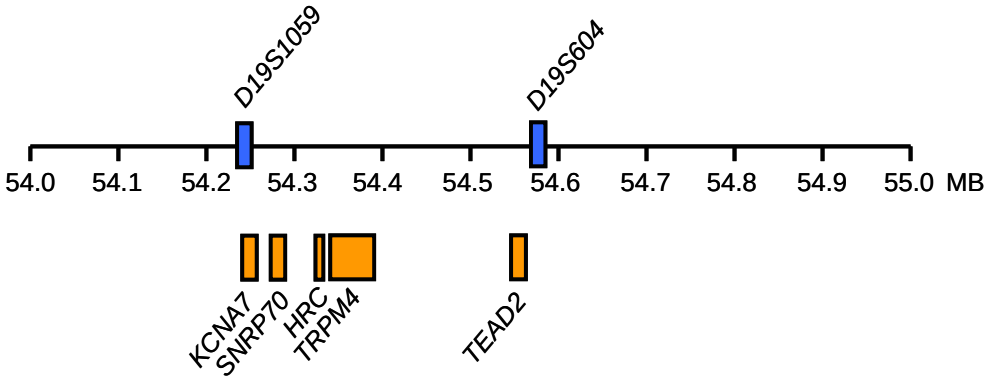
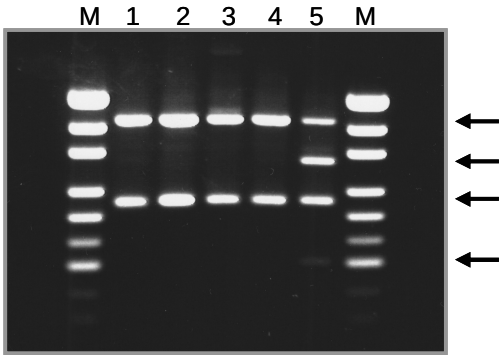


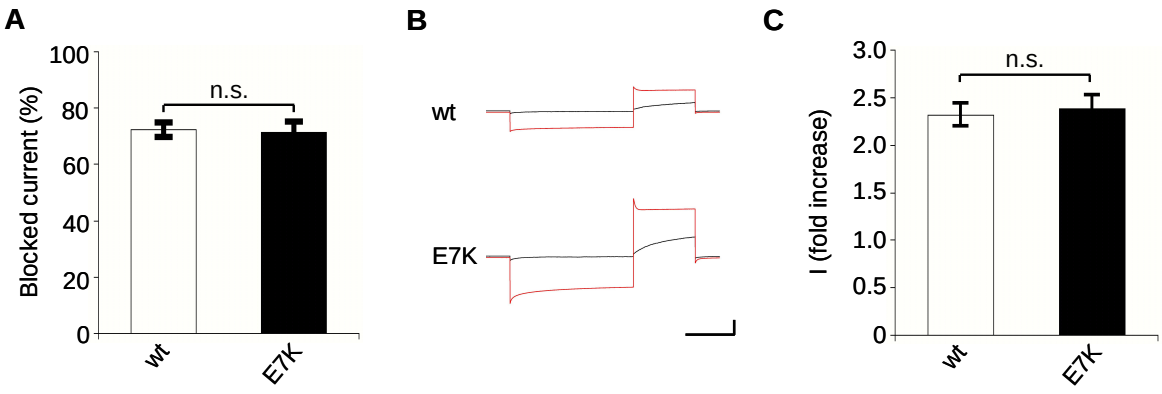
Supplemental Figure 1

PFHBI locus at chromosome 19q13.33. Scale bar refers to basepairs of DNA in megabases (MB) at chromosome locus 19q13.33 according to GenBank. Horizonzal blue bars – DNA localization of microsatellite markers *D19S1059* and *D19S604* used to delineate PFHBI locus. For haplotype analysis 2-point lod score analysis was performed with MLINK (see (1) for additional microsatellite markers and genotyping). Horizontal orange bars beneath scale bar – localization of genes expressed in human cardiac tissue(2-6). *KCNA7*: potassium voltage-gated channel, *Shaker*-related subfamily, member 7; *SNRP70*: U1 small nuclear ribonucleoprotein 70 kDa; *HRC*: sarcoplasmic reticulum histidine-rich calcium-binding protein precursor; *TRPM4*: transient receptor potential cation channel subfamily M member 4; *TEAD2*: transcriptional enhancer factor TEF-4.



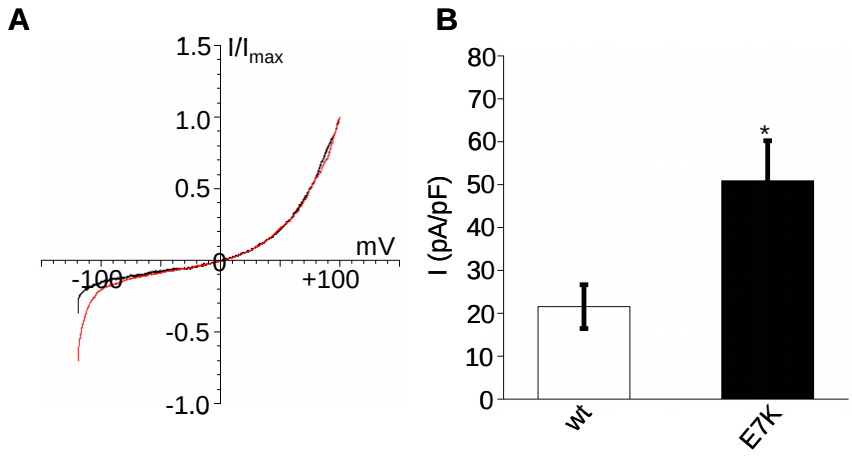
Supplemental Figure 2

RFLP analysis of *TRPM4* exon 1 after PCR amplification. Amplicon was obtained by PCR using primers TRPM4_Ex01+02-up and TRPM4_Ex01+02-low (see Supplemental Table 2) and genomic DNA as template. *Mbo*II cuts wild-type amplicon (662 bp) into fragments of 415, 217, 27 and 3 bp (lane 1-4). The 27 and 3 bp fragments were too small for detection. Presence of the heterozygous 19 G>A TRPM4-mutation creates two additional fragments (307 and 108 bp, lane 5). M: reference length standard for gel electrophoresis (*Msp*I-digested pUC19-DNA, Fermentas Inc., Canada). Arrows indicate position of *Mbo*II restriction-fragments.



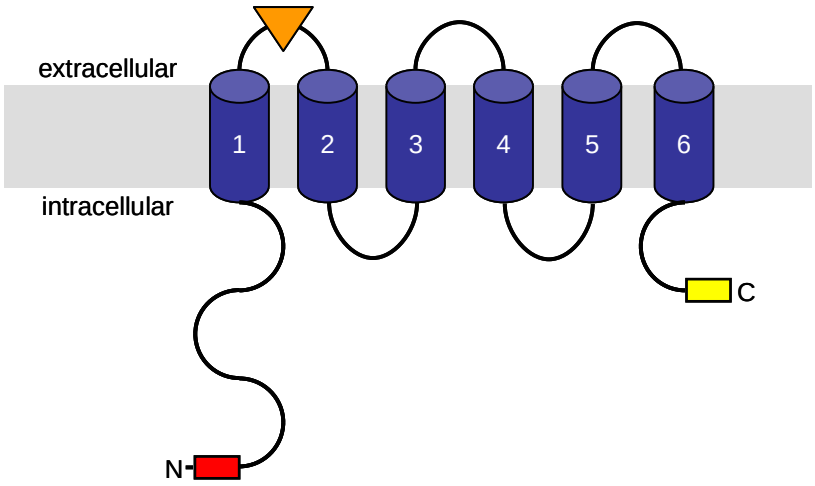
Supplemental Figure 3

Influence of AMP-PNP and PtdIns(4,5)P_2 on TRPM4b-channel activity. **(A)** TRPM4b (wt) and TRPM4b^{E7K} (E7K) currents were recorded in the inside-out patch-clamp configuration. Holding potential was 0 mV, currents were elicited by a 500 ms pulse to -100 mV followed by a 250 ms pulse to +100 mV. Bar graph displays percentages of current block by bath application of 500 µM AMP-PNP (means ± s.e.m., n = 3). **(B)** TRPM4b (wt) and TRPM4b^{E7K} (E7K) currents were recorded as described in **A**. Black traces were recorded before, red traces after application of 10 µM diC8-PtdIns(4,5)P_2 (Echelon Biosciences, USA). Scale bars: 200 ms, 2 nA. **(C)** Bar graph displays fold change of current increase by bath application of 10 µM PtdIns(4,5)P_2 (means ± s.e.m; n = 3). n.s. – not significant.



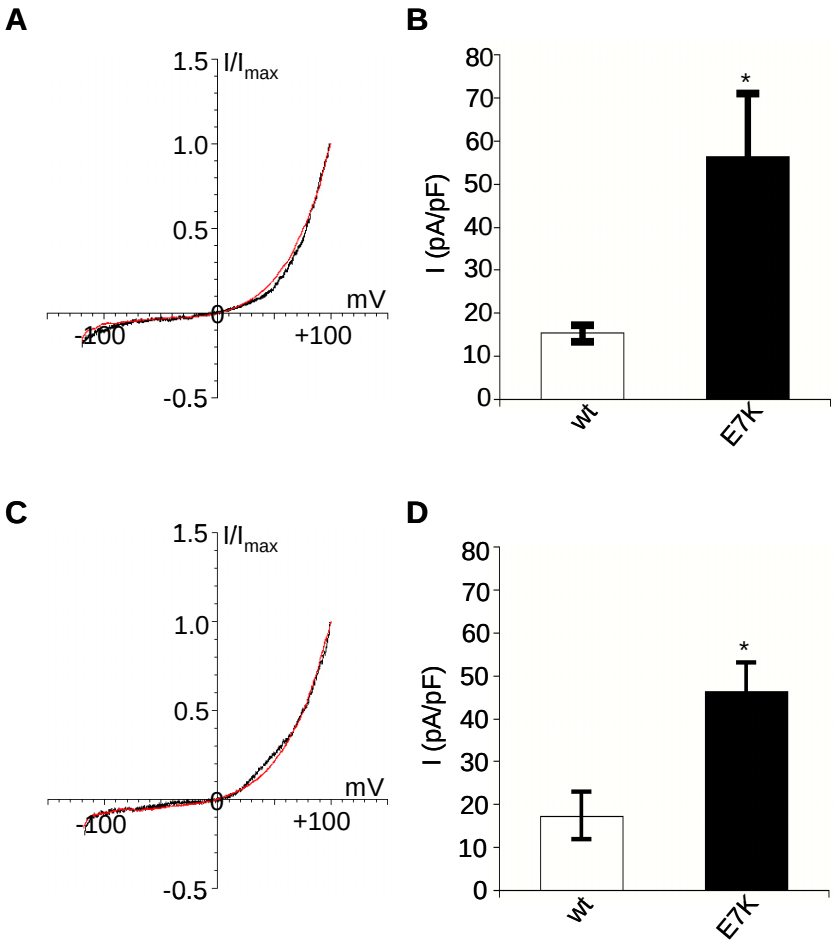
Supplemental Figure 4

Expression of TRPM4b and TRPM4b^{E7K} in CHO-cells. **(A)** Voltage-dependence of wild-type (black) and mutant (red) TRPM4b current measured on transiently transfected CHO-cells in the whole-cell patch-clamp configuration with 250 ms voltage ramps from -120 to +100 mV. Holding potential was -60 mV. Current amplitudes were normalized to those at + 100 mV. **(B)** Current densities (pA/pF) at a test potential of +80 mV given as mean ± s.e.m. (TRPM4b (wt) n = 7, TRPM4b^{E7K} (E7K) n = 6). Statistical significance (*) was tested by unpaired Student's t-test. $P < 0.05$.



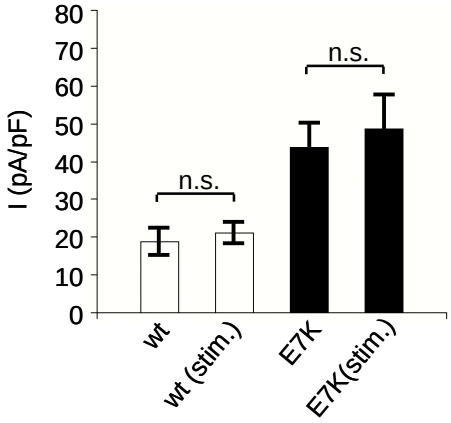
Supplemental Figure 5

FLAG- and Myc-Tag sites introduced into TRPM4b protein. Putative membrane topology is diagrammed schematically with six transmembrane domains (1-6, blue cylinders) flanked by cytoplasmic N- (N) and C-terminal (C) sequence. Gray bar symbolizes plasma membrane. Red or yellow box: FLAG-tag (introduced either after Met 1 (red) or after Asp 1214 (yellow)). Orange triangle: Myc-Tag (introduced after Leu 722).



Supplemental Figure 6

Expression of tagged TRPM4b channels. **(A)** Voltage-dependence of Myc-tagged TRPM4b (black) and, respectively, TRPM4b^{E7K} (red) current measured on transiently transfected HEK-293 cells in the whole-cell patch-clamp configuration with 250 ms voltage ramps from -120 to +100 mV. Holding potential was -60 mV. Current amplitudes were normalized to those at +100 mV. **(B)** Current densities (pA/pF) of Myc-tagged TRPM4b channels are given as mean ± s.e.m. (TRPM4b (wt) n = 5; TRPM4b^{E7K} (E7K) n = 7). Statistical (*) significance was tested by unpaired two-tailed Student's t-test. $P < 0.05$. **(C)** Voltage dependence of C-terminally FLAG-tagged wild-type (black) and mutant channel (red) was determined as described in **a**. **(D)** Current densities (pA/pF) of C-terminally FLAG-tagged TRPM4b-channels are given as mean ± s.e.m. (TRPM4b (wt) n = 7; TRPM4b^{E7K} (E7K) n = 7). Statistical (*) significance was tested by unpaired two-tailed Student's t-test. $P < 0.05$.



Supplemental Figure 7

TRPM4b-mediated current density is insensitive to protein kinase A stimulation in HEK-293 cells. Transiently transfected HEK-293 cells expressing TRPM4b- or TRPM4b^{E7K}-channel were subjected to whole-cell patch-clamp recordings as described in Material and Methods either with or without stimulation of protein kinase A by application of 300 µM 8-Br-cAMP, 1 µM okadaic acid (OA)(7) and 10 µM 3-isobutyl-1-methylxanthine (IBMX)(8). Current densities (pA/pF) are given as mean ± s.e.m. (wt - n = 13; wt/OA/IBMX - n = 11). Statistical significance was tested by unpaired two-tailed Student's t-test. n.s. – not significant.

References:

1. Brink, P.A., et al. 1995. Gene for progressive familial heart block type I maps to chromosome 19q13. *Circulation* **91**:1633-1640.
2. Launay, P., et al. 2002. TRPM4 is a Ca2+-activated nonselective cation channel mediating cell membrane depolarization. *Cell* **109**:397-407.
3. Bardien-Kruger, S., et al. 2002. Characterisation of the human voltage-gated potassium channel gene, KCNA7, a candidate gene for inherited cardiac disorders, and its exclusion as cause of progressive familial heart block I (PFHBI). *Eur J Hum Genet* **10**:36-43.
4. Gregory, K.N., et al. 2006. Histidine-rich Ca binding protein: a regulator of sarcoplasmic reticulum calcium sequestration and cardiac function. *J Mol Cell Cardiol* **40**:653-665.
5. Heidecker, B., et al. 2008. Transcriptomic biomarkers for individual risk assessment in new-onset heart failure. *Circulation* **118**:238-246.
6. Kaneko, K.J., Rein, T., Guo, Z.S., Latham, K., and DePamphilis, M.L. 2004. DNA methylation may restrict but does not determine differential gene expression at the Sgy/Tead2 locus during mouse development. *Mol Cell Biol* **24**:1968-1982.
7. Ishihara, H., et al. 1989. Calyculin A and okadaic acid: inhibitors of protein phosphatase activity. *Biochem Biophys Res Commun* **159**:871-877.
8. Weston, M.C., Anderson, N., and Peachell, P.T. 1997. Effects of phosphodiesterase inhibitors on human lung mast cell and basophil function. *Br J Pharmacol* **121**:287-295.

Supplemental Table 1

Clinical presentation of mutation carriers. The table represents a compilation of the conduction states of the mutation carriers, stratified by sex. "ECGs available" represents the number of individuals for whom AV conduction was intact or where an ECG prior to onset of complete heart block or pacemaker implantation was available (note: less than the total number of mutation carriers as ECGs were not available for all). We were not privy to the indications for all pacemaker implants. Data from an ECG most proximate to the date of pacemaker insertion is shown. Heart rate is presented as the mean beats per minute with standard deviation. RBBB associated with a LAHB or LPHB have been combined into one column as bifascicular block. Of these, only one person had a LPHB. Note that a number of mutation carriers did not have evidence of a conduction defect.

Male and female were compared for significant differences, Chi^2 statistics for categorical variables and Students T-test for continuous variables (SPSS 15). * = statistical significant differences $P < 0.05$, PM = Pacemaker, ECG = electrocardiogram, n = number, HR = heart rate, bpm = beats/minute, ms = milliseconds, V1/2 and V5/6 = measurement in ECG lead V1 or V2 and V5 or V6 whichever is the wider, ˚ = degrees, QTc = QT interval corrected for heart rate with Bazett's formula, RBBB = right bundle branch block, LAHB/LPHB = left anterior or posterior hemi block.

INDIVIDUALS	Mutation carriers (n)	Pacemaker implanted (PM)	Proportion with PM (%)	Age at first PM implantation	ECGs available (n=)	HR (bpm)	Mean P-wave duration (ms)	Mean PR interval (ms)	Mean QRS duration in V1/2 (ms) *	Mean QRS duration in V5/6 (ms)	QTc interval > 440 ms (n=)	QRS axis (˚)	RBBB	Bifascicular Block	NORMAL ECG
All	71	48	68	29+/-19.5	34	66.3+/-17.9	77+/-22	153+/-28	134+/-31	133+/-30	6	57+/-50.3	19	8	7
Female	32	20	63	28+/-18.9	17	65.1+/-14.4	81+/-10	151+/-16	124+/-28	121+/-26	2	61+/-41.8	9	3	5
Male	39	28	72	30+/-20.3	17	67.3+/-20.8	74+/-24	155+/-35	143+/-31	143+/-31	4	53+/-58.6	10	5	2

Supplemental Table 2

Primer for *TRPM4b* amplification and genomic sequencing. Primer sequences for amplification of exons of *TRPM4*. *TRPM4* is located on human chromosome 19 at 54.35 MB. Primer name suffices: -up, forward; -low, reverse; -seq.: additional sequencing primer.

TRPM4b Exon	Primer (PCR, Sequencing)	Sequence	Amplicon Size (bp)
1+2	TRPM4_Ex01+02-up	CTCTGGGCGGGTCTGGAAGC	662
	TRPM4_Ex01+02-low	CGAATGGGGAATGAGGGGTGTCTA	
3	TRPM4_Ex03-up	GTCGACAGCCCCCTCTACAG	347
	TRPM4_Ex03-low	GGCGTCCCGGCTCCACT	
4	TRPM4_Ex04-up	GGTCTCTGTCCCCCTCCCTGTG	430
	TRPM4_Ex04-low	GCCGATGCCCGTGTGC	
	TRPM4_Ex04seq-low	CACACTGGGCGGCGGGGG	
5+6	TRPM4_Ex05+06-up	CCCCGCCGCCCAGTGTG	661
	TRPM4_Ex05+06-low	TTCCCCAAATCTCGACCAGACCAA	
	TRPM4_Ex06seq-up	CTGGGGTGTGGTCCGGAATAGAGA	
7+8+9	TRPM4_Ex07-09-up	GCAAAGGCTGATGGGAGGTAA	1367
	TRPM4_Ex07-09-low	GGAATTTTCGGTGGATGTGTAGAT	
	TRPM4_Ex08seq-low	CCCGGAACCTGGAAGTC	
	TRPM4_Ex09seq-low	GCATGGGCCTTGGATTAGAGA	
10	TRPM4_Ex10-up	CACCCGGGCCTTTGCTTCTG	1385
	TRPM4_Ex10-low	GGCAGGGTTGAGGGGCGTCTAAGA	
	TRPM4_Ex10seq-up	GCCCAGTAGCAACCCCTTTAT	
11+12	TRPM4_Ex11+12-up2	TCATACCCTTGCCCATCTCTTGTC	1856
	TRPM4_Ex11+12-low2	AGGGGTCGGGGAATTGTATGGTAA	
	TRPM4_Ex11seq-low2	CAGCAAAAGGAGGGGTGGTGA	
	TRPM4_Ex12-up	GAAGGACCTGGGGGCGGGATTACA	
13+14	TRPM4_Ex13+14-up	TCCTTGGCCTCTGATGACCCCAGTTA	1639
	TRPM4_Ex13+14-low	CTAAGACGCCCAGCAAGGGTTGGACT	
	TRPM4_Ex14seq-low	AGGGAGAGTGGCTGGTCAAG	
15+16	TRPM4_Ex15+16-up	GTGTTTCTTTGGGCCGTTTCCTTA	1165
	TRPM4_Ex15+16-low	ACTGGCTCCTCCCTCACTTTCTGC	
	TRPM4_Ex16seq-up	CCTCCCTGTGGTATCTCTAGTG	
17	TRPM4_Ex17-up	GATGTGCCAAGCCAAAAGTCTCG	697
	TRPM4_Ex17-low	TTACCCTGGGGCAATCGTCAT	
18+19	TRPM4_Ex18+19-up	CTGAAGGATGGCATTGGGAAGACT	757
	TRPM4_Ex18+19-low	AGAGGGGGTGGGGTAGGCAGAGA	
20	TRPM4_Ex20-up	ACCGCGCCCGGCAGTCTT	388
	TRPM4_Ex20-low	TGGGGAGAAGGCAGCATTAGATTG	
21	TRPM4_Ex21-up	TGACCCTTGGCCCTTCTAAACTGA	809
	TRPM4_Ex21-low	AAACCCCGCCCCTCGCTACCTAAG	
22+23	TRPM4_Ex22+23-up	AACGGGCGTGGCTTAGGTAG	671
	TRPM4_Ex22+23-low	CTGCGGAATGGGAGGAATGT	
24	TRPM4_Ex24-up	GACACATCCGCGAGTACGAACAGC	432
	TRPM4_Ex24-low	AGAGCAGGTGATTGGCAGGGAAGG	
25	TRPM4_Ex25-up	GTTCCTGTATTTTTGCGTGTTTTT	397
	TRPM4_Ex25-low	AGGCTGGGACTGGTTCTGG	