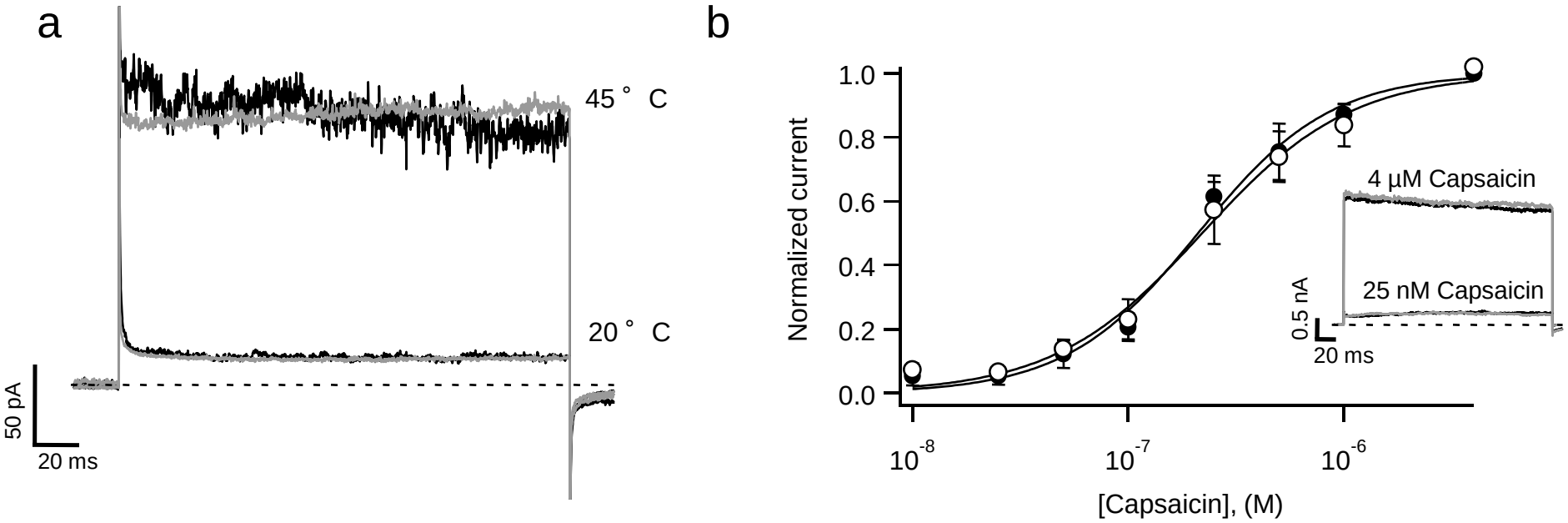
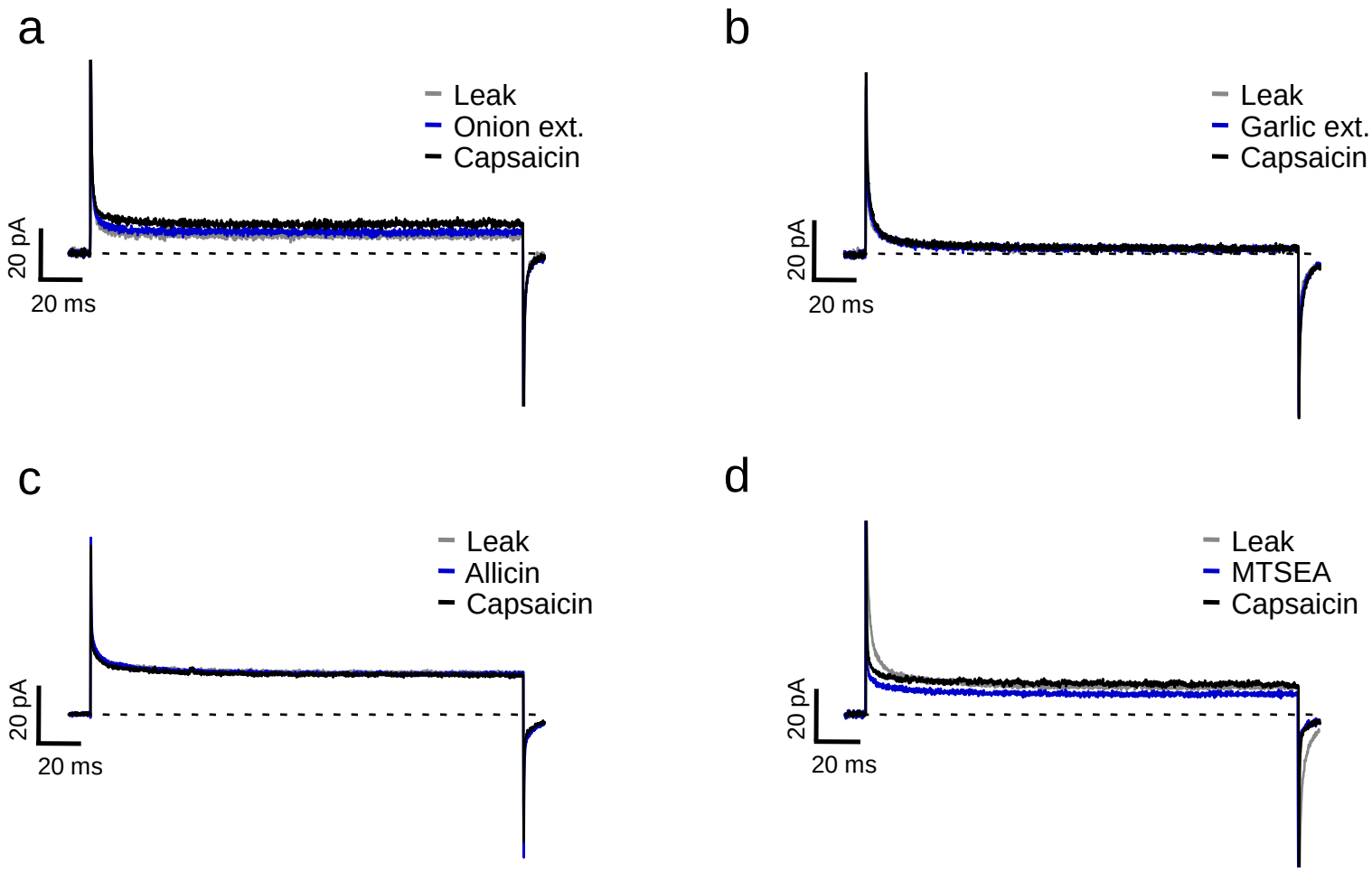
A single N–terminal cysteine in TRPV1 determines activation by pungent compounds from onion and garlic

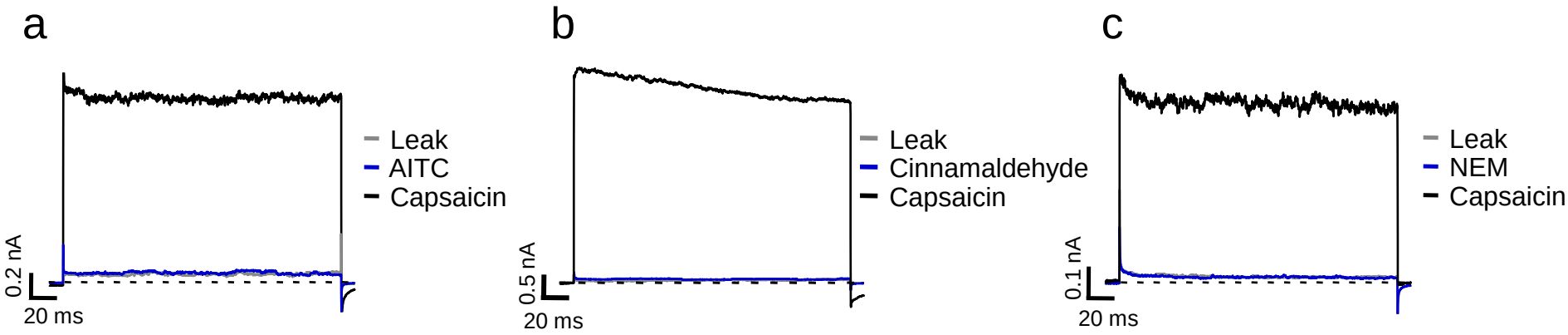
Héctor Salazar, Itzel Llorente, Andrés Jara–Oseguera, León D. Islas, Refugio García–Villegas, Mika Munari, Sharona E. Gordon & Tamara Rosenbaum.



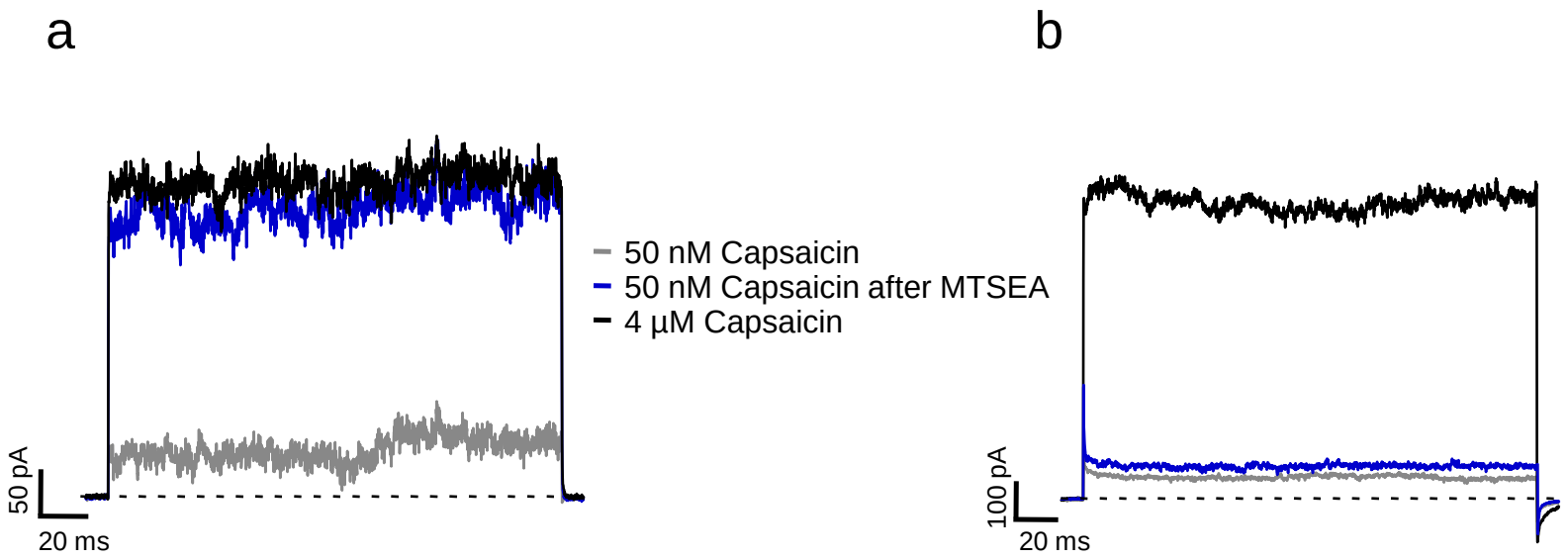
Supplementary Figure 1. DTT does not affect activation of TRPV1 in response to temperature or capsaicin. (a) Representative traces from 5 equal experiments obtained at +100 mV. The black traces depict current activation in TRPV1–expressing HEK293 membrane patches in response to the temperatures indicated. The grey traces represent currents after treatment with 10 mM DTT in the same patch. (b) Dose–response for activation by capsaicin before (filled symbols) and after treatment with 10 mM DTT (empty symbols). Smooth lines are fits to the Hill equation yielding K_D of 178 ± 7.6 nM before and 145 ± 3.1 nM after DTT. The inset depicts representative currents obtained after activation with 25 nM or 4 µM capsaicin before and after exposure to 10 mM DTT. The response of TRPV1–expressing HEK293 membrane patches to temperature was obtained by stepping the voltage from a holding potential of 0 mV to +100 mV for 200 ms. Temperature was varied using a CL–100 bipolar temperature controller (Warner Instruments, Hamden, CT). Membrane patches were exposed to a solution at 20 ˚C and to 45 ˚C for initial current measurements. Subsequently, the patches were treated with 10 mM DTT for 10 min and currents were re–measured at the same temperatures.



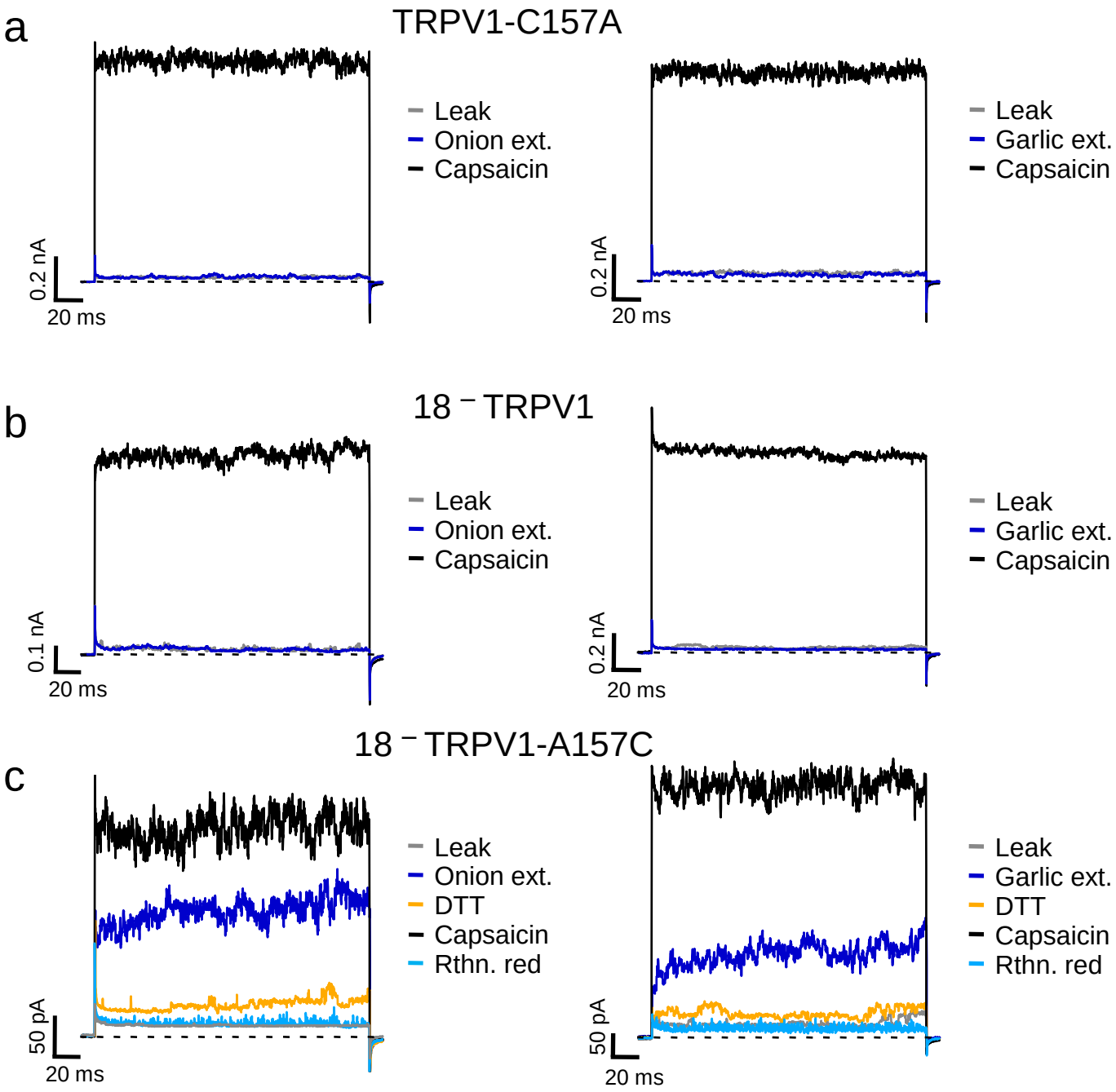
Supplementary Figure 2. HEK 293 cells transfected only with GFP do not respond to cysteine–modifying reagents. Voltage was stepped from a holding potential of 0 mV to 110 mV for 200 ms. After leak currents were measured, the patch was exposed to onion extract (a), to garlic extract (b), 200 µM allicin (c) or 2 mM MTSEA for 5 minutes (d), all shown in blue. Finally, 4 µM capsaicin was applied to all different patches. There was no current–activation under any of the described conditions. Recordings shown here are representative of 4 equal experiments.



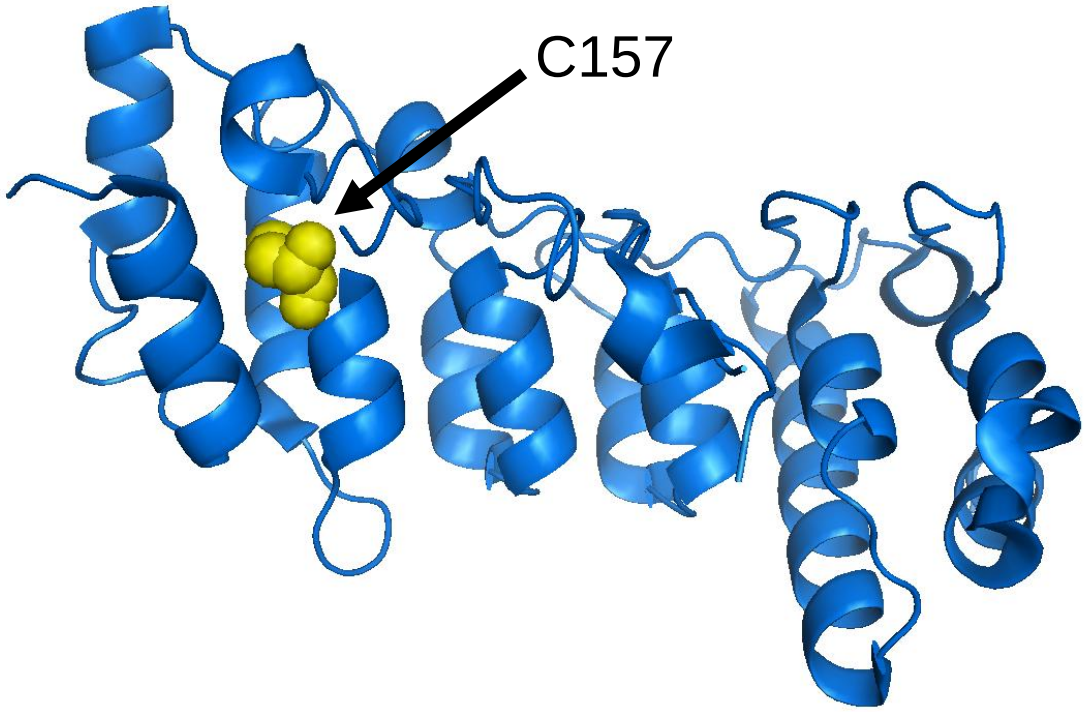
Supplementary Figure 3. Not all cysteine–modifying reagents activate TRPV1 channels. Representative traces shown here were obtained as in Fig. 4 from TRPV1–expressing HEK293–cell membrane patches. Addition of 200 µM AITC (a), 100 µM cinnamaldehyde (b) or 2 mM NEM (c) failed to activate TRPV1–mediated currents while capsaicin always activated considerable currents (n= 5 equal experiments).



Supplementary Figure 4. Activation of TRPV1 currents in HEK 293 cells is due to reaction of cysteine–modifying agents with an intracellular cysteine. Pipettes were filled with recording solution and 20 mM L–cysteine in order to react with any MTSEA permeating the membrane. (a) Application of capsaicin (50 nM) to an inside–out patch activated a small current and application of MTSEA (2 mM) induced current potentiation. Exposure to 4 µM capsaicin induced activation of larger TRPV1 currents. (b) Application of MTSEA to an outside–out patch failed to produce any current increase. Application of 4 µM capsaicin induced current activation. Representative currents of 6 equal experiments.



Supplementary Figure 5. Onion and garlic extracts activate TRPV1 channels through modification of C157. Representative traces shown were obtained as in Fig. 4 from HEK293 membrane patches. The point mutation C157A (a) and the substitution of all cysteines in the channel sequence (b) produce extract–insensitive TRPV1 channels. (c) Reinsertion of C157 into 18⁻TRPV1 regains channel sensitivity to the extracts. Effects of extracts on 18⁻TRPV1–C157 are reversible by application of DTT (10 mM).



Supplementary Figure 6. Localization of C157 in the N–terminus of TRPV1. Ribbon diagram rendering of the published structure of the N–terminus of TRPV1. Based on this structure, C157 (shown in yellow) is found at the inner helix 2 of the ANK–2 repeat of TRPV1.