Supplemental Data

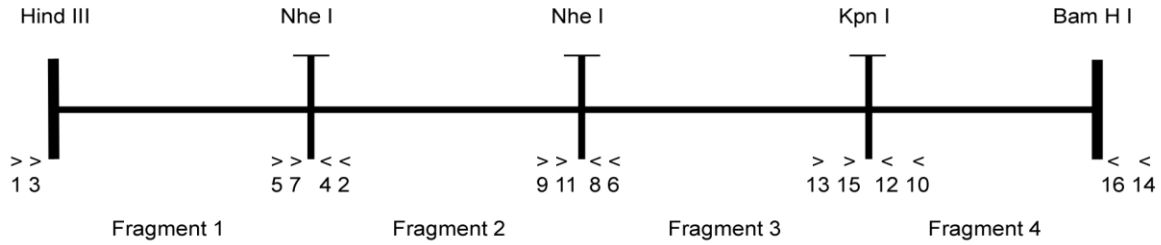
Informed Consent. This study was approved by the Institutional Review Board of the Yale University School of Medicine.

Sanger Sequencing. The exon–intron boundaries of the *FAM38A* gene were determined using the University of California, Santa Cruz (UCSC) Genome Browser (http://genome.ucsc.edu). Exon-flanking primer pairs flanking exons of interest were designed with Primer 3 software (http://primer3.sourceforge.net/webif.php)(Table 1). These coding exons and flanking DNA were amplified from patient genomic DNA by polymerase chain reaction (PCR). PCR amplicons were subjected to capillary-based nucleotide sequence analysis.

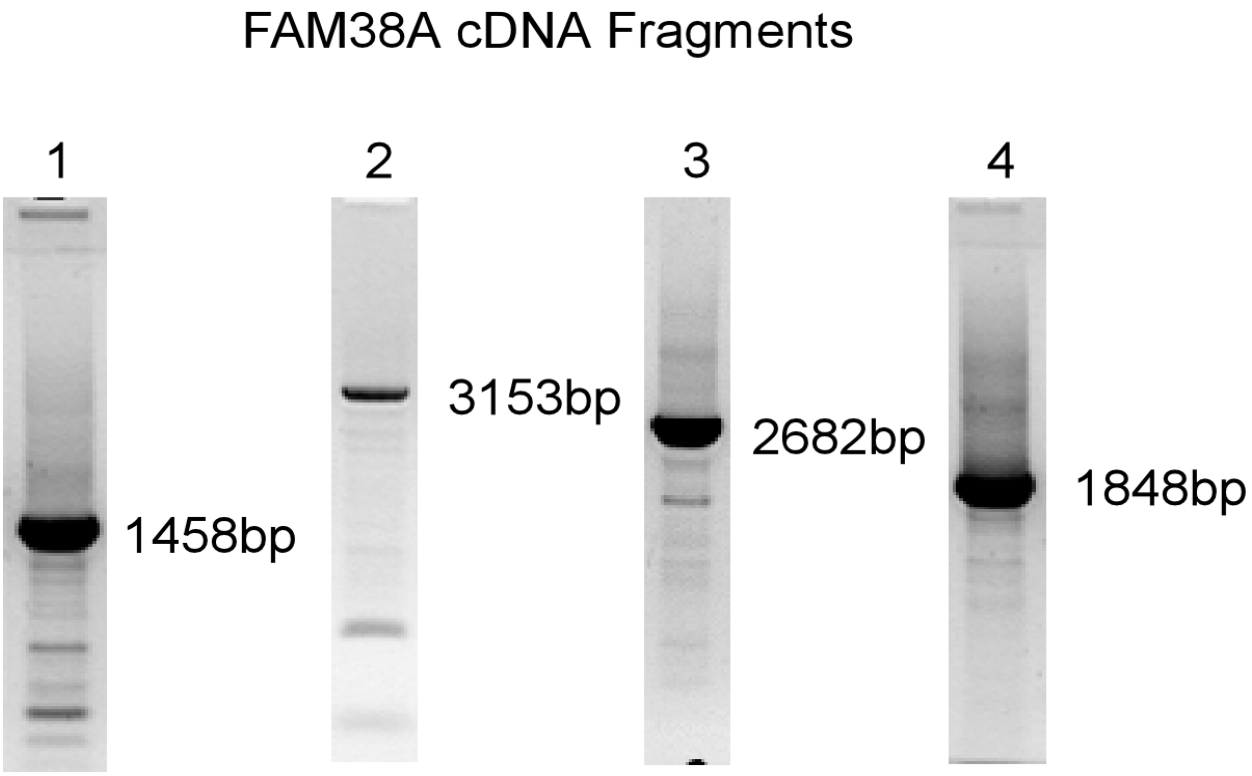
Discovery Proteomics. Materials. Urea, Tris-HCl, $CaCl_2$, iodoacetamide (IAA) were from Sigma-Aldrich (St. Louis, MO). Chloroform and Dithiothretitol (DTT) were from American Bioanalytical (Natick, MA). Methanol, acetonitrile (ACN), trifluoroacetic acid (TFA), formic acid (FA) and HPLC grade water were obtained from Burdick and Jackson (Morristown, NH). Sequencing grade modified trypsin was from Promega (Madison,WI). UltraMicroSpin columns (C_{18}) were from The Nest Group Inc. (Southborough, MA).

Supplemental Figure S1. Identification of *FAM38A* mRNA in human erythroid cell RNA. mRNA was prepared from cultured, primary human erythroid progenitor cells, cDNA prepared by reverse transcription, then amplified with primers tiled across the *FAM38A* locus to amplify four cDNA fragments. The initial PCR reaction utilized the outside, flanking primers, with the second PCR reaction utilizing the internal primers. The four *FAM38A* cDNA fragments were analyzed by agarose gel electrophoresis on ethidium stained gels. These fragments were subcloned and sequenced. The overlapping cDNA fragments corresponded to the human full length *FAM38A* cDNA (GI:257196141). A. Location of primers in the *FAM38A* cDNA. The sequences of these primers are provided in Supplemental Table S1. B. The *FAM38A* cDNA fragments amplified by RT-PCR from human erythroid cell mRNA.

Supplemental Figure S1A.



Supplemental Figure S1B.



Supplemental Figure S2. Amino acid sequence of human PIEZO1 and location of peptides identified in human erythrocyte membrane ghosts by mass spectrometry. The location of peptides identified in wild type erythrocyte membrane ghosts are shown in red.

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   1 MEPHVLGAVL YWLLLPCALL AACLLRFSGL SLVYLLFLLL LPWFPGPTRC
  51 GLQGHTGRLL RALLGLSLLF LVAHLALQIC LHIVPRLDQL LGPSCSRWET
 101 LSRHIGVTRL DLKDIPNAIR LVAPDLGILV VSSVCLGICG RLARNTRQSP
 151 HPRELDDDER DVDASPTAGL QEAATLAPTR RSRLAARFRV TAHWLLVAAG
 201 RVLAVTLLAL AGIAHPSALS SVYLLLFLAL CTWWACHFPI STRGFSRLCV
 251 AVGCFGAGHL ICLYCYQMPL AQALLPPAGI WARVLGLKDF VGPTNCSSPH
 301 ALVLNTGLDW PVYASPGVLL LLCYATASLR KLRAYRPSGQ RKEAAKGYEA
 351 RELELAELDQ WPQERESDQH VVPTAPDTEA DNCIVHELTG QSSVLRRPVR
 401 PKRAEPREAS PLHSLGHLIM DQSYVCALIA MMVWSITYHS WLTFVLLLWA
 451 CLIWTVRSRH QLAMLCSPCI LLYGMTLCCL RYVWAMDLRP ELPTTLGPVS
 501 LRQLGLEHTR YPCLDLGAML LYTLTFWLLL RQFVKEKLLK WAESPAALTE
 551 VTVADTEPTR TQTLLQSLGE LVKGVYAKYW IYVCAGMFIV VSFAGRLVVY
 601 KIVYMFLFLL CLTLFQVYYS LWRKLLKAFW WLVVAYTMLV LIAVYTFQFQ
 651 DFPAYWRNLT GFTDEQLGDL GLEQFSVSEL FSSILVPGFF LLACILQLHY
 701 FHRPFMQLTD MEHVSLPGTR LPRWAHRQDA VSGTPLLREE QQEHQQQQQE
 751 EEEEEEDSRD EGLGVATPHQ ATQVPEGAAK WGLVAERLLE LAAGFSDVLS
 801 RVQVFLRRLL ELHVFKLVAL YTVWVALKEV SVMNLLLVVL WAFALPYPRF
 851 RPMASCLSTV WTCVIIVCKM LYQLKVVNPQ EYSSNCTEPF PNSTNLLPTE
 901 ISQSLLYRGP VDPANWFGVR KGFPNLGYIQ NHLQVLLLLV FEAIVYRRQE
 951 HYRRQHQLAP LPAQAVFASG TRQQLDQDLL GCLKYFINFF FYKFGLEICF
1001 LMAVNVIGQR MNFLVTLHGC WLVAILTRRH RQAIARLWPN YCLFLALFLL
1051 YQYLLCLGMP PALCIDYPWR WSRAVPMNSA LIKWLYLPDF FRAPNSTNLI
1101 SDFLLLLCAS QQWQVFSAER TEEWQRMAGV NTDRLEPLRG EPNPVPNFIH
1151 CRSYLDMLKV AVFRYLFWLV LVVVFVTGAT RISIFGLGYL LACFYLLLFG
1201 TALLQRDTRA RLVLWDCLIL YNVTVIISKN MLSLLACVFV EQMQTGFCWV
1251 IQLFSLVCTV KGYYDPKEMM DRDQDCLLPV EEAGIIWDSV CFFFLLLQRR
1301 VFLSHYYLHV RADLQATALL ASRGFALYNA ANLKSIDFHR RIEEKSLAQL
1351 KRQMERIRAK QEKHRQGRVD RSRPQDTLGP KDPGLEPGPD SPGGSSPPRR
1401 QWWRPWLDHA TVIHSGDYFL FESDSEEEEE AVPEDPRPSA QSAFQLAYQA
1451 WVTNAQAVLR RRQQEQEQAR QEQAGQLPTG GGPSQEVEPA EGPEEAAAGR
1501 SHVVQRVLST AQFLWMLGQA LVDELTRWLQ EFTRHHGTMS DVLRAERYLL
1551 TQELLQGGEV HRGVLDQLYT SQAEATLPGP TEAPNAPSTV SSGLGAEEPL
1601 SSMTDDMGSP LSTGYHTRSG SEEAVTDPGE REAGASLYQG LMRTASELLL
1651 DRRLRIPELE EAELFAEGQG RALRLLRAVY QCVAAHSELL CYFIIILNHM
1701 VTASAGSLVL PVLVFLWAML SIPRPSKRFW MTAIVFTEIA VVVKYLFQFG
1751 FFPWNSHVVL RRYENKPYFP PRILGLEKTD GYIKYDLVQL MALFFHRSQL
1801 LCYGLWDHEE DSPSKEHDKS GEEEQGAEEG PGVPAATTED HIQVEARVGP
1851 TDGTPEPQVE LRPRDTRRIS LRFRRRKKEG PARKGAAAIE AEDREEEEGE
1901 EEKEAPTGRE KRPSRSGGRV RAAGRRLQGF CLSLAQGTYR PLRRFFHDIL
1951 HTKYRAATDV YALMFLADVV DFIIIIFGFW AFGKHSAATD ITSSLSDDQV
2001 PEAFLVMLLI QFSTMVVDRA LYLRKTVLGK LAFQVALVLA IHLWMFFILP
2051 AVTERMFNQN VVAQLWYFVK CIYFALSAYQ IRCGYPTRIL GNFLTKKYNH
2101 LNLFLFQGFR LVPFLVELRA VMDWVWTDTT LSLSSWMCVE DIYANIFIIK
2151 CSRETEKKYP QPKGQKKKKI VKYGMGGLII LFLIAIIWFP LLFMSLVRSV
2201 VGVVNQPIDV TVTLKLGGYE PLFTMSAQQP SIIPFTAQAY EELSRQFDPQ
2251 PLAMQFISQY SPEDIVTAQI EGSSGALWRI SPPSRAQMKR ELYNGTADIT
2301 LRFTWNFQRD LAKGGTVEYA NEKHMLALAP NSTARRQLAS LLEGTSDQSV
2351 VIPNLFPKYI RAPNGPEANP VKQLQPNEEA DYLGVRIQLR REQGAGATGF
2401 LEWWVIELQE CRTDCNLLPM VIFSDKVSPP SLGFLAGYGI MGLYVSIVLV
2451 IGKFVRGFFS EISHSIMFEE LPCVDRILKL CQDIFLVRET RELELEEELY
2501 AKLIFLYRSP ETMIKWTREK E
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Supplemental Tables

Supplemental Table S1. *Primers for amplification across the FAM38A cDNA*.

Primer 1	TATAAAGCTTCCGAAGGAGAAGGAGGAAGA	Hind III linker.
Primer 2	TATAGGATCCGCTCTGGTCCATGATGAGGT	Bam HI linker.
Primer 3	TATAAAGCTTCCAGCCATGGAGCCGCACGT	Hind III linker
Primer 4	TATAGGATCCGCAGTTATCAGCCTCGGTGT	Bam HI linker
Primer 5	TATAAAGCTTCTAGGGTGCTGGGTCTCAAG	Hind III linker.
Primer 6	TATAGGATCCTAGCTGTCCTGCCTGTTCCT	Bam HI linker.
Primer 7	TATAAAGCTTCTGGACTGGCCTGTGTATGC	Hind III linker
Primer 8	TATAGGATCCACGGATACGCTCCATCTGTC	Bam HI linker
Primer 9	TATAAAGCTTGTGGGACTGCCTCATTCTGT	Hind III linker.
Primer 10	TATAGGATCCCTGGAAGAGGAAGAGGTT	Bam HI linker.
Primer 11	TATAAAGCTTAAGAACATGCTGTCGCTCCT	Hind III linker
Primer 12	TATAGGATCCACAGGGCGAAGTAGATGCAC	Bam HI linker
Primer 13	TATAAAGCTTCATGTTCCTGGCTGATGTTG	Hind III linker.
Primer 14	TATAGGATCCGCAGTGTCCTTCTCTGACA	Bam HI linker.
Primer 15	TATAAAGCTTACGTCCTCCCTATCAGACGA	Hind III linker
Primer 16	TATAGGATCCTCTCTGACAGCAGCATCAGG	Bam HI linker

Supplemental Table S2. *Primers flanking exons 46 and 51 of the human FAM38A gene*.

Exon 46	tgtaaaacgacggccagtGCCAGCTGGGTACAAGTGAC
Exon 46	caggaaacagctatgaccAGAATGCGGTTGTGTGACC
Exon 51	tgtaaaacgacggccagTGCCCATGGTCATTTTCAGT
Exon 51	caggaaacagctatgacCGGGAGGATGCATCACAG

Supplemental Table S3. Coverage across the *FAM 38A* locus in patient exome samples.

Sample:	HA-006	HA-032	HA-128	HA-135	HA-136	M1-24	M1-38	M1-39	M1-40	
Source Location:	Manitoba	Manitoba	Manitoba	Manitoba	Manitoba	New York	New York	New York	New York	
Xerocytosis:	+	+	+	-	-	+	+	+	+	
Total Reads	207,969,448	182,763,722	221,020,790	198,350,788	190,200,202	76,573,978	74,589,346	68,595,106	75,324,274	
Non duplicated Reads	143,326,370	137,773,325	170,214,149	155,290,377	152,454,208	72,721,380	68,878,929	64,516,906	70,917,040	
Mapped Reads	133,994,834	129,220,739	159,566,254	145,781,211	142,780,790	67,444,842	64,455,562	59,940,234	65,954,551	
Mapped Bases	9,889,997,216	9,544,514,665	11,779,921,597	10,765,779,161	10,545,271,824	4,977,902,513	4,755,045,595	4,421,276,255	4,863,508,180	
On Target Bases	4,511,414,200	4,895,080,184	4,414,613,257	3,937,459,838	3,979,840,555	1,940,838,879	1,863,597,226	2,310,563,920	1,983,913,273	
Mean Base Coverage	126.904164	137.255354	169.305243	151.077232	152.665215	74.66408	71.760007	64.893513	76.396277	
No Coverage Bases	1.7%	1.4%	1.4%	1.4%	1.4%	1.7%	1.8%	1.6%	1.7%	
>= 2 Coverage Bases	96.5%	97.2%	97.4%	97.5%	97.6%	96.9%	96.8%	96.7%	96.8%	
>= 10 Coverage Bases	92.9%	94.8%	94.7%	95.3%	95.3%	92.6%	92.9%	92.1%	93.0%	
>= 20 Coverage Bases	89.0%	92.0%	92.1%	92.9%	93.0%	86.2%	87.0%	84.9%	87.2%	87.3%