Supplementary Material

Functional Characterisation of a TRPM2 orthologue from the sea anemone *Nematostella vectensis* in human cells

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<u>GGCGCGCC</u>GCCACC**ATG**GGAAAAGACTCTTTTACTCCCTTGTATGACGGAGGGGATTCTAGC
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CGGATTATCCTGAGTCTTGACATAATATTCTTCATCGTCCGCAGCCTCCAGATCTTTAGCGT
CAACAGGCTGCTTGGACCCAAGCTTGTGATGATTCAGAAGATGATGCAAGACCTGGCACAGT
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CCTAGTCCAGGCATTTATGCCCGCAATAATACGTGGGTGACAATTACATCCGTCGTGCAATA
TCCCTATTGGCAGATGTACGGCGAGCTGTTTCTCGACGAAATCCAGGGTGAAAAGCCCAAGG
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GATCTGTTCGGGGACATAACTCTGCAGGCAGGAGATGATGCGGCAGCAGTCAGATGGCAGAG
AGTATCAGGCAACATTCCCCTCTACGCTAGTCACGTTTCCATCCTTGAGAAGGTCGCAAAGA
TGCGAGATGCCGCGTTT**TGA**TCTAGA
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Supplementary Figure 1. DNA sequence of *nv*TRPM2 as synthesized by commercial gene synthesis

The synthesized DNA sequence includes the open reading frame (4656 bp) of *nv*TRPM2 (start and stop codons are highlighted in red) as well as the Kozak consensus sequence immediately before the start codon. The underlined sequences represent the corresponding recognition sites for *Asc* I, *Sph* I and *Xba* I, which were used for subcloning purposes (see Methods). The codon usage of the original *nv*TRPM2 open reading frame was adapted to the human expression system without changing the original amino acid sequence.

MGKDSFTPLYDGGDSSHVHLNKFGSNQLSQSKKSWIARNFSRRECIRFVPKSHDVSRCKCGR
PRERHSQQALESGQGSEEWNVASCTTKHPTNAYGEIDFEGYGGQKRAPYLRMSHDTDANLVI
TLMLKRWNLEIPNLVISVTGGAKSFVLKPRLREMFRRGLIKAAKTTGAWIITGGTNTGVMKH
VGEAVKEQQLMFGSDTQVNVIGIATWGIVDKQSDLISEKNGKYPALYSMEPTPGHQGAMLDP
NHSHFFLVDDGTEGKYGVEIGMRSRIEEAIMKVKTDSRSEAGSIGVPVVLLVLEGGPNTVAT
MYELIKKKVPAVVIDGSGRAASVVGFAYNHTIKRNVDGQTINVIDPQYEDEVRAKVVEVFGA
KGADKTYSMIKDVLEDEKMISVYSLDGEISQDIDLAILKALLKANRSSPVAQLNLALAWNRI
DLAKSDIFTEEQQWTTETLSAAMLTALLDDKAEFAELFLQNGLSMREFLSLDILCKLYAEVP
GNTTIKPLLQKEMGKRQVKTIDMDVVGEVIEELMGDMFESYYRKDGHYFGELASYAEGLVLK
NRKSSKDLLANINRIDPLPTPYLDVFLWAVLCNRRELARVLWEAGREPMAAALMASRLLKRM
ASRAQEDNTITDISSDLYDHARLFEERAVGVLDECFNENETLSQTLLVRELDHYSRMTALEL
AVSAESQDFIAHTSCQVLLTRLWMGTMAMNTRWWKVLVCLYLPVLIFPIIYFVPDEQHERQA
AEREHQKSLNQKSSKVKSHKEKNDAPVVPVYRSKEEKAVSNDEEARVGTENEEEDFQLEDYI
PEIREDDSMEVIMRNKKLGFCDRIMHFYSAPFSKFVGNVVGYLAFIFLYAYVVLFNFPRFDP
AKTLGGIHPTEIVLYFWVFTILIEEIRQLAAKPPKYIKDKVSVYFSDTWNFVDIFSLTVFII
AIILRFFTNSRIFTASRIILSLDIIFFIVRSLQIFSVNRLLGPKLVMIQKMMQDLAQFIIIL
AVFTIAYGIALHAVMFPSPGIYARNNTWVTITSVVQYPYWQMYGELFLDEIQGEKPKEFGEV
DPDGRWLSPLLLAIYMVFTNILLLNLLIAIFNYTFERVQEDSDKVWKFQRYDLVQEYHSRPV
FAPPLVLLGHILIFIRWVWRMCRCGHPPRGSTMKIGLSPAEMEQMDNWEFQAAEMYIHQQQQ
KNSGTLEERVRALGDRVDCINSQLNRVLDSMSGTRAHALTDGNGLEGGHDSEGRLARMEVEL
SSNSESLQKILALLQQQPPVKGQAAVPIQLTLLHYKARSSPYPGSTAKRFAVQDNMVDWQVP
FPDYKPVNYTAPVVLANPVWADKDLMAMSPRPELPYNQMDHTCNVNRVSYNGTYVVKDGLPL
NPMGRTGMQGRGLLGRFGPNHAADPVVTRWKRTSAGVMLQGGKKVLEFVAIQRKDNNQWAIP
GGMVEPGQLVTQALKAEFGEEAMAKLNVSQEEKERIAKQIERLFQQGQEIYKGYVDDPRNTD
NAWMETVAVNFHDDKGDLFGDITLQAGDDAAAVRWQRVSGNIPLYASHVSILEKVAKMRDAA
F

Supplementary Figure 2. Sequence similarity between *nv*TRPM2 and *h*TRPM2

Amino acid sequence of *nv*TRPM2 (1551 aa) shown in single letter code. Residues identical to *h*TRPM2 (1503 aa) are given in green. The short sequence motif of the predicted pore loop, which determines cation selectivity of TRPM channels is highlighted in bold red letters (for comparison, the corresponding sequence motif in *h*TRPM2 is QIP). Putative transmembrane segments as derived from the topology of *h*TRPM2 are underlined in red. The C-terminal NUDT9H domain is grayed out and the active site homologous to the NUDIX sequence motif $GX_5EX_7REUXEEXGU$ is boxed. A methionine residue within the N-terminal part (boxed in red), which has been shown in *h*TRPM2 to be sensitive to oxidation by H_2O_2 is also conserved in *nv*TRPM2.

DSYHVNARHLLYPNCPVT*RFPVPNEKVPWETEFLIYDPPFYTAERKDAAAMDPMGDTLEPL
STIQYNVVDGLRDRRSFHGPYTVQAGLPLNPMGRTGLRGRGSLSCFGPNHTLYPMVTRWRRN
EDGAICRKSIKKMLEVLVVKLPLSEHWALPG GSREPGEMLPRKLKRILRQEHWP SFENLLKC
GMEVYKGYMDDPRNTDNAWIETVAVSVHFQDQNDVELNRLNSNLHACDSGASIRWQVVDRRI
PLYANHKTLLQKAAAEFGAHY

TLLHYKARSSPYPGSTAK*RFAVQDNMVDWQVPFPDYKPVNYTAPVVLANPVWADKDLMAMS
PRPELPYNQMDHTCNVNRVSYNGTYVVKDGLPLNPMGRTGMQGRGLLGRFGPNHAADPVVTR
WKRTSAGVMLQGGKKVLEFVAIQRKDNNQWAIPG GMVEPGQLVTQALKAEFGEEAMAKLNVS
QEEKERIAKQIERLFQQGQEIYKGYVDDPRNTDNAWMETVAVNFHDDKGDLFGDITLQAGDD
AAAVRWQRVSGNIPLYASHVSILEKVAKMRDAAF

Supplementary Figure 3. Sequence similarity between *h*NUDT9 enzyme and the NUDT9 domain of *h*TRPM2 and *nv*TRPM2, respectively

Amino acid sequence of the NUDT9H domain from *h*TRPM2 (aa 1236-1503; upper sequence) and *nv*TRPM2 (aa 1271-1551; lower sequence) given in single letter code. Shown is the similarity of both sequences with the corresponding region of the *h*NUDT9 enzyme (aa 59-350), which was demonstrated to be sufficient for enzymatic function. Identical residues are depicted in green letters and the NUDIX sequence motif of the catalytic active site is boxed. The red asterisks indicate the cut-paste limits for the generation of the channel chimeras with alternative NUDT9 domains. Residue Q1438 of *nv*TRPM2 of which the mutation to arginine induces H_2O_2 sensitivity is given in red. The stretch of 15 amino acid residues immediately downstream of the NUDIX box, which is absent in wild-type *h*TRPM2 is highlighted in yellow. The sequence of *h*TRPM2 underlined with a dashed red line was replaced by the corresponding sequence of *nv*TRPM2 (underlined in red) to obtain the chimera *h*TRPM2-(+Δ15), which shows normal sensitivity to H_2O_2 but a strongly reduced response to ADPR.

***nv*TRPM2:**

S1◄◄PIIYFVPDEQHERQAAEREHQKSLNQKSSKVKSHKEKNDAPVVPVYRSKEEKAVSNDE
EARVGTENEEEDFQLEDYIPEIREDDSMEVIMRNKKLGFCDRIMHFYSAPFSKFVG►►S2

***h*TRPM2:**

S1◄◄TGLISFREKRLQDVGTPAARARAFFTAPVVVFHL►►S2

***h*TRPM3:**

S1◄◄SLEFKNKDDMPYMSQAQEIHLQEKEAEEPEKPTKEKEEEDMELTAMLGRNNGESSRKK
DEEEVQSKHRLIPLGRKIYEFYNAPIVKFWF►►S2

Supplementary Figure 4. Sequence similarity between the putative S1-S2 linker of *nv*TRPM2, *h*TRPM2 and *h*TRPM3

Amino acid sequences (*nv*TRPM2: aa 730-843; *h*TRPM2: aa 768-801; *h*TRPM3: aa 812-900) of the putative extracellular linker region between transmembrane segments S1 and S2. The respective sequence matches are highlighted (red for *nv*TRPM2 and *h*TRPM2, green for *nv*TRPM2 and *h*TRPM3).
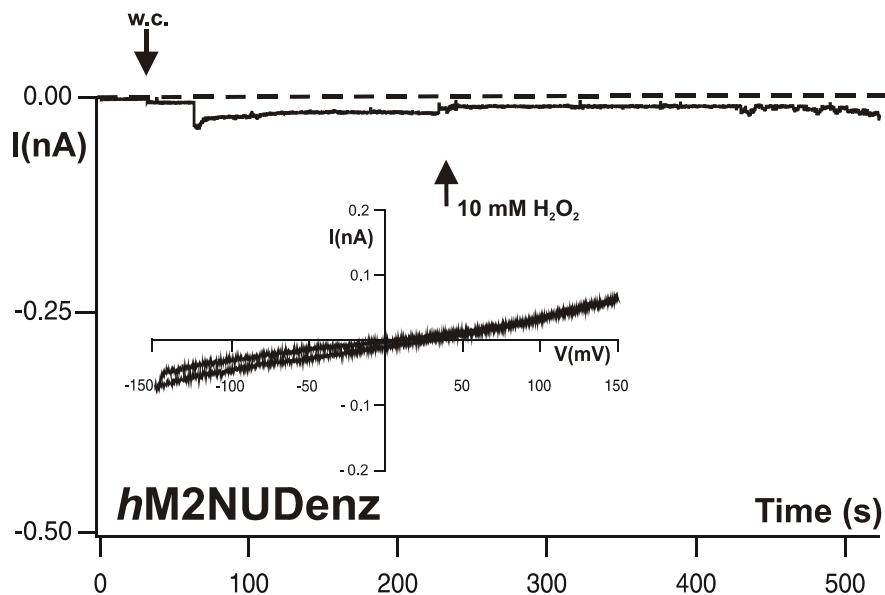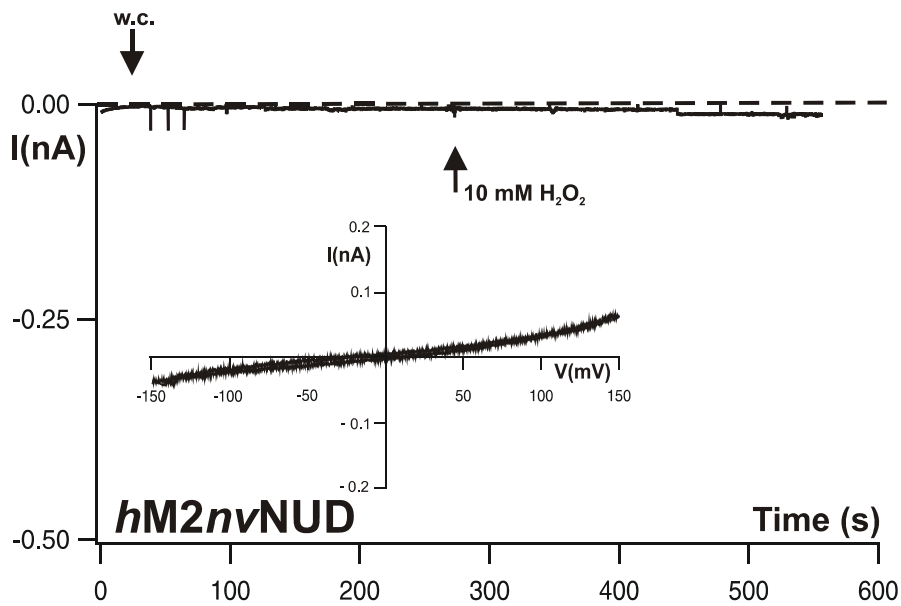
Supplementary Figure 5. Sensitivity of *h*TRPM2 chimeras with alternative NUDT9 domains to intracellular ADPR and extracellular H_2O_2.

Representative whole-cell patch clamp experiments in which the variants *h*TRPM2-*nv*NUD (*h*TRPM2 with NUDT9 domain of *nv*TRPM2) or *h*TRPM2-NUDenz (*h*TRPM2 with NUDT9 domain of the human NUDT9 enzyme) were stimulated with 0.6 mM ADPR and 1 μM Ca^{2+} in the pipette solution and after several minutes additionally stimulated by extracellular application of 10 mM H_2O_2 (as indicated by an arrow). The corresponding I/V curves are given in the inset figure. Similar results were obtained from at least 6 independent experiments.