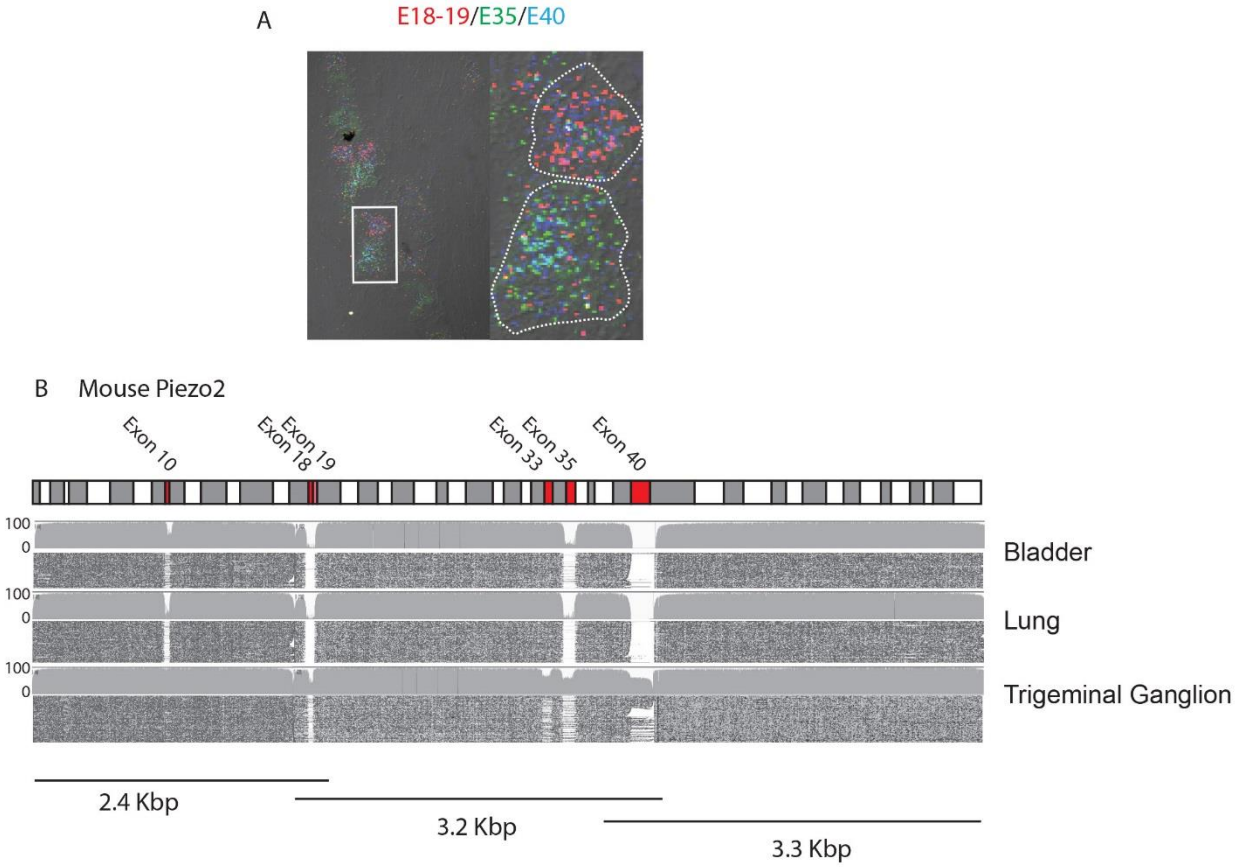

Figure S1 Somatosensory neurons express alternatively spliced Piezo2, related to Figure 2. A, ISH reveals that TG neurons differentially express exons 18-19 (red), exon 35 (green), and exon 40 (blue). Left panel shows a field of TG neurons and the right panel is a magnification of the boxed area which contains two neurons with dramatically different patterns of expression of alternatively spliced exons. The upper boxed neuron expresses higher levels of E18-19 and less E35 and E40, and the lower neuron expresses more E35 and E40, and less E18-19. B, Sequence reads from TG, lung and bladder are aligned with the complete coding sequence of mouse Piezo2 (exons 1-56; top); the positions of alternatively spliced exon are highlighted in red. The complete coding sequence of Piezo2 was sequenced by independently sequencing three PCR amplified long overlapping fragments; the sequences from these three fragments have been overlapped in this representation. The sizes and positions of PCR fragments are illustrated in the scheme at the bottom (see methods for details). Note, the region in the middle of Piezo2 contains all the alternatively spliced exons that are present in mouse TG and therefore, these alternatively spliced exons were the focus of our work.

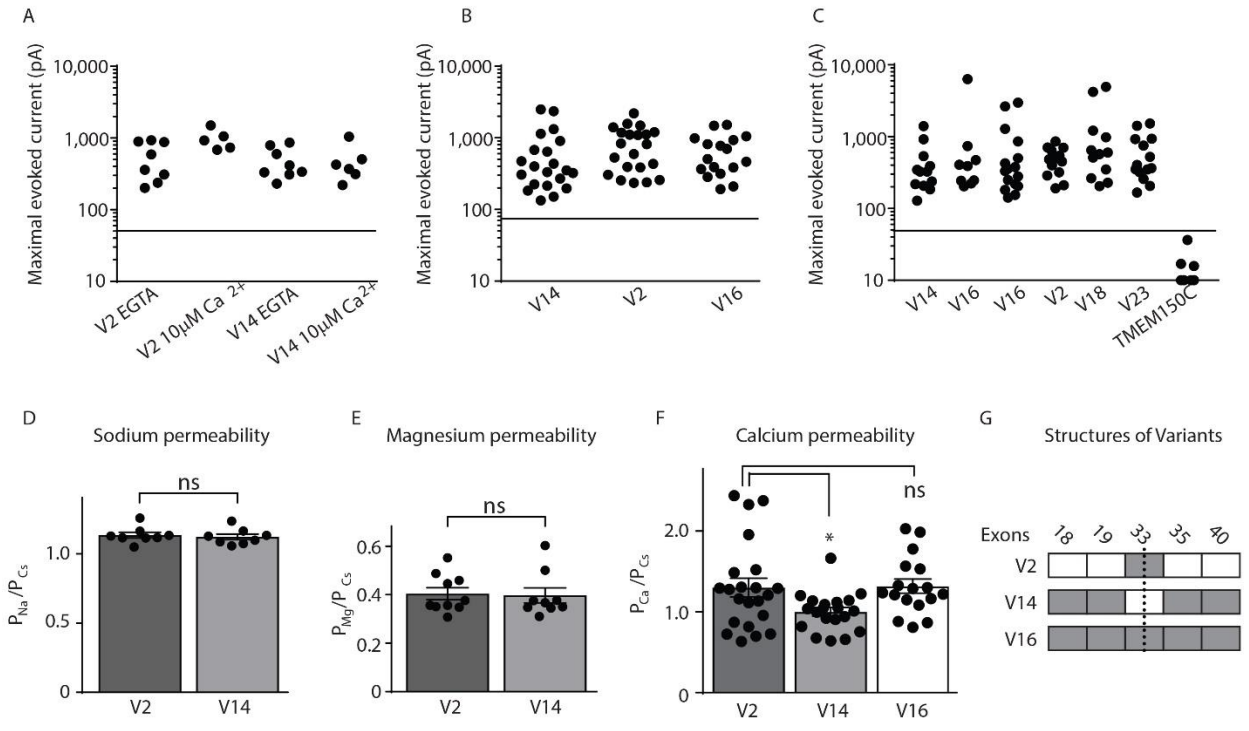

Figure S2 Splice variants of Piezo2 produce robust mechanically activated currents and exhibit different calcium permeability, related to Figures 4 and 5.

To ensure that artefactual currents from Piezo1 in HEK293-cells did not contaminate our recordings, we set threshold cut-offs for inclusion. Since we used a holding voltage of -40 mV (unless stated otherwise), not -80 mV at which large endogenous currents up to 100 pA were recorded previously (Coste et al., 2010; Dubin et al., 2017a, b), we set our cut-off threshold for inclusion of currents to those with maximal magnitudes greater than 50 pA. A, in experiments of calcium induced receptor sensitization (Figure 4C-D), the approximate average maximum current was 600 pA. B, for calcium permeability recordings (Figure 4 A-B), cells we poked with the same indentation over a range of holding voltages (only responses closest to the reversal potential were used to calculate permeability). At the most negative voltage tested (nominally -60 mV), the mean currents were approximately 700 pA (the inclusion threshold for currents was >75 pA). C, in the experiments investigating kinetics of Piezo2 splice variants (Figure 5) smaller amounts of plasmid was used (400 ng instead of 1000 ng) to limit responses (currents above 2 nA were deemed unreliable for kinetic analysis). At larger indentations, mean maximal evoked current ranged from over 400 pA to more than 1 nA. As previously reported, few HEK293-cells

transfected with TMEM150C displayed small currents (Coste et al., 2010; Dubin et al., 2017a, b). The Piezo2 variants V2 and V14 have similar permeability for sodium (D) and magnesium (E). Data are means ±SEM (n=8 for sodium permeability and n=9 for magnesium permeability); no significant difference in permeability between splice variants (Student's t-test). F, The higher calcium permeability of V2 (contain E33, but not contain E18, E19, E35, and E40) and V8 (contains E33, E35, and E40, but lacks E18 and E19) relative to the lower calcium permeability of V14 (contains all spliced exons except E33) suggests that the presence of E33 confers higher calcium permeability. Data represent means ±SEM (n=19 for V2, n=16 for V14, and n=10 for V8); significant difference in calcium permeability between variants * (one-way ANOVA with Dunnett's multiple comparison test) V2 vs V14 p= 0.0387 and V2 vs V16 p= 0.985. G, Schematic of the exon usage of the indicate splice variants.

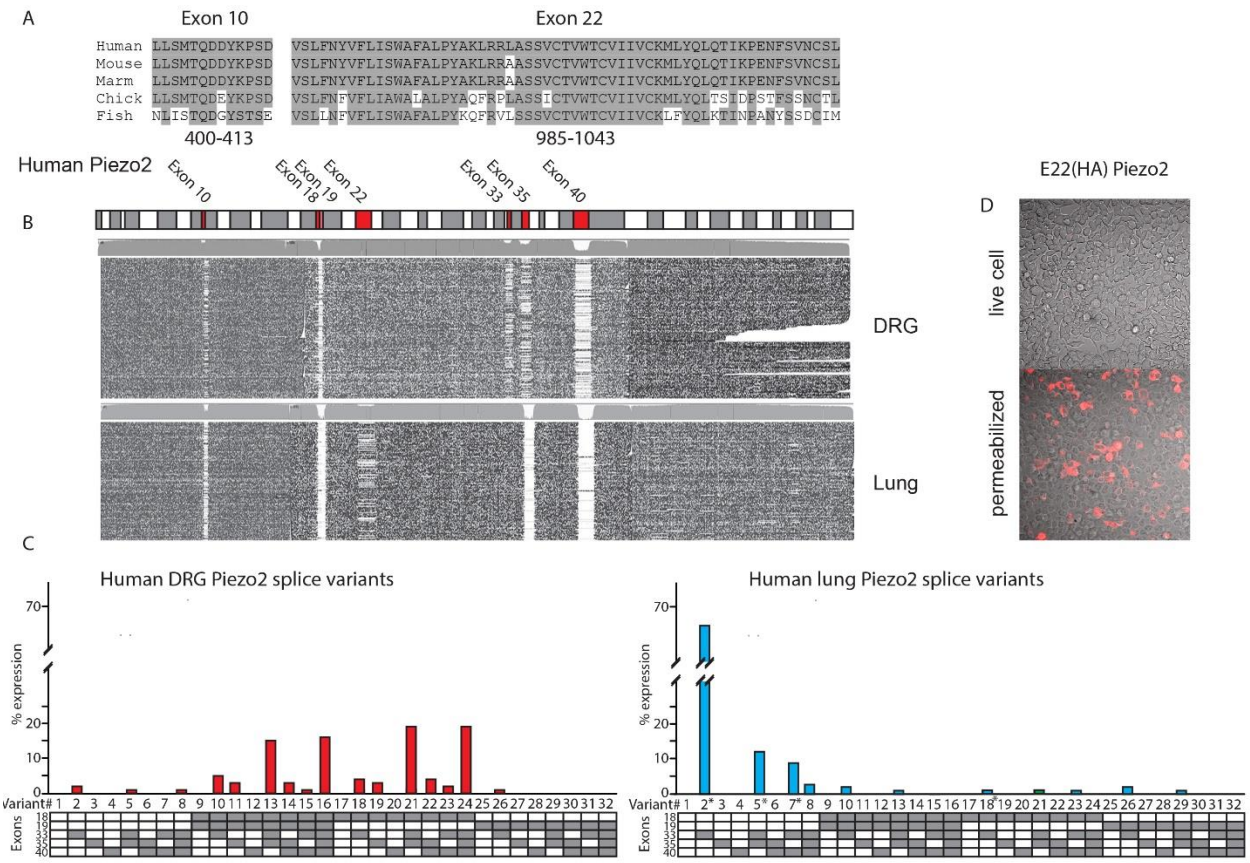

Figure S3 Alternative splicing of Piezo2 is a conserved process, related to Figure 7.

A, Alignment of the coding sequence of alternatively spliced exons 10 and 22 show their high level of sequence similarity. Sequences of alternatively spliced exons of Piezo2 from various species are compared; amino-acids identical to those of human Piezo2 are shaded grey. Numbering below sequence refers to the position in the V16 mouse Piezo2 sequence. Exon22 is only alternatively spliced in human Piezo2 transcripts. B, Sequencing of human Piezo2 transcripts reveals that seven exons are alternatively spliced. Exons 1-56 are colored in alternating grey and open blocks with alternatively spliced exons highlighted in red. Sequence reads from DRG and from lung are displayed as plots, black dots indicate sequence identity and open areas represents regions of sequence lacking similarity. C, Analysis of reads from DRG (left panel; red bars) showed that 16 variants are expressed at levels ≥1%, while lung (right panel; blue bars) expresses one predominant forms of Piezo2, named variants 2. Lower panel is a schematic of the numbering system, based on the five alternately splice exons E18, E19, E33, E35, and E40, used to name variants. * indicates that some human lung Piezo2 transcripts lack exon 22. D, Fields of HEK-cells transfected with HA-epitope tagged E22 (HA) Piezo2 construct

immune-stained for HA live (upper panels) and following permeablization (lower panels). The HA epitope was engineered into the sequences coding for exon 22 and was expressed in HEK293 cells. HA epitope was detected (red stained cells) only after membrane disruption in cells expressing confirming the intracellular location of sequences encoded by this exon.

Table S1, Mouse Piezo2 central region splice variants, related to Figures 2 and 6.

Variant #	Exon 18	Exon 19	Exon 33	Exon 35	Exon 40	Mouse TG Fraction	Mouse lung Fraction	Mouse bladder Fraction	Mouse Merkel cell Fraction
1									
2			■			0.02	0.75	0.64	0.24
3				■					
4					■				
5			■	■		0.01	0.19	0.26	0.61
6				■	■				
7			■		■	0.01	0.02	0.02	
8			■	■	■	0.01	0.01	0.02	0.02
9	■	■							
10	■	■	■			0.03	0.01	0.01	
11	■	■		■		0.05			
12	■	■			■	0.01			
13	■	■	■	■		0.04	0.01	0.01	0.01
14	■	■		■	■	0.09			0.01
15	■	■	■		■	0.07			0.01
16	■	■	■	■	■	0.20			0.02
17	■								
18	■		■			0.05	0.01	0.02	0.01
19	■			■		0.04			0.01
20	■				■				
21	■		■	■		0.06		0.01	0.02
22	■			■	■	0.06			
23	■		■			0.12			0.01
24	■		■	■	■	0.14			0.01
25		■							
26		■	■				0.01	0.01	
27		■		■					
28		■			■				
29		■	■	■					0.01
30		■		■	■				
31		■	■		■				
32		■	■	■	■				
					Total reads	1721	4800	2373	2615

Only full-length reads were aligned and only reads >1% of the total are included.

Table S2 Cation permeabilities (reversal potentials), related to Figure 4.

	Cation:	**Calcium**	
Variant*	**mV**	**SEM**	**n**
V2	**7.76**	**1.37**	**22**
V14	**3.97**	**0.9**	**20**
V16	**9.83**	**1.29**	**10**
	Cation:	**Sodium**	
Variant**	**mV**	**SEM**	**n**
V2	**2.84**	**0.303**	**8**
V14	**2.93**	**0.456**	**8**
	Cation:	**Magnesium**	
Variant***	**mV**	**SEM**	**n**
V2	**-18.2**	**3.57**	**11**
V14	**-17.27**	**1.54**	**9**

*V2 versus V14 p=0.0363, and V2 versus V16 p=0.7792 (Dunnett multiple comparison).
**V2 versus V14 p=0.87 (Student's t-test).
***V2 versus V14 p=0.77 (Student's t-test).

Table S3: human Piezo2 central region splice variants, related to Figure 7.

Variant #	Exon 18	Exon 19	Exon 33	Exon 35	Exon 40	Human DRG Fraction	Human lung Fraction
1							
2			■			0.02	0.50 (2* = 0.13)
3				■			
4					■		
5			■	■		0.01	0.11 (5*= 0.01)
6				■	■		
7			■		■		0.09 (7* =0.02)
8			■	■	■	0.01	0.03
9	■	■					
10	■	■	■			0.05	0.02
11	■	■		■		0.03	
12	■	■			■		
13	■	■	■	■		0.15	0.01
14	■	■		■	■	0.03	
15	■	■	■		■	0.01	
16	■	■	■	■	■	0.16	
17	■						
18	■		■			0.04	18* = 0.01
19	■			■		0.03	
20	■				■		
21	■		■	■		0.19	0.01
22	■			■	■	0.04	
23	■		■			0.02	0.01
24	■		■	■	■	0.19	
25		■					
26		■	■			0.01	0.02
27		■		■			
28		■			■		
29		■	■	■			0.01
30		■		■	■		
31		■	■		■		
32		■	■	■	■		
					Total reads	2750	4729

Only full-length reads were aligned and only reads >1% of the total are included. * indicates that exon 22 is absent.

Table S4: Barcoded primers for amplification of Piezo2 fragments, related to Figure 2, 6, and 7.

Species and tissue	Fragment amplified	Primer sequence - Forward	Primer sequence - Reverse
Mouse bladder	5’	CATCACGCATGGCTTCGGAAGTGGTGTGCGGG	GCGTGATGAGTTCAGCGACCGTGAACTGCTTC
Mouse bladder	middle	CATCACGCCCTCCTTTCTACTCGTCTGCATCC	GCGTGATGTTCCTTGGCGCGAATACAGCATGG
Mouse bladder	3’	CATCACGCTATCCACAGTCCTGCGGATTGAAAG	GCGTGATGTCAGTTTGTTTTTTCTCTAGTCCACTTG
Mouse lung	5’	GTCGTATCATGGCTTCGGAAGTGGTGTGCGGG	CAGCATAGAGTTCAGCGACCGTGAACTGCTTC
Mouse lung	middle	GTCGTATCCCTCCTTTCTACTCGTCTGCATCC	CAGCATAGTTCCTTGGCGCGAATACAGCATGG
Mouse lung	3’	GTCGTATCTATCCACAGTCCTGCGGATTGAAAG	CAGCATAGTCAGTTTGTTTTTTCTCTAGTCCACTTG
Mouse TG	5’	TGATGTCCATGGCTTCGGAAGTGGTGTGCGGG	GGACATCAAGTTCAGCGACCGTGAACTGCTTC
Mouse TG	middle	TGATGTCCCCTCCTTTCTACTCGTCTGCATCC	GGACATCATTCCTTGGCGCGAATACAGCATGG
Mouse TG	3’	TGATGTCCTATCCACAGTCCTGCGGATTGAAAG	GGACATCATCAGTTTGTTTTTTCTCTAGTCCACTTG
Mouse Merkel cell	5’	ATGGCTTCGGAAGTGGTGTGCGGG	AGTTCAGCGACCGTGAACTGCTTC
Mouse Merkel cell	middle	CCTCCTTTCTACTCGTCTGCATCC	TTCCTTGGCGCGAATACAGCATGG
Mouse Merkel cell	3’	TATCCACAGTCCTGCGGATTGAAAG	TGCGGCCGCTCAGTTTGTTTTTTCTCTAGTCCACTTG
Human lung	5’	TCAGACGATGCGTCATATGGCCTCAGAAGTGGTGTGCGGG	GCAGAGTCATGTATAGAGTTCAGCCACAGTAAACTGCTTT
Human lung	middle	ACAGTCTATACTGCTGCCCAACCTCCTTTCTGCTGGTGTGCA	AGATGTAGCACATCATTCCCCTGGCGTGAGTACAGCATGG
Human lung	3’	ACACTGACGTCGCGACGGCTTAACTCCATTTCAAGGGAGC	TATACGTATATAGACGGATCAAATGGACTAGAGAAAAAACAAATTGA
Human DRG	5’	TACTAGAGTAGCACTCATGGCCTCAGAAGTGGTGTGCGGG	CATGTACTGATACACAAGTTCAGCCACAGTAAACTGCTTT
Human DRG	middle	CTGCGTGCTCTACGACCCCAACCTCCTTTCTGCTGGTGTGCA	AGTCATCGTATCGCGCTCCCCTGGCGTGAGTACAGCATGG
Human DRG	3’	ATAGAGACTCAGAGCTGGCTTAACTCCATTTCAAGGGAGC	TCTACTCTCGCATCTAGATCAAATGGACTAGAGAAAAAACAAATTGA

Table S5: Numbers of SMRT sequences aligned, related to Figure 2, 6, and 7.

Location	Human	Mouse
Mapped to 5’ fragment	41,434	10,951
Mapped to middle fragment	24,269	27,545
Mapped to 3’ fragment	38,337	12,561