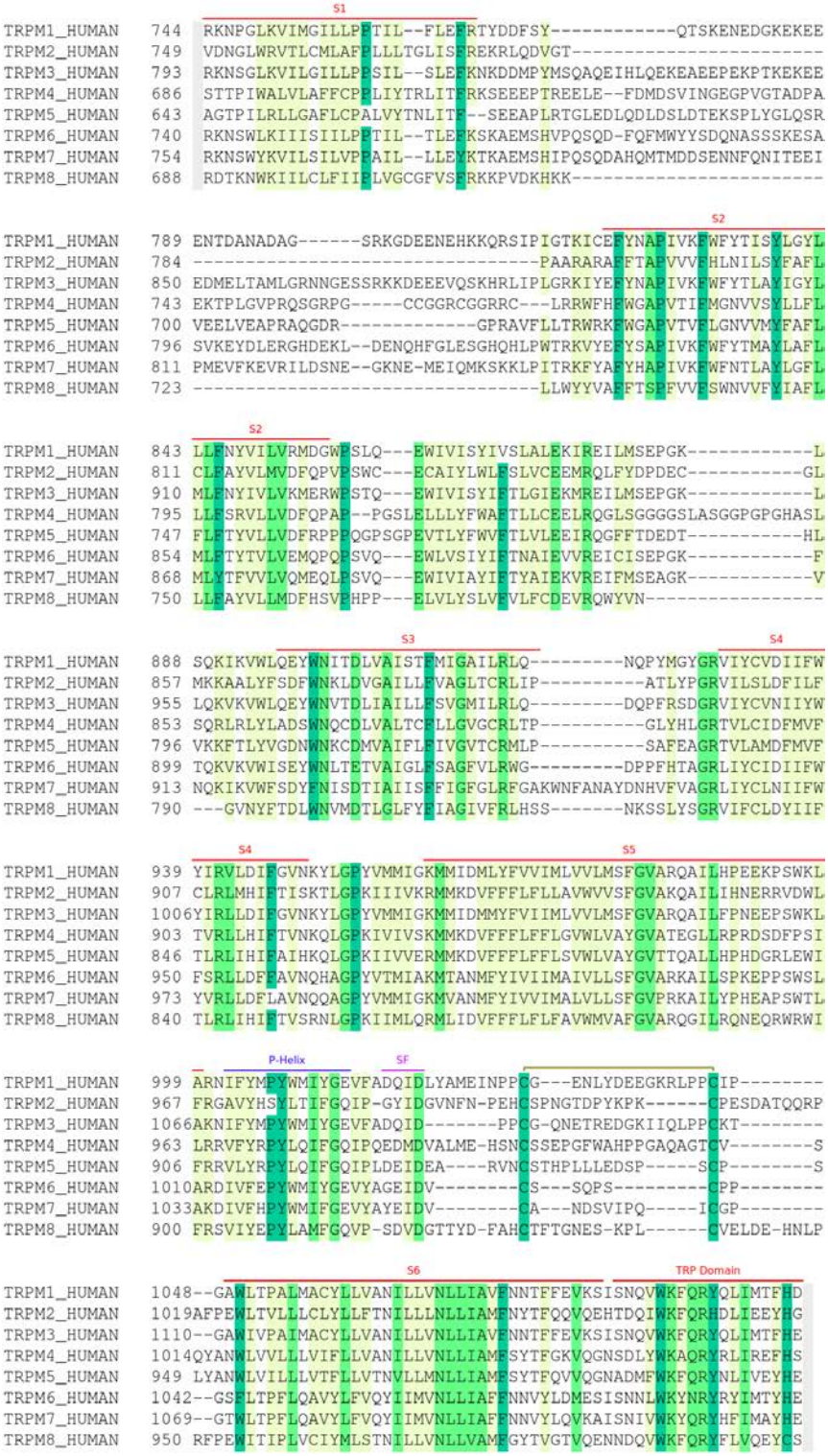
Functional and Modelling Studies of the Transmembrane Region of the TRPM8 channel

Gabriel Bidaux et al.

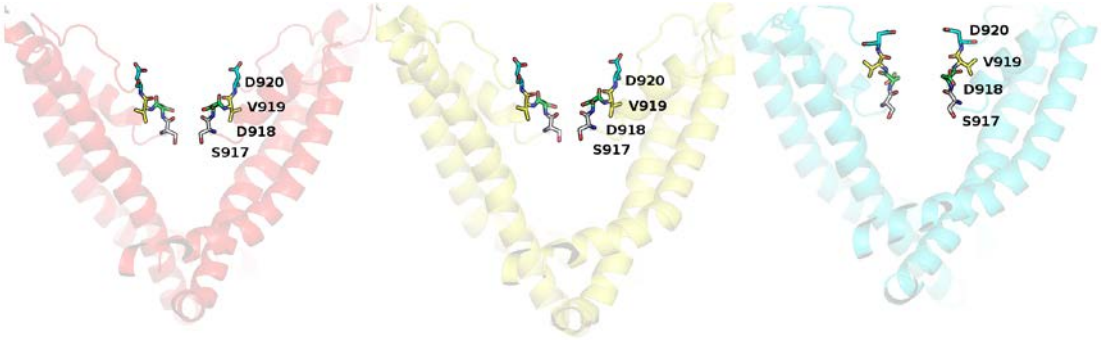
Supplementary Information

Supplementary Figure S1:



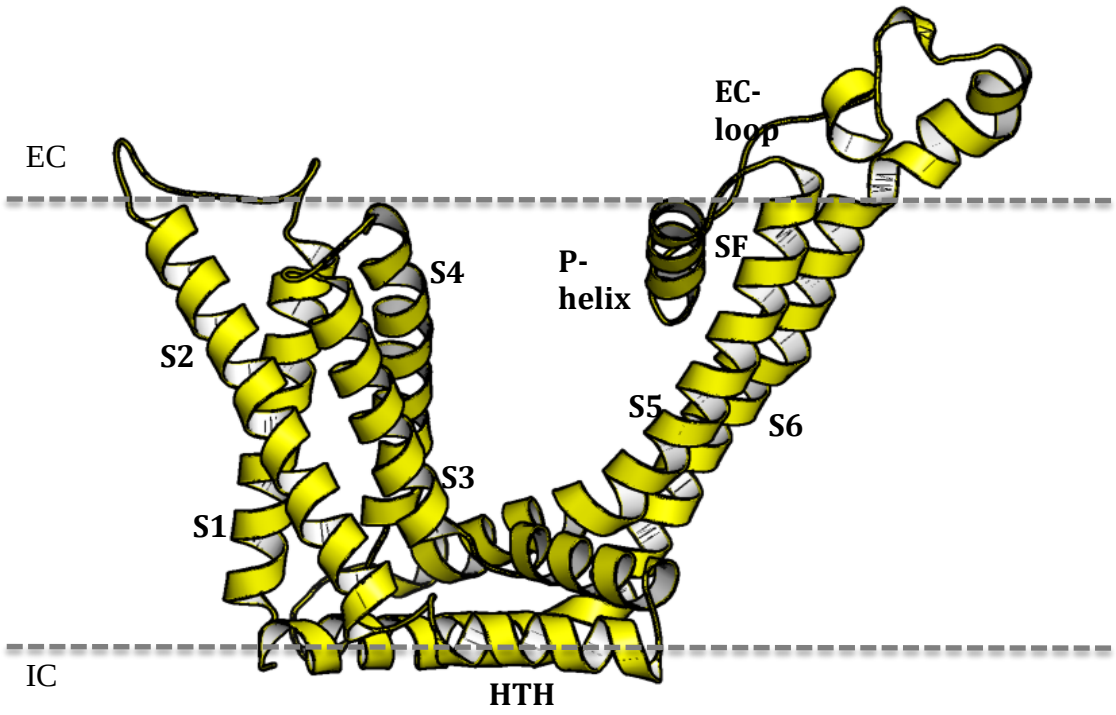
Supplementary Figure S1. Multiple sequence alignment of the transmembrane region within the TRPM ion channel family.

Supplementary Figure S2:



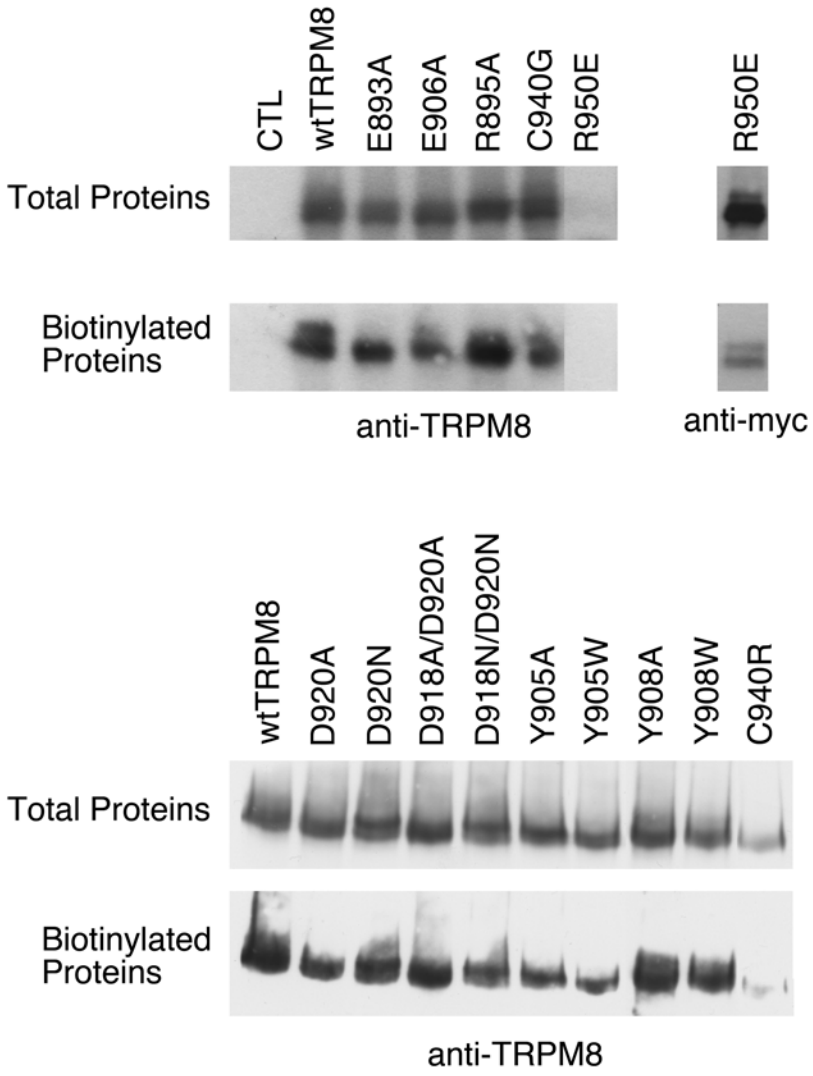
Supplementary Figure S2. Side view of the pore and the conformation of residues in the selectivity filter. The three conformations represent the closed (red), intermediate (yellow) and closed (cyan) states. Only two, diagonally opposite subunits have been shown for clarity.

Supplementary Figure S3:



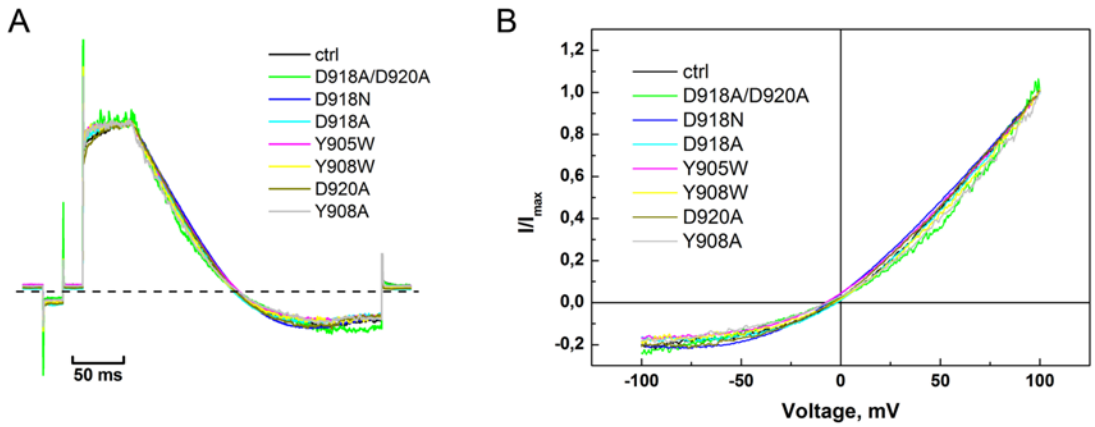
Supplementary Figure S3. A monomeric chain of TRPM8 TM region (yellow) as positioned in the lipid bilayer. The top and the lower boundary of the lipid bilayer have been illustrated as a grey line. Four identical chains come together to form a functional channel

Supplementary Figure S4:



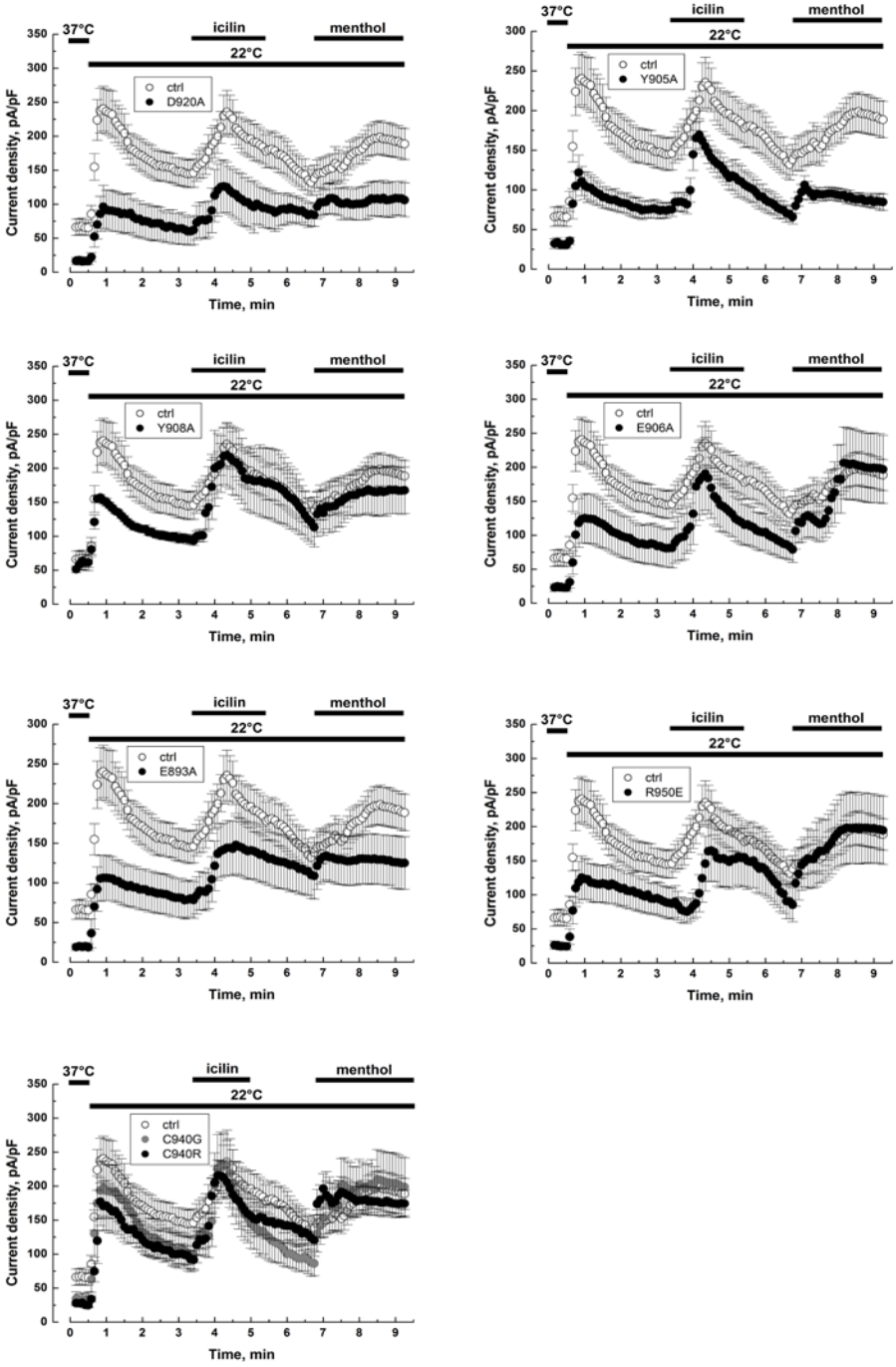
Supplementary Figure S4. Cell surface biotinylation of *wild-type* and mutant TRPM8 proteins in HEK cells. Immunoblottings showing the detection of TRPM8 proteins in total protein extracts (Total Proteins) after pull-down of biotinylated proteins with neutravidin beads (Biotinylated Protein). Proteins were detected with antiTRPM8 antibody except TRPM8 ($R^{950}A$), which was revealed with anti-myc antibody.

Supplementary Figure S5:



Supplementary Figure S5. TRPM8 mutant proteins exhibit unaltered electrophysiological properties. **A**: Normalized whole-cell traces of representative cells transfected with wild type (ctrl) or mutant TRPM8 proteins and stimulated with the voltage ramp protocol presented above. Corresponding current/voltage relationships are shown in **B**.

Supplementary Figure S6:



Supplementary Figure S6. Whole-cell recordings at +100mV of TRPM8 currents induced with either cold (22°C), or Icilin (10 µM), or Menthol (500 µM) for HEK cells concomitantly transfected with wild type TRPM8 and one specific TRPM8 mutant at a ratio 1:3. Cells transfected with wild type TRPM8, alone, were used as control (ctrl).

Supplementary Table S1:

Plasmid	**Peak current (pA/pF), Cold (22°C)**	**Peak current (pA/pF), Icilin (10 µM)**	**Peak current (pA/pF), Menthol (500 µM)**
CTL	187.1±40	183.2±75.8	168.5±56.2
D918A	162.4±54	165.4±52	173.9±51.7
D918N	159.1±37.6	154±33.6	145±32.8
D918E	139.7±43.2	190.3±49	110.6±19.9
D920A	128.8±34.5	119.1±40.2	130.7±23.5
D920N	13.7±6.3	9.4±2.1	8.8±1.4
D918A/D920A	28.2±10.4	31.6±9.3	21.4±4.7
D918E/D920E	223.8±30.5	164.8±13.9	170.7±23.1
D918N/D920N	6.3±1.1	31.6±9.3	21.4±4.7
V919I	222.1±69.2	228.6±53	166.5±33.9
Y905A	4.4±0.4	7.1±1.5	6.7±2.1
Y905W	57.9±16.7	64.4±23.2	84.1±23.2
E893A	7.5±2.3	23.4±9.8	26.4±11.1
E906A	38.2±23.6	24.6±17.4	17.4±11.2
R950E	5.9±0.6	6.2±0.8	11.5±3.5
Y908A	62.8±25.3	241.8±92.3	102.5±8.9
Y908W	87.7±25	157.2±48	109.2±31.6
C940G	4.3±0.6	3.9±1.5	3±0.1
C940R	13.4±9.2	3.5±0.3	3.3±0.5

Supplementary Table S1.

Summary of our patch-clamp results on single TRPM8 mutants when expressed in HEK293. Control (CTL) represents wild-type TRPM8 expressed in the same model. All data are presented as mean±SEM and show current density values obtained at +100mV following application of cold, icilin or menthol (n=3-8).