SUPPLEMENTAL INFORMATION

A ROLE OF THE TRP DOMAIN OF VANILLOID RECEPTOR I IN CHANNEL GATING

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Methods

Intracellular Ca^{2+} imaging. Transfected cells (10^6 cells/cm^2) were incubated with 5 μM fura-2 AM dissolved in standard extracellular solution and 0.02% pluronic (both from Invitrogen) for 40 min at 37°C in darkness in isotonic 0 Ca^{2+} solution (in mM: 140 NaCl, 4 KCl, 4 $MgCl_2$, 5 glucose, 10 HEPES pH 7.4). For Ca^{2+} imaging, cells were continuously perfused (1 ml/min) with isotonic standard solution at 20-22ºC. Fluorescence measurements were made with a Leica (Nussloch, Germany) DM IRE2 inverted microscope fitted with a 12-bit cooled CCD camera (Imago QE Sensicam; T.I.L.L. Photonics, Graefelfing, Germany). Fura-2 was excited at 340 and 380 nm with a Polychrome IV monochromator (T.I.L.L. Photonics), and the emitted fluorescence was filtered with a 510 nm long-pass filter. Calibrated ratios (0.5 Hz) were displayed on-line with T.I.L.L. Vision software version 4.01 (T.I.L.L. Photonics).

Electrophysiology measurements in *Xenopus* oocytes. Capped cRNA was synthesized using the mMESSAGE mMACHINE™ from AMBION. cRNA (5 ng for each species) was microinjected (V=50 nl) into defolliculated oocytes (Stage V and VI) as described (Ferrer-Montiel and Montal, 1999). Oocytes were functionally assayed 48-72h after cRNA injection. Whole-cell currents from oocytes were recorded with a two-microelectrode voltage-clamp amplifier (Garcia-Martinez et al., 2000;Garcia-Sanz et al., 2004). Oocytes were continuously perfused (2 ml min^{-1}) with Mg^{2+}-Ringer's solution (in mM: 10 HEPES pH 7.4, 115 NaCl, 2.8 KCl, 0.1 $BaCl_2$, 2.0 $MgCl_2$) at 20ºC. TRPV1 currents were activated with acidic solution (Mg^{2+}-Ringer´s solution with 10 mM MES, pH 6.0) or 10 μM capsaicin. The holding potential was kept at -60 mV unless otherwise indicated. Data acquisition and processing was carried out in a NPI TEC10 two microelectrode voltage clamp amplifier (NPI Electronic) with the Pulse/PulseFit 8.5v software package (HEKA Elektronik).

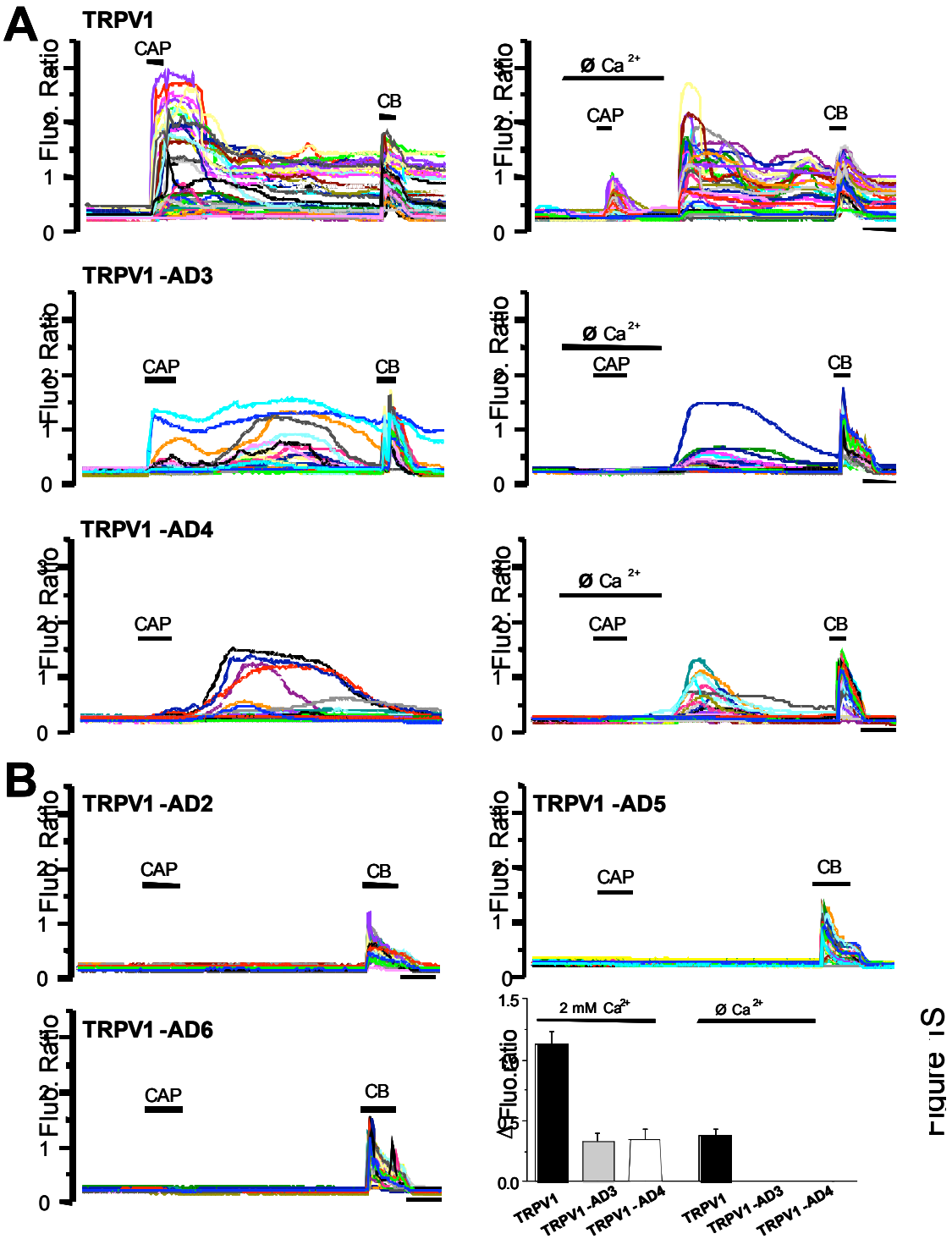
A
TRPV1
CAP
CB
Ø Ca 2+
Fluo. Ratio
TRPV1 -AD3
TRPV1 -AD4
B
TRPV1 -AD2
TRPV1 -AD5
TRPV1 -AD6
2 mM Ca2+
Ø Ca 2+
ΔFluo.Ratio
TRPV1
TRPV1 -AD3
TRPV1 -AD4

Figure S1

Figure S1. Ratiometric Ca^{2+} responses (fura-2) to 100 μM capsaicin (CAP) of the different constructs transfected into HEK293 cells. Following capsaicin application, the field was probed with 100 μM carbachol (CB) to evoke Ca^{2+} release from internal stores. Each graph presents 50 randomly chosen cells. Wild type TRPV1 was the only construct producing a Ca^{2+} elevation with capsaicin in the absence of external calcium (with 1 mM EGTA). The delayed response (i.e. after Ca^{2+} addition) observed in TRPV1-AD3 and TRPV1-AD4 is due to residual and or bound capsaicin remaining in the cells despite extensive wash. The graph at the bottom right summarizes the Ca^{2+} elevation in a representative experiment (n = 25 to 60) for the different conditions. Only cells with a response >0.05 have been included in the average calculation. The calibration bar applies to all records and equals 2 min.

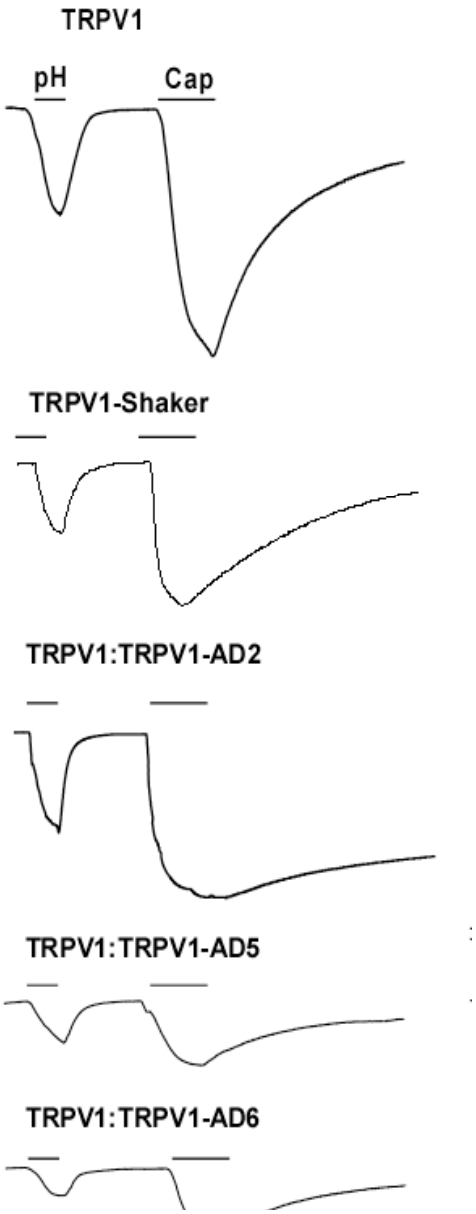

Figure S2. pH and vanilloid responses from oocytes co-injected with TRPV1, TRPV1:Shaker, TRPV1:TRPV1-AD2, TRPV1:TRPV1-AD5 and TRPV1:TRPV1-AD6 at a ratio of 1:1 (w:w). The holding potential was -60 mV. Currents were activated with pH 6.0 or 10 μM Capsaicin. Traces are representative of at least 5 oocytes.

Reference List

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Garcia-Sanz N, Fernandez-Carvajal A, Morenilla-Palao C, Planells-Cases R, Fajardo-Sanchez E, Fernandez-Ballester G, Ferrer-Montiel A (2004) Identification of a tetramerization domain in the C terminus of the vanilloid receptor. J Neurosci 24:5307-5314.