows that the tether force increased when pulling rate was increased. We fit data for different pulling rates to exponential function and obtained asymptotic value of the steady state force for the different pulling segments. A linear function (3) was used to fit the steady state force at each tether pulling rate to get $F_{ss(0)}$ and η_{eff} (Fig. 3 (B)):

$$F_{ss}(V_{pull}) = F_{ss(0)} + 2\pi\eta_{eff} V_{pull}$$

Measurements of electromechanical force (EMF)

For EMF measurements, the cell was first voltage clamped by the patch clamp system. Then the protocol described in tether relaxation section was used pull a membrane tether to 30 μm. The length constant for the HEK tether is greater than 30 μm as long as the frequency is <1 kHz (5), so we can assume that voltage

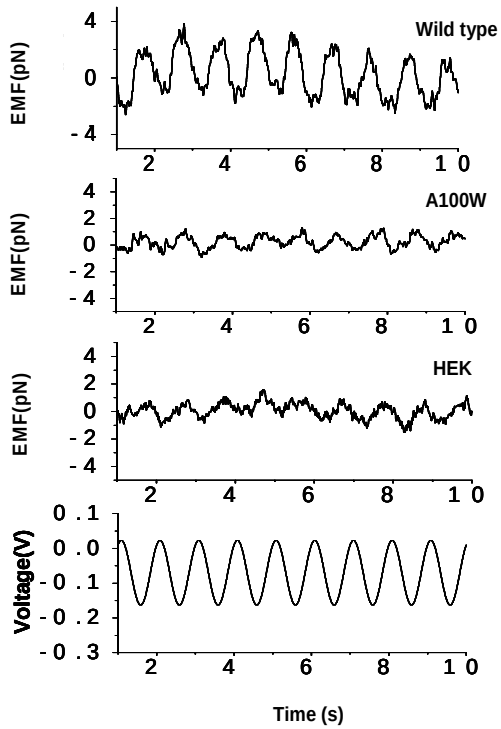

Figure 4 Typical plots of EMF in response to 1Hz sinusoid voltage stimulus for three cell types. (The zero value of EMF represents the tether equilibrium force.)

stimulus is the same across the plasma membrane of the cell body and the tether. Once the tether force reached F_{eq}, the patch clamp was used to change the

transmembrane potential through a 1Hz sinusoid voltage wave protocol (Sinusoidal waves with peak to peak voltage of 200mV, holding potential –70mV). The resulting EMF was measured by recording the displacement of the trapped bead and calibrating those measurements for force (4).

Fig. 4 shows typical plots of EMF for different test cells when a sinusoid voltage protocol at 1Hz was applied. The tether force increases with hyperpolarization, and decreases with depolarization.

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