The vanilloid transient receptor potential channel TRPV1 is a tetrameric six-transmembrane segment (S1-S6) channel that can be synergistically activated by various proalgesic agents such as capsaicin, protons, heat or highly depolarizing voltages. In TRPV1 channel, the charged residues in the S4 region and intracellular S4-S5 linker have been proposed to constitute a part of a voltage sensor that acts in concert with other stimuli to regulate channel activation. Molecular basis of this gating event are poorly understood. We mutated charged residues all along the S4 and the S4-S5 linker of TRPV1 and related vanilloid receptors to identify potential voltage-sensing residues. The functionality of most of the TRPV1 mutants was altered with respect to voltage, capsaicin, heat and/or their interactions. We identified two amino acid residues (R557 and D576) that could potentially constitute part of TRPV1 voltage sensor. The non-functional charge-reversing mutations R557E and R579E were partially rescued by charge-swapping mutations R557E/E570R and D576R/R579E, indicating that electrostatic interactions contribute to allosteric coupling between the voltage-, temperature- and capsaicin-dependent activation mechanisms. The mutant K571E was normal in all aspects of TRPV1 activation except for 2aminoethoxydiphenyl borate (2APB), a common activator of TRPV1, TRPV2 and TRPV3, revealing the specific role of K571 in chemical sensitivity. Surprisingly, substitutions at homologous residues in TRPV2 or TRPV3 were without effect on temperature, voltage and 2APB induced activity. Thus, the charged residues in S4 and the S4-S5 linker, despite their highly conserved nature, regulate differentially the temperature, voltage and chemical gating among the TRPV channels.