

## Online Supplemental Data

for

### IDENTIFICATION OF DIRECT AND INDIRECT EFFECTORS OF THE TRANSIENT RECEPTOR POTENTIAL MELASTATIN 2 (TRPM2) CATION CHANNEL\*

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Running head: Direct effectors of TRPM2 channels

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**Figure S1. Correction for channel rundown.** Inward current at -20 mV from an inside-out patch excised from an oocyte injected with TRPM2 cRNA, elicited by repeated exposures to various concentrations of ADPR (*bars*), in the presence of 125  $\mu\text{M}$   $[\text{Ca}^{2+}]_i$  (*bars*). Mean currents (*horizontal blue lines*) were calculated for quasi-steady segments of record (highlighted by *light blue squares*) in the presence of 1, 3.2, and 32  $\mu\text{M}$  ADPR. To estimate fractional activation by various  $[\text{ADPR}]$ , mean currents in 1 or 3.2  $\mu\text{M}$  ADPR (*red and green bars*, respectively) were normalized to the maximal currents (adjacent *gray bars*) estimated for the respective segments. Because in inside-out patches TRPM2 currents continuously decline, these maximal currents were obtained by linear interpolation (*blue lines*) of the mean currents in the presence of saturating (32  $\mu\text{M}$ ) ADPR, obtained in bracketing segments of record in the same patch. For test applications which preceded exposure to 32  $\mu\text{M}$  ADPR (cf., first exposure to 1  $\mu\text{M}$  ADPR), maximal current for the test segment was estimated by linear extrapolation of the mean currents obtained subsequently, in the same patch, upon repeated exposures to 32  $\mu\text{M}$  ADPR.

**Figure S2. Replacement of intracellular  $\text{Na}^+$  with  $\text{K}^+$  does not affect activation by ADPR or NAADP alone, or co-activation by ADPR+NAADP, in sub-saturating  $\text{Ca}^{2+}$ .** A, Dose response curve for fractional activation by ADPR in the presence of 15  $\mu\text{M}$   $[\text{Ca}^{2+}]_i$ , using a  $\text{Na}^+$ -gluconate based pipette solution and a  $\text{K}^+$ -gluconate based bath solution. Currents were normalized to the maximal current obtained in the same patch in the presence of 32  $\mu\text{M}$  ADPR and 15  $\mu\text{M}$   $[\text{Ca}^{2+}]_i$ . Solid line represents a fit to the Hill equation; predicted midpoint is printed in the panel, Hill coefficient is  $n_H = 1.5 \pm 0.3$ . B, Fractional activation by 50  $\mu\text{M}$  (*left*) and 1 mM (*right*) NAADP, in the absence (*black bars*) and presence (*white bars*) of 0.1  $\mu\text{M}$  ADPR, using a  $\text{Na}^+$ -gluconate based pipette solution and a  $\text{K}^+$ -gluconate based bath solution containing 15  $\mu\text{M}$  free  $[\text{Ca}^{2+}]_i$ ; currents were normalized to that obtained in the same patch in the presence of 15  $\mu\text{M}$   $\text{Ca}^{2+}$  + 32  $\mu\text{M}$  ADPR.

Fig. S1

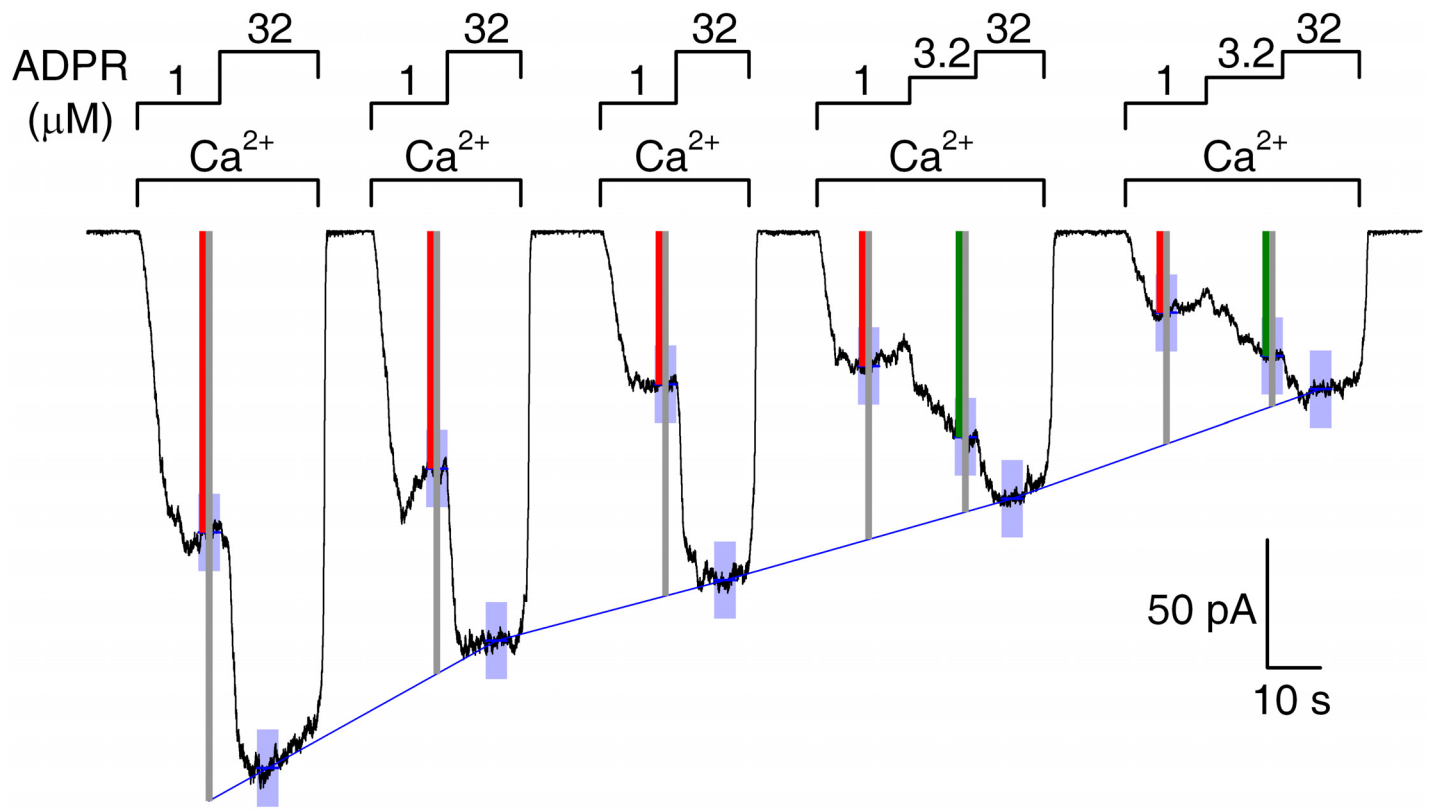


Fig. S2

