Supplemental Data

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Recessive Mutations of the Gene *TRPM1* Abrogate ON Bipolar Cell Function and Cause Complete Congenital Stationary Night Blindness in Humans

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Figure S1. Positions of Rare Missense Variants Found in this Study and Their Context within Partial Alignments of TRPM1 Orthologues as well as Human TRP Channel Protein Paralogs

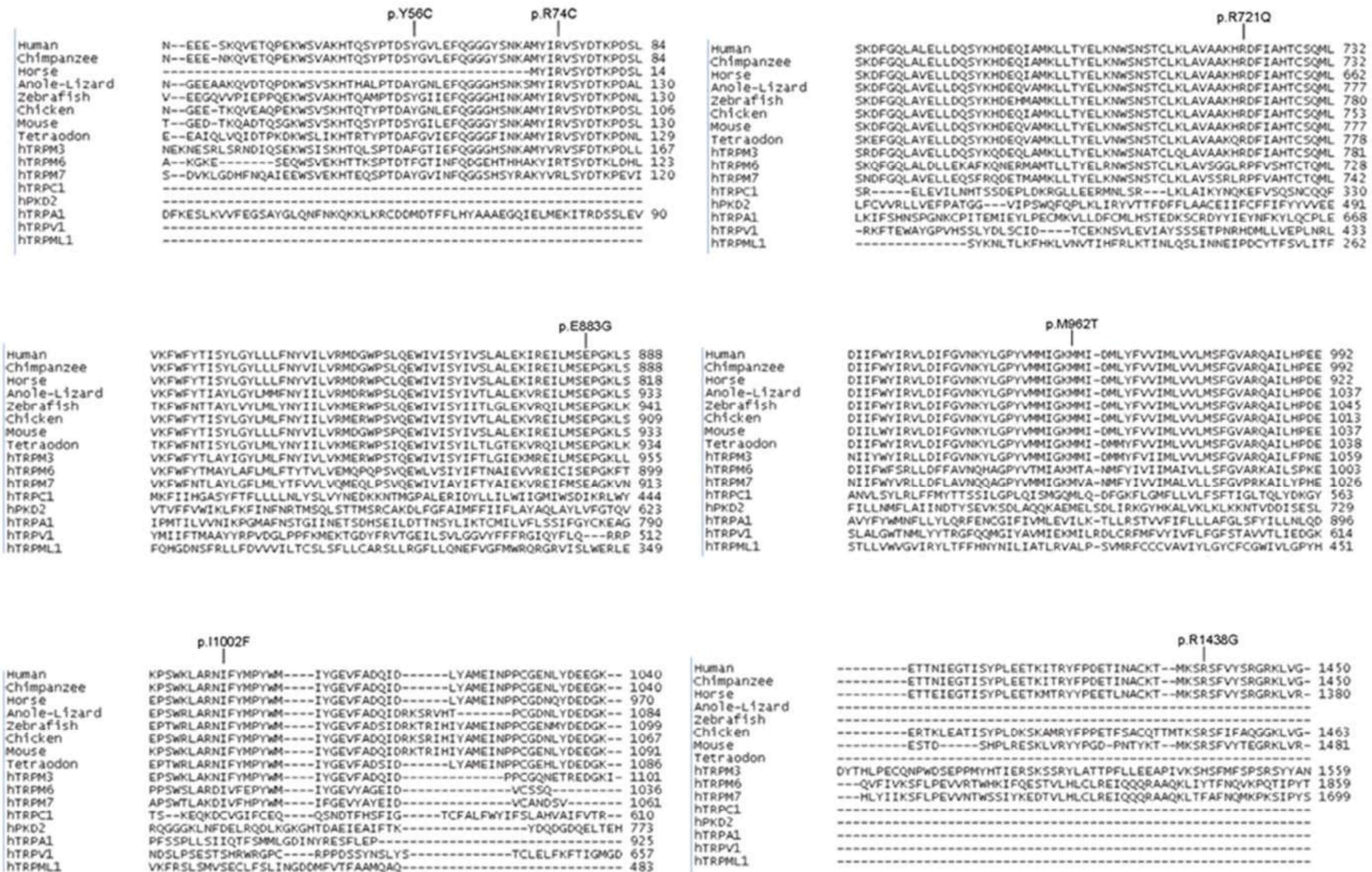

Figure S2. Alignment of Novel Transcript of Human TRPM1 with other Vertebrate Species

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Anole-Lizard      ---------GLLRRMNGFFKRNSLKGSASGSQKGQKAWIDKTFFKRECIYIIANSKDATR
Chicken*          MKGRGGKFTGSLRRMSSSFKRTSFKGSASGSQKGQKAWIEKTFSKRECIYVIANNKDISR
Human             ---------------MSSFKRGSLKSSTSGSQKGQKSWIEKTFCKRECIFVIPSMKDSNR
Mouse             --------MGSMRKMSSSFKRGSIKSSTSGSQKGQKAWIEKTFCKRECIFVIPSTKDPNR
Tetraodon         --------IMDPKGPSAAFKRSSLKRSTSGSQKAQKVWIERNFLKRECIHIFP-TKDPTR
Zebrafish         --------FGAKKAKEGSFKRASIKRTSSGSQKAQRAWIERTFLKRECNHIFP-SKEPNK
                                  . *** *:* ::*****.*: **::.* **** .::.  *: .:

Anole-Lizard      CCCGQLLTQHTPIPAITTTNKNGEEAAKQVDTQPDKWSVSKHTHALPTDAYGNLEFQGGG
Chicken*          CCCGQLITQHIPPPPSTTANKNGE-ETKQVEAQPEKWSVSKHTQTYPTDAYGNLEFQGGG
Human             CCCGQFTNQHIPPLPSATPSKNEE-ESKQVETQPEKWSVAKHTQSYPTDSYGVLEFQGGG
Mouse             CCCGQLTNQHIPPLPSGAPSTTGE-DTKQADTQSGKWSVSKHTQSYPTDSYGILEFQGGG
Tetraodon         CACGQLTTQHVAIPPGAN-SVEEAIQLVQIDTPKDKWSLIKHTRTYPTDAFGVIEFQGGG
Zebrafish         CCCGQLVNQHVAILPGSTNKNVEEGQVVPIEPPQEKWSVAKHTQAMPTDSYGIIEFQGGG
                  *.***: .** .  .    .          :.   ***: ***:: ***::* :******

Anole-Lizard      HSNKSMYIRVSYDTKPDALLHLMVKEWQLELPKLLISVHGGLQNFELQPKLKQVFGKGLI
Chicken*          HSNKAMYIRVSYDTKPDSLLHLMVKDWQLELPKLLISVHGGLQNFEMQPKLKQVFGKGLI
Human             YSNKAMYIRVSYDTKPDSLLHLMVKDWQLELPKLLISVHGGLQNFEMQPKLKQVFGKGLI
Mouse             YSNKAMYIRVSYDTKPDSLLHLMVKDWQLELPKLLISVHGGLQSFEMQPKLKQVFGKGLI
Tetraodon         FINKAMYIRVSYDTKPDNLLHLMVKDWQLELPTLLISVHGGLQNFDLQPKLKQVFGKGLI
Zebrafish         HINKAMYIRVSYDTKPDNLLHLMVKDWQLELPTLLISVHGGLQNFDLQPKLKQVFGKGLI
                  . **:************ *******:******.**********.*::*************

Anole-Lizard      KAAMTTGAWIFTGGVSTG--VIRHVGDALKDHSSKSRGRICAIGIAPWGIVENKEDLIGK
Chicken*          KAAMTTGAWIFTGGVSTG--VIRHVGDALKDHSSKSRGRICAIGIAPWGIVENKEDLIGK
Human             KAAMTTGAWIFTGGVSTG--VISHVGDALKDHSSKSRGRVCAIGIAPWGIVENKEDLVGK
Mouse             KAAMTTGAWIFTGGVSTG--VVSHVGDALKDHSSKSRGRLCAIGIAPWGMVENKEDLIGK
Tetraodon         KAAVTTGAWIFTGGVNTAPGVIRHVGDALKDHSSKSRGKVCAIGIAPWGILENKEDLIGK
Zebrafish         KAAVTTGAWIFTGGVSTG--VIRHVGDALKDHSSKSRGKVCAIGIAPWGIVENKEDLIGR
                  ***:***********.*.  *: ***************::*********::******:*:
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Exon1a is shown in the square, and is highly conserved within diverse vertebrate species. *All sequences were from cDNA sequences apart from chicken which was derived from a BLAT interrogation of *Gallus gallus* genomic sequence (NW_001471425).

Table S1. Sequence of the Mutation Screening Primers

exon	sense	anti-sense	product size (bp)
1a	CCAGACGCCCAAATCCTTCCCATTT	GCCCCATGCCCACCCAGCACAGTTT	584
1	CTCAATTATGCAAACCCTGCTGACATTT	GCACCTGAGTTTGTCCACGCTTGAGTTT	466
2	GGGCAGACATATTGTTTTATAAAGGTAT	CCTTCTGGACTCCTCTTTCTGCCTCTTA	279
3	GGGAAAGGGGGAGATTGTTGTAGAAA	GCCACAGCCATGAAGAAGACCAGGTAT	352
4	GCCTCACTCCCCTTTGACACGAGAA	CACAGTGAGTTCTGGGTGGTACATTGATTA	339
5	GGGAATGTAGGGACTCAGGGCAAGT	GGCTGCAAGGGAGCTCTCTTATTATTCTTA	335
6	GGCAGGGTAGAGATGTGAGAATAAGTTTTA	CCCAGGAGGACTGCGTGCCTTT	332
7	CCAGATAGAACATCCCCCAAGTCGTAAT	GGAGATCTAGCAAATCTTCCTGATTTAA	423
8	CCTCCCACAGCAAAGTCTCAAATCAAA	CCGGACTAAAATATGAATAACCCTGTCAT	334
9	CCCCATATCTCCTTCTTGTTTTCAACTT	CCAACATCAGAGGGACAGGAGTCACCAT	174
10	CCAGGCGTGGAGTGAAAAATCATT	GGGAGAACATACTAATAGGTCACTTGTACAAA	217
11	GCACATACAGAGGTGGGAGGGTCAAT	GGTGGGACTGAAGCAAGGACAAGAA	352
12	GGAATCTGGCGTTCGGAATACCCTTT	GCACAATATGCACCAGTGACAAACACATT	313
13	GGCCTCAAACTACTGACCTCAAGTGAT	CCTGTGCCTGGAATAATAATACATTTTGAAA	283
14	CCACTTACCCCCTAGACGTGTGATGAA	CTCTGAACCGGCCAAGTTGTTGAAAA	309
15	GACAGAAGAAGAGTTTCTATATTAAAGCACTT	GGGCTATGTATATTTGACCAGGATATTAT	267
16	GGAGTGGAAATGTAAGATGTACTTCAGAA	CCACCCCTCCCTGCAGAGACAAGTA	504
17	GTCCTTGGTGGATATGCCTGTCTAAGAAA	CCTCAGGGGGTTGCTAGAAGGAATAAAT	426
18-19	GGGATAACATTCCAGGGCTCCTAGGTT	CCAACATATCAAAGCATTCAAAATATACTGAAT	640
20	CCCAAACACGGCTAACAGCTTGTCTTT	CGCCTGGCCCAACATGCATAATTT	272
21	GAATGTTCCATCAGTCTTATTGTTCCAA	CCCAAGGCCTGTTCTTATGTCCTAACTA	414
22	CCCACAAGCCAAGACAGTGAGAGACAAA	GCCTCAGAAGCAGGGAAGAGAATCTTA	265
23	CCTCCCCTCCTGTCAAAACAAAACAAA	CTGGCACCAAAAAACAAGAGAAGTATTT	201
24	GCTGGGGCTACAGAGTTTATTTTTT	CTGCAAAAATTGGATCTACTTAAAAACCCTAAT	446
25	GGCCATCTCATTGAGTATTTTCACTTAAAT	CACACGAGCAAGTAGTTGAGTGAGATTT	409
26	GGAGTGGCGGAGAAGAGAGCTTAATTAAA	CAGGAGAACTCGGAGTGCATGTTTAAAT	356
27	GGGGATTGTGAAGCTTGTAAATATACTCAAA	CCTGTCCATGTATCACATCTGTTTTAT	751
27-1a	GGAAGATGATGAAAGACAGACAGACTCTAA	GACACCCATTAGTGGTTCTGACTGTTAAA	782

Genomic DNA for each individual was extracted from peripheral blood leukocytes using Gentra® Puregene Blood Kit (Qiagen, Duesseldorf, Germany). 192 European Collection of Cell Cultures (ECACC) DNA samples, derived from anonymised UK blood

donors was used as controls. PCR primers were designed to amplify each coding exon with flanking intron sequence. PCRs were setup using MolTaq polymerase Kit according to manufacturer's instruction (Molzym, Bremen, Germany). Briefly, 3.0µl of 10x PCR buffer, 0.6µl of 10mM dNTP mix, 0.6µl of each 10µm primer, 0.3µl of PCR Enhancer and 1.5units of MolTaq was added to molecular grade water into a final volume of 30µl. PCR cycles were as follows: denaturation at 94°C for 3 minutes, then cycled: denaturation at 94°C for 1 minute, annealing at 58°C for 30 seconds and extension at 72°C for 30 seconds. A final extension was carried out for 10 minutes at 72°C. All amplimers were purified using Multiscreen µ96 PCR clean up kit (Millpore, Billerica, MA). Subsequent sequencing reactions were setup using BigDye Terminator v3.1 sequencing kit and run on an automated capillary sequencer ABI3730 (Applied Biosystem, Forster City, CA). Electropherograms were analyzed using the "Seqman Pro" alignment algorithm (DNASTAR, Madison,WI). The pathogenic impact of the *TRPM1* sequence variants caused by non-synonymous changes of the protein sequence was evaluated through SIFT and PolyPhen sequence homology-based programs.

Table S2. Primer Sequences for RACE and RT-PCR

RACE and RT-PCR Primers	
1aF1	ACCAAAAGCCAGCACATGCTCCTCCTA
1aF2	CCCGAGGGAGTCAGCAGGGTGGCTCACA
1F1	GCCTGAGCTGTGCCCTCTCCATT
1F2	GCCCTGGCCAAGGAGGAGGCTGAAA
1F3	GCTCCTCATGGGGACTGCTCCTCTTAAA
3R	GGCTGAGTCTCCACCTGTTTGCTTTCCTCTT
6R	CAGGATGAAGTGGGTGTGGGAGTTGTT
TRPM1-27R	CCTGTCCATGTATCACATCTGTTTTAT
GeneRacer™ 5' Primer	CGACTGGAGCACGAGGACACTGA
GeneRacer™ 5' Nested Primer	GGACACTGACATGGACTGAAGGAGTA
TRPM1 5'GSP-REV	GGAGCAGTGAGTCTGGCTTGGTGTCATA
TRRM1 5'GSP-nested	GGCAGAGGGGGGATATGCTGGTTGGTGAA
TRPM1 3'GSP-FOR	CCCAGATGGCAGTCACCTTGCAGTAGAT
TRPM1 3'GSP-nested	CCTCGAATCCCTCGCTTGTCCCTAA
GeneRacer™ 3' Primer	GCTGTCAACGATACGCTACGTAACG
GeneRacer™ 3' Nested Primer	CGCTACGTAACGGCATGACAGTG

5’ and 3’ RACE experiments were performed using the protocol supplied by the GeneRacer™ Kit manufacturer (Invitrogen, Carlsbad, CA). Primers were designed on the basis of previous published reference sequence for *TRPM1* (NM_002420.4). Typically 1µg total RNA from both the retina (Clontech, Mountain View, CA) and total skin RNA (Stratagene, La Jolla, CA) were used as template. For 5’RACE, a *TRPM1* gene-specific primer TRPM1 5’GSP-REV was used together with GeneRacer™(Invitrogen, Carlsbad, CA) primers to capture mRNA 5' ends. All PCR reactions were setup using Platinum® Taq

DNA polymerase High Fidelity (Invitrogen, Carlsbad, CA) according to the GeneRacer Kit instructions. The PCR conditions were: 94°C for 2 minutes followed by 5 cycles of 94°C for 30 seconds, 72°C for 2 minutes, 5 cycles of 94°C for 30 seconds, 70°C for 2 minutes, followed by 20 cycles of 94°C for 30 seconds, 68°C for 30 seconds and 72°C for 2 minutes. Nested-PCR was performed on this cDNA resource using the GeneRacer™ 5' Nested Forward primer (Invitrogen, Carlsbad, CA) and the TRPM1 5'GSP-nested primer (table S2). PCR cycles for the nested reactions were as follows: 94°C for 2 minutes, followed by 25 cycles of 94°C for 30 seconds, 65°C for 30 seconds and 68°C for 2 minutes, finally 68°C for 10 minutes. For 3'RACE, two reverse transcription modules were tested to prime the first-strand cDNA synthesis, SuperScript™ III RT Module and Cloned AMV RT Module (Invitrogen, Carlsbad, CA). One contained a GeneRacer™ Oligo dT Primer with a dT tail of 24 nucleotides. A second GeneRacer™ Oligo dT Primer had a dT tail of 18 nucleotides. The SuperScript™ III RT Module was used for 3'RACE experiment. Two rounds of PCR for 3'RACE were setup using the conditions as described for the 5'RACE PCR experiment, with the gene-specific forward primer: TRPM1 3'GSP-FOR and a nested gene-specific forward primer: TRPM1 3'GSP-nested. PCR products were purified from the gel using the QIAGEN Gel extraction Kit (Qiagen, Duesseldorf, Germany) and cloned into pCR4-TOPO vector (Invitrogen, Carlsbad, CA). DNA mini-preparations from different clones were purified using Qiaprep® Miniprep kit (Qiagen, Duesseldorf, Germany). Sanger sequencing reactions were performed on the ABI sequencing system as previously described. RT-PCRs described in figure 5 (main text), were performed using a primer complimentary to exon 27 (TRPM1 27R – table S2), SuperScript™ III reverse-transcriptase and 1.0µg of retinal or skin RNA for cDNA synthesis. Reaction setup was as follows: 1µg of total RNA, 2µM of primer TRPM1-27R, 1µl of dNTP mix (10mM), 7µl of DEPC-treated water to total of 10µl. The mixture was incubated at 65°C for 5 minutes,

then placed on ice for 2 minutes. The following cDNA Synthesis Mix was added to each RNA/primer mixture: 10X RT buffer 2µl, 4µl of 25 mM $MgCl_2$, 2µl of 0.1 M DTT, 1µl of RNaseOUT™ (40 U/µl), 1µl of SuperScript™ III RT (200U/µl). The mixed reaction was incubated at 50°C for 50 minutes, terminated at 85°C for 5 minutes, and chilled on ice. Then, 1µl of RNase H (2U/µl) was added to each tube and incubated for 20 min at 37°C. The cDNA was used as template for the ensuing PCRs. PCR cycles using the primer pairs as described in Figure 5 (main text) were as follows: denaturation at 94°C 3 minutes, 5 cycles of 94°C for 30 seconds and 72°C 1 minute, 5 cycles of 94°C for 30 seconds and 70°C 1 minute, followed by 25 cycles at 94°C for 1 minute, 58°C-64°C for 30 seconds and 72°C for 1 minute, with a final extension of 10 minutes at 72°C. The PCR for confirming the existence of exon 1a in a full length TRPM1 transcript used primer pair 1aF1 and TRPM1-27R, and cycles were as follows: 94°C 3 minutes, 5 cycles of 94°C for 30 seconds and 72°C 6 minutes, 5 cycles of 94°C for 30 seconds and 70°C 6 minutes, followed by 25 cycles at 94°C for 1 minute, 64°C for 30 seconds and 72°C for 6 minute, with a final extension of 10 minutes at 72°C