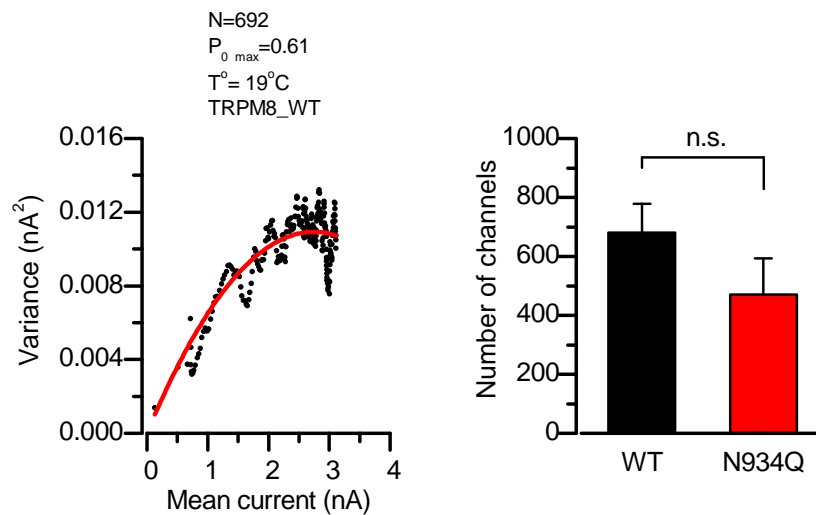## SUPPLEMENTARY FIGURE AND METHODS

Variance Analysis
To estimate the number of TRPM8-wt and TRPM8-N934Q channels in the plasma membrane, we used non-stationary noise analysis(1;2). HEK293 cells were transfected with the constructs and recorded 48 h later. About 100 current records in whole-cell configuration were collected in each cell, during activation of the channels by 150 ms depolarizing voltage steps from 0 to +180 mV, at 19°C. Ensemble averaged current (<I>) and its variance (σ^2) on each isochrone were calculated. The variance as a function of <I> was fitted using the equation:

$$\sigma^2 = i*<I> - (<I>^2/N) \text{ (ii)}$$

, where i is the single channel unitary current and N is the number of channels in the plasma membrane. The maximum open probability ($P_{o\ max}$) was estimated using the relation $P_{o\ max} = I_{max} / i*N$, where I_{max} is the mean maximal current in each experiment. Data for variance analysis where acquired at 20 KHz and filtered at 5 KHz.

Supplementary Figure 1. Determination of the number of channels in the plasma membrane using variance analysis. A. Dot plot of variance versus mean current obtained from whole-cell current of a HEK293 cell expressing wilt type TRPM8 channels. Solid line corresponds to the fit of the data to a parabola (equation ii), were N (the number of active channels in the plasma membrane) and i (the unitary single channel current) were free parameters. **B.** Bar plot of the mean number of active channels for both TRPM8WT and TRPM8N934Q estimated by variance analysis. These values were obtained by fitting experimental data from seven (WT) and nine (N934Q) independent experiments.

REFERENCES

1. Alvarez, O., Gonzalez, C., and Latorre, R. (2002) *Adv. Physiol Educ.* **26,** 327-341

2. Brauchi, S., Orio, P., and Latorre, R. (2004) *Proc. Natl. Acad. Sci. U. S. A* **101,** 15494-15499