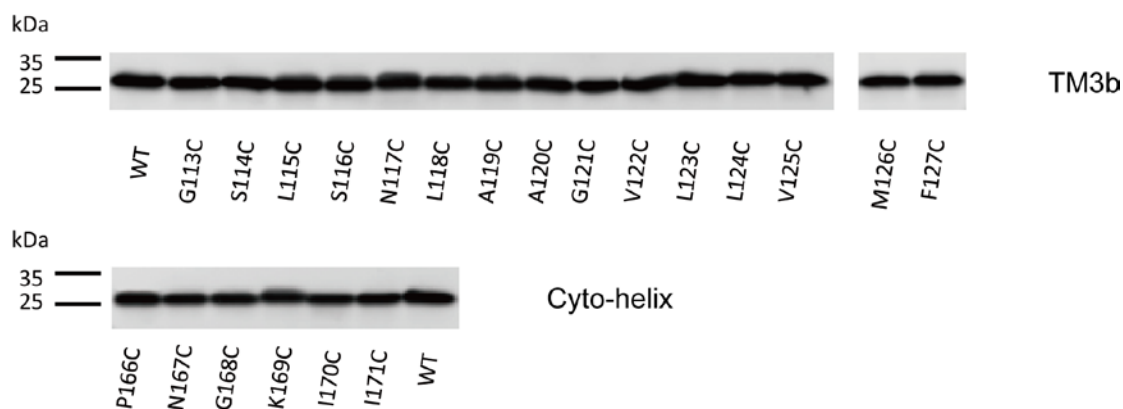


## Supplemental Figures



**Figure S1 Western blot of *E.coli* MscS channels with cysteine mutation in TM3b and Cyto-helix.** Cultures grown in the LB medium were induced with 1mM IPTG for 1 hour. Samples were immediately spun down at 5000rpm for 10min. The pellet was resuspended with non-reducing SDS-PAGE loading buffer and boiled at 85°C for 10min. Proteins were separated in 12% SDS-PAGE gels and electrotransferred to a PVDF membrane at 300 pA for 45min. The membrane was then blocked, by incubation in 2% non-fat powdered milk in TBS buffer, for 1 hour. After blocking, MscS-His6 was binding by incubation with monoclonal anti-His antibody overnight at 4°C reducing. After washing, the secondary antibody, Donkey anti-Rabbit, was added and incubated at room temperature for 2 hours.