

## Supplementary Material

# Molecular basis for the sensitivity of TRP channels to polyunsaturated fatty acids

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**Supplementary Table 1** Oligonucleotides used for targeting mutagenesis of TRPL (multiple mutants)

<b>Mutation</b>	<b>Primer</b>	<b>Sequence</b>
TRPL-GQ367-386AK	Sense	atgtgcccccgaattgccggacggCcAagttgatgcgcaagccctttatgaa
TRPL-GQ367-386AK	Antisense	ttcataaagggttcgcatcaactTgGccgtccggcaattcggggcgcacat
TRPL-VIG438IIA	Sense	ctcatcgtggtgatgtacAtCattgCgttcgtgtgggaggaggt
TRPL-VIG438IIA	Antisense	acctctcccacacgaacGcaatGaTgtacatcaccacgatgag
TRPL-WEEV443FEEL	Sense	acgtgattgggttcgtgTCgaggagCtCcaggagatatttggcgtg
TRPL-WEEV443FEEL	Antisense	cacggcaaatatctctgGaGctcctcGAaacgaaccaatcacgt
TRPL-LRNM458IRNL	Sense	atttggcgtgggatgaagagttatAtCcgGaacCtgtggaactcatcgactttctgc
TRPL-LRNM458IRNL	Antisense	gcagaaagtcgatgaagttccacaGgttCcgGaTataactcttcatcccacggcaat
TRPL- IDFLRNSL465VDYLRNMF	Sense	tatttgcgaacatgtggaactcGtcgactAtctgcgcaacaTGTtctactgtgagtgat gtgttaa
TRPL- IDFLRNSL465VDYLRNMF	Antisense	ttaaacacatcacactcacgtagaACAgttgcgcagaTagtccaCgaagttccacatgtt gcgcaata
TRPL-SL471MF	Sense	gaacttcatcgactttctCcgGaacaTGTtctactgtgagtgatgtgt
TRPL-SL471MF	Antisense	acacatcacactcacgtagaACAtgttCcgGagaaagtcgatgaagttc
TRPL-QQATEE486IQATD	Sense	tgtttaagagcctttgcctacatcATCcaggccacagaCatCgcGagggatccgcagat ggcttacata
TRPL-QQATEE486IQATD	Antisense	tatgtaagccatctcgggatccctCgcGatGtctgtggcctgGATgatgtaggcaaagg ctcttaaaca
TRPL-AN521GM	Sense	cgaaggtctctttgcggcCgGcaTGgtcttctcggccttgaag
TRPL-AN521GM	Antisense	cttcaaggccgagaagacCAtgCcGgcccaaaagaccttcg
TRPL-IAEGL513LAEGA	Sense	gattttgaccgcaactaCtagccgaaggCGCctttgcggcgccaatgt
TRPL-IAEGL513LAEGA	Antisense	acattggccgccgcaaagGCGccttcgggctaGtagttgcgggtcaaaaac
TRPL-RSK729SSM	Sense	gtctagcaagaccatcgategtcaaTCctccaTgaaacgaaaggagcaggagcagttta
TRPL-RSK729SSM	Antisense	aactgtcctcgtcctttcgtttcAtggagGAttgacgatcgatggtcttctgtagactt
TRPL-YDNI742HDNV	Sense	caggagcagtttagcgagCatgataaCGtTatgcgctccctggtttgg
TRPL-YDNI742HDNV	Antisense	ccaaccaggagcgcgatAaCGttatcatGctcgtaaactgctcctg
TRPL-KE760RD	Sense	tgccgccatgcatcgcaGgttcgaCaataatcccgtatcggag
TRPL-KE760RD	Antisense	ctccgatacgggattattGtcgaacCtgcgatgcatggcgcca
TRPL-SGM792NSW	Sense	atgctcgagatattcgagaacaAcAgCTGggatgttctcggccaacaaaagg
TRPL-SGM792NSW	Antisense	cccttttgtggccgaggaaacatccCAGcTgTgttctcgaatatctcgagcat
TRPL-LM818-819IL	Sense	cggegtatcaaagtgtgggaacggcgcAtTCtAaagggttccaggtggccagtgcc aaa
TRPL-LM818IL	Antisense	ttgcactggcggcacttgaagcccttTaGAATgcgccgttcccacactttgatacggc
TRPL-AP825GF	Sense	acggcgcttgatgaaggcctccaggtAgGATTcgtgcaaaatggctgtgagctcgat gcc
TRPL-AP825GF	Antisense	ggcatcgagctcacagccattttgcacGAATCcTacctggaagcccttcatcaagcggc gt

**Supplementary Table 2** Oligonucleotides used for targeting mutagenesis of TRPL (single mutants)

<b>Mutation</b>	<b>Primer</b>	<b>Sequence</b>
TRPL-F389M	Sense	tgccctcctctattgttcttctctgAtGatcttgattctggtgtctcagcgag
TRPL-F389M	Antisense	ctcgcctgagacaccagaatcaagatCaTcaggaagaacaataggaggaggca
TRPL-L393A	Sense	ttgttcttctgttcatcttgattGCggtgtctcagcgagcggacgatgact
TRPL-L393A	Antisense	agtcacgtccgctcgtgagacaccGCaatcaagatgaacaggaagaacaaa
TRPL-F406L	Sense	cggacgatgactttgtacgcatcttGgggacgaccaggatgaagaaggagc
TRPL-F406L	Antisense	gtccttcttcatcctggtcgtcccCaagatgctgacaagatcatcgtccg
TRPL-L420K	Sense	aagaaggagctggcggagcaggagAAgcgtcagcgaggccagacaccagc
TRPL-L420K	Antisense	gctgggtgtctggcctcgtgacgcTTctctgctccgccagctccttct
TRPL-V438I	Sense	tggagctcatcgtggtgatgtacAtCattgggtcgtgtgggaggaggt
TRPL-V438I	Antisense	acctctcccacacgaaccaatGaTgtacatcaccacgatgagctcca
TRPL-G440A	Sense	tcatcgtggtgatgtacgtgattgCgttcgtgtgggaggaggtgcagga
TRPL-G440A	Antisense	tctgcacctctcccacacgaacGcaatcacgtacatcaccacgatga
TRPL-W443F	Sense	acgtgattgggttcgtgtTCgaAgagggtgcaggagatattt
TRPL-W443F	Antisense	aaatatctcctgcacctTtcGAacacgaaccaatcacgt
TRPL-V446L	Sense	tgggttcgtgtgggaggagCtCaggagatatttgccgtg
TRPL-V446L	Antisense	caggcaaatatctctgGaGctcctcccacacgaacca
TRPL-V452D	Sense	gcgaggtgaagcaactgtgggacgAtggCctgcaggagtatctcaacgacatgt
TRPL-V452D	Antisense	acatgctgtgagatactcctgcagGccaTcgtcccacagttgcttccctcgc
TRPL-L458I	Sense	tccgtgggcatgaagagttatAtCcgcaacatgtggaacttcatcga
TRPL-L458I	Antisense	tcgatgaagttccacatgttgcgGaTataacttctcatgcccacggca
TRPL-M461L	Sense	gcatgaagagttatttgcgaacTtgtggaacttcatcacttctg
TRPL-M461L	Antisense	cagaaagtcgatgaagtccacaAgttgcgcaataacttctcatgc
TRPL-I465V	Sense	cgcaacatgtggaactcGtcgacttctgcgcaaca
TRPL-I465V	Antisense	tgttgcgcagaaagtcgaCgaagttccacatggttgcg
TRPL-F467Y	Sense	gcaacatgtggaactcactgactAtctgcgcaacagtctctactgtgag
TRPL-F467Y	Antisense	actcacgtagagactgttgcgcagaTagtcgatgaagttccacatggttc
TRPL-S471M	Sense	gaacttcatcgaacttctCcgGaacaTGctctactgtgagtgatg
TRPL-S471M	Antisense	catcacactcacgtagagCAgttCcgGagaaagtcgatgaagttc
TRPL-L472F	Sense	ttcatcgacttctgcgcaacagTtctactgtgagtgatgtgttaa
TRPL-L472F	Antisense	ttaaacacatcacactcacgtagaAactgttgcgcagaaagtcgatgaa
TRPL-L479C	Sense	acagtcctactgtgagtgatgtTAttaagagcctttgcctacatcca
TRPL-L479C	Antisense	tggatgtaggcaaaaggctcttaaTAacatcacactcacgtagagactgt
TRPL-Q486I	Sense	gctttgcgggtgtatcctctttATTgtAcaaaaagagatgatatacaactcg
TRPL-Q486I	Antisense	cgagttgtatatcatctctttttgTacAATAaagaagatacaacccgcaaagc
TRPL-E490D	Sense	atccttcttcaggtccaaaagaTatgatatacaactgcatgcgacgga
TRPL-E470D	Antisense	tccgtcgcagtcgagttgtatatcatAtcttttggacctgaaagaaggat
TRPL-A521G	Sense	tagccgaaggtctcttgcggcggGcaatgtcttctcggccttgaagct

TRPL-A521G	Antisense	agcttcaaggccgagaagacattgCccgcccaaagagacctcggcta
TRPL-N522M	Sense	ccgaaggtctctttgcggcggccaTGgtcttctcggccttgaagctggtgc
TRPL-N522M	Antisense	gcaccagcttcaaggccgagaagacCAtgccgccgcaaagagacctcgg
TRPL-V550I	Sense	atctccctaggtcgcgatgAtcatc gatata gtaaaat
TRPL-V550I	Antisense	atcttactatc gatgaTcatcgcacctagggagat
TRPL-K689Q	Sense	acggagtggaagtttgcgccgaaccCagCtgtgatgagctatttcgaggaca
TRPL-K689Q	Antisense	tgtcctcgaaatagctcatccacaGctGggttcgggcaaactccactccgt
TRPL-R729S	Sense	agtctagcaagaccatc gatcgtcaaTCctccaagaaacgaaaggagcaggag
TRPL-R729S	Antisense	ctcctgctccttctgtttcttgagGAttgacgatc gatggtcttctagact
TRPL-K731M	Sense	caagaccatc gatcgtcaacgtccaTgaaacgaaaggagcaggagcagttta
TRPL-K731M	Antisense	taaactgctcctgctccttctgttcAtggagcgttgacgatc gatggtcttg
TRPL-Y742H	Sense	aggagcaggagcagtttagcgagCaCgataatataatcgcctccctggt
TRPL-Y742H	Antisense	accagggagcgcattatattatcGtGctcgtaaactgctcctgctcct
TRPL-I745V	Sense	agcagtttagcgagatgataatGtaatgcgtccctggttggcga
TRPL-I745V	Antisense	tcgccaaccaggagcgcattaCattatcatactcgtcaaacgtct
TRPL-V754I	Sense	ataatgcgtccctggttggcgatatAtCgccccatgcatc gcaagttcgagaat
TRPL-V754I	Antisense	attctcgaactgc gatc gatggcggcGaTatatcgccaaccaggagcgcattat
TRPL-V766I	Sense	caagttcgagaataatccGAtatcggaggacgatatca
TRPL-V766I	Antisense	tgatcgtcctccgataTCggattattctgaacttg
TRPL-N772I	Sense	atcccgtatcggaggacgatatcaTtgaggtaagagt gagaatcaatcaga
TRPL-N772I	Antisense	cgtattgatctactcttgacctcaAtgatcgtcctccgatacgggatt
TRPL-K775R	Sense	cggaggacgatatcaatgaggtcaGgagtgagatcaatac gatcgtgta
TRPL-K775R	Antisense	taacgatcgtattgatctactcCtgacctcattgatcgtcctccg
TRPL-S792N	Sense	agatgctcgagatattcgagaacaAcggaatggatgttctcggccaa
TRPL-S792N	Antisense	ttggccgaggaaacatccattccgTgttctcgaatatctc gagcatct
TRPL-G793S	Sense	atgctcgagatattcgagaacagcTCAatggatgttctcggccaaca
TRPL-G793S	Antisense	tgttgccgaggaaacatccattGAgctgttctcgaatatctc gagcat
TRPL-M794W	Sense	tcgagatattcgagaacagcgggaTGggatgttctcggccaacaaa
TRPL-M794W	Antisense	ttgttgccgaggaaacatccCAatccgtgttctcgaatatctcga
TRPL-G821D	Sense	agtgtgggaacggcgttgatgaaggActtccaggtggcggcagtgcaaaatg
TRPL-G821D	Antisense	cattttgcactggcggcactggaagTccttcatcaagcggcgttcccact

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**Supplementary Table 3** Oligonucleotides used for targeting mutagenesis of TRP

<b>Mutation</b>	<b>Primer</b>	<b>Sequence</b>
TRP-IMA431VMG	Sense	ATTGAACTGGCAATCATTACCTATgTAATGGgTCTAATATTTGA GGAAGTCAAATCT
TRP-IMA431VMG	Antisense	AGATTTTCAGTTCCTCAAATATTAGAcCCATTAcATAGGTAATGA TTGCCAGTTCAAT
TRP-L439V	Sense	ATAATGGCTCTAATATTTGAGGAAgTGAAATCTTTATATTCGGA CGGCT
TRP-L439V	Antisense	AGCCGTCCGAATATAAAGATTTCAcTTCCTCAAATATTAGAGC CATTAT
TRP-L454M	Sense	ACGGCTTGTTGAGTACATCATGGATaTgTGGAACATAGTGGAC TACATATCGA
TRP-L454M	Antisense	TCGATATGTAGTCCACTATGTTCCAcAtATCCATGATGTACTCA AACAAAGCCGT
TRP-V458I	Sense	AGTACATCATGGATCTTTGGAACATAaTtGACTACATATCGAAC ATGTTCTAT
TRP-V458I	Antisense	ATAGAACATGTTTCGATATGTAGTCaAtTATGTTCCAAAGATCCA TGATGTACT
TRP-MF464SL	Sense	ACATAGTGGACTACATATCGAACAgccTCTATGTGACGTGGATT CTTTGTAGG
TRP-MF464SL	Antisense	CCTACAAAGAATCCACGTCACATAGAggcTGTTTCGATATGTAGT CCACTATGT
TRP-C472L	Sense	ACAgtcTCTATGTGACGTGGATTCTTTtaAGGGCCACCGCTTGGG TAATCGTCCATC
TRP-C472L	Antisense	GATGGACGATTACCCAAGCGGTGGCCCTtaAAAGAATCCACGT CACATAGAgacTGT
TRP-GM514AN	Sense	TATCAGAGGGCGCCTTTGCTGCCGcAAacGTCTTCTCCTATCTAA AGCTCGTC
TRP-GM514AN	Antisense	GACGAGCTTTAGATAGGAGAAGACgfTTgCGGCAGCAAAGGCG CCCTCTGATA
TRP-H734Y	Sense	AGTCCATGGAACGGGCACAGACGCTGtATGACAAAaTcATGAA GCTGCTGGTC
TRP-H734Y	Antisense	GACCAGCAGCTTCATgAtTTTGTcATaCAGCGTCTGTGCCCGTTC CATGGACT
TRP-V737I	Sense	ACGGGCACAGACGCTGCATGACAAAaTcATGAAGCTGCTGGTC AGGAGGTACA
TRP-V737I	Antisense	TGTACCTCCTGACCAGCAGCTTCATgAtTTTGTcATGCAGCGTCT GTGCCCGT
TRP-R767K	Sense	ATTACCGAGGATGATATCATTGAGGTGaagCAGGACATCAGCTC CTTGCGGTTTCGAG

TRP-R767K	Antisense	CTCGAACCGCAAGGAGCTGATGTCCTGcttCACCTCAATGATAT CATCCTCGGTAAT
TRP-NSW784SGM	Sense	GAGTTGCTGGAGATTTTCACCAACAgTgGCatGGATGTACCCGA CATTGAGAAGAAG
TRP-NSW784SGM	Antisense	CTTCTTCTCAATGTCGGGTACATCCatGCcAcTGTTGGTGAAAAT CTCCAGCAACTC

**Supplementary Table 4** Gene Bank numbers for TRP channels from different species

Channel	Specie	Trivial name	Gen Bank Number
TRP	<i>Aedes aegypti</i>	yellow fever mosquito	XP_001650890
TRP	<i>Bombus impatiens</i>	common eastern bumble bee	XP_003489572
TRP	<i>Culex quinquefasciatus</i>	southern house mosquito	XP_001869048
TRP	<i>Danaus plexippus</i>	monarch butterfly	EHJ65374
TRP	<i>Harpegnathos saltator</i>	Jerdon's jumping ant	EFN82264
TRP	<i>Musca domestica</i>	house fly	XP_005183118
TRP	<i>Nasonia vitripennis</i>	jewel wasp	XP_001605329
TRP	<i>Periplaneta americana</i>	American cockroach	AGG86916
TRP	<i>Tribolium castaneum</i>	red flour beetle	XP_968670
TRPL	<i>Bombus impatiens</i>	common eastern bumble bee	XP_003490000
TRPL	<i>Bombyx mori</i>	domestic silkworm	XP_004922702
TRPL	<i>Ceratitis capitata</i>	mediterranean fruit fly	XP_004517522
TRPL	<i>Culex quinquefasciatus</i>	southern house mosquito	XP_001847164
TRPL	<i>Harpegnathos saltator</i>	Jerdon's jumping ant	EFN80329
TRPL	<i>Musca domestica</i>	house fly	XP_005190181
TRPL	<i>Nasonia vitripennis</i>	jewel was	XP_001604491
TRPL	<i>Periplaneta americana</i>	American cockroach	AGG86915
TRPL	<i>Tribolium castaneum</i>	red flour beetle	XP_968598
TRPgamma	<i>Aedes aegypti</i>	yellow fever mosquito	XP_001650221
TRPgamma	<i>Bombus impatiens</i>	common eastern bumble bee	XP_003490670
TRPgamma	<i>Bombyx mori</i>	domestic silkworm	XP_004927991
TRPgamma	<i>Ceratitis capitata</i>	mediterranean fruit fly	XP_004536237
TRPgamma	<i>Danaus plexippus</i>	monarch butterfly	EHJ68691
TRPgamma	<i>Harpegnathos saltator</i>	Jerdon's jumping ant	EFN85066
TRPgamma	<i>Musca domestica</i>	house fly	XP_005191407
TRPgamma	<i>Nasonia vitripennis</i>	jewel wasp	XP_001604587
TRPgamma	<i>Periplaneta americana</i>	American cockroach	AAR26327

**Supplementary Table 5** Prediction characteristics and comparison with the prediction algorithm SNAP2 (<https://rostlab.org/owiki/index.php/Snap2>) depending on the conservative versus non-conservative classification and experimental results obtained in the study

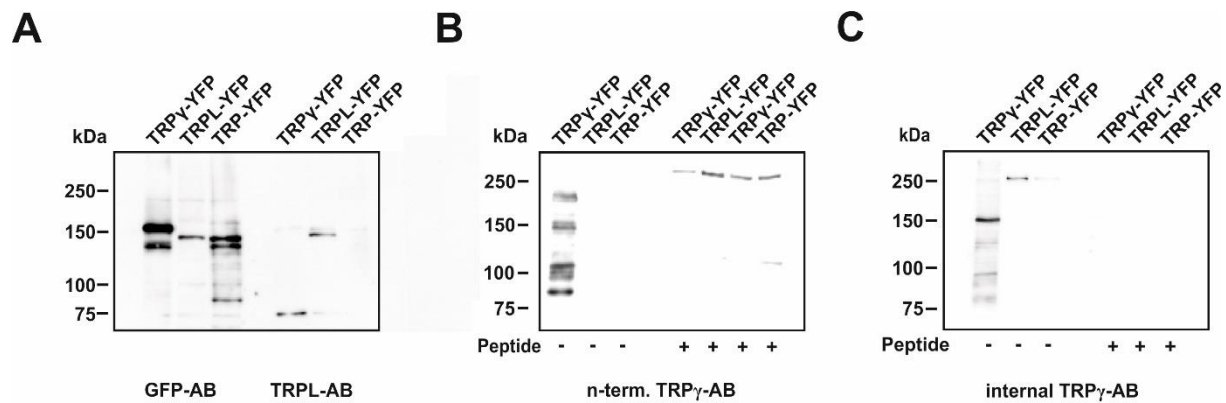
<b>Position</b>	<b>Wildtype Amino Acid/ side chain class</b>	<b>Side chain polarity</b>	<b>Side chain charge (ph 7.4)</b>	<b>Variant Amino Acids/ side chain class</b>	<b>Side chain polarity</b>	<b>Side chain charge (ph 7.4)</b>	<b>Type of Substitution: Conservative/ Non-Conservative</b>	<b>Predicted Effect (snap2)</b>	<b>Score (snap2)</b>	<b>Expected Accuracy (snap2)</b>	<b>Observed exp. effect (our data)</b>
367	G/aliphatic	nonpolar	neutral	A/aliphatic	nonpolar	neutral	Conservative	effect	27	63%	no
368	Q/ amide	polar	neutral	K/basic	basic polar	positive	Non-conservative	neutral	-27	61%	no
389	F/aromatic	nonpolar	neutral	M/sulfur containing	nonpolar	neutral	Conservative	neutral	-52	78%	no
393	L/aliphatic	nonpolar	neutral	A/aliphatic	nonpolar	neutral	Conservative	neutral	-19	57%	no
406	F/aromatic	nonpolar	neutral	L/aliphatic	nonpolar	neutral	Conservative	neutral	-24	61%	no
420	L/aliphatic	nonpolar	neutral	K/basic	basic polar	positive	Non-conservative	neutral	-17	57%	no
438	V/aliphatic	nonpolar	neutral	I/aliphatic	nonpolar	neutral	Conservative	neutral	-6	53%	yes
440	G/aliphatic	nonpolar	neutral	A/aliphatic	nonpolar	neutral	Conservative	effect	21	63%	yes
443	W/aromatic	nonpolar	neutral	F/aromatic	nonpolar	neutral	Conservative	neutral	0	53%	no
446	V/aliphatic	nonpolar	neutral	L/aliphatic	nonpolar	neutral	Conservative	neutral	35	66%	yes
452	V/aliphatic	nonpolar	neutral	D/acid	acidic polar	negative	Non-conservative	neutral	-26	61%	no
458	L/aliphatic	nonpolar	neutral	I/aliphatic	nonpolar	neutral	Conservative	neutral	-82	93%	yes

461	M/sulfur-containing	nonpolar	neutral	L/aliphatic	nonpolar	neutral	Conservative	neutral	-54	78%	yes
465	I/aliphatic	nonpolar	neutral	V/aliphatic	nonpolar	neutral	Conservative	neutral	-78	87%	yes
467	F/aromatic	nonpolar	neutral	Y/aromatic	nonpolar	neutral	Conservative	effect	12	59%	yes
471	S/hydroxyl-containing	polar	neutral	M/sulfur-containing	nonpolar	neutral	Non-conservative	neutral	-23	61%	yes
472	L/aliphatic	nonpolar	neutral	F/aromatic	nonpolar	neutral	Conservative	neutral	-53	78%	yes
479	L/aliphatic	nonpolar	neutral	C/sulfur-containing	nonpolar	neutral	Conservative	neutral	-65	82%	yes
486	Q/amide	polar	neutral	I/aliphatic	nonpolar	neutral	Conservative	neutral	-57	78%	no
490	E/acid	acidic polar	negative	D/acid	acidic polar	negative	Conservative	effect	21	63%	no
513	I/aliphatic	nonpolar	neutral	L/aliphatic	nonpolar	neutral	Conservative	neutral	53	78%	no
517	L/aliphatic	nonpolar	neutral	A/aliphatic	nonpolar	neutral	Conservative	neutral	-8	53%	no
521	A/aliphatic	nonpolar	neutral	G/aliphatic	nonpolar	neutral	Conservative	neutral	-26	61%	yes
522	N/amide	polar	neutral	M/sulfur-containing	nonpolar	neutral	Non-conservative	effect	25	63%	yes
550	V/aliphatic	nonpolar	neutral	I/aliphatic	nonpolar	neutral	Conserative	neutral	-57	78%	no
689	K/ basic	basic polar	positive	Q/amide	polar	neutral	Non-conservative	neutral	-55	78%	no
729	R/basic	basic polar	positive	S/hydroxyl-containing	polar	neutral	Non-conservative	neutral	-7	53%	no
731	K/ basic	basic polar	positive	M/sulfur-containing	nonpolar	neutral	Non-conservative	neutral	-10	53%	no
742	Y/aromatic	polar	neutral	H/basic aromatic	basic polar	positive(10%)	Non-conservative	effect	38	66%	yes



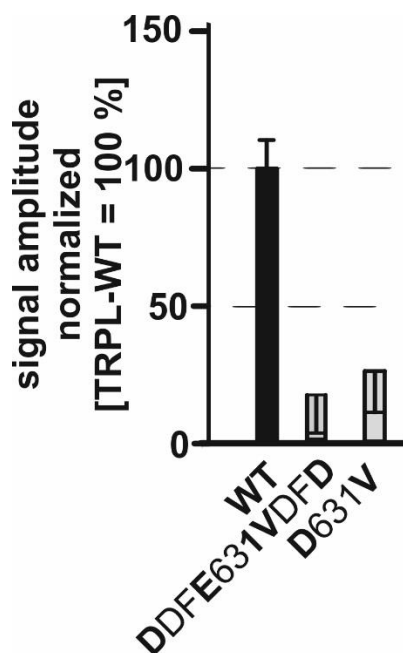
						neutral( 90%)						
745	I/aliphatic	nonpolar	neutral	V/aliphatic	nonpolar	neutral	Conservative	neutral	-89	93%	yes	
754	V/aliphatic	nonpolar	neutral	I/aliphatic	nonpolar	neutral	Conservative	neutral	-48	72%	no	
760	K/basic	basic polar	positive	R/basic	basic polar	positive	Conservative	neutral	-62	82%	no	
762	E/acid	acidic polar	negative	D/acid	acidic polar	negative	Conservative	neutral	-32	66%	no	
766	V/aliphatic	nonpolar	neutral	I/aliphatic	nonpolar	neutral	Conservative	neutral	-33	66%	no	
772	N/amide	polar	neutral	I/aliphatic	nonpolar	neutral	Non-conservative	neutral	-70	82%	no	
775	K/basic	basic polar	positive	R/basic	basic polar	positive	Conservative	neutral	-48	72%	yes	
792	S/hydroxyl-containing	polar	neutral	N/amide	polar	neutral	Conservative	neutral	-72	87%	yes	
793	G/aliphatic	nonpolar	neutral	S/hydroxyl-containing	polar	neutral	Non-conservative	effect	39	66%	yes	
794	M/sulfur-containing	nonpolar	neutral	W/aromatic	nonpolar	neutral	Non-conservative	effect	18	59%	yes	
818	L/aliphatic	nonpolar	neutral	I/aliphatic	nonpolar	neutral	Conservative	neutral	-25	61%	no	
819	M/sulfur-containing	nonpolar	neutral	L/aliphatic	nonpolar	neutral	Conservative	neutral	-41	72%	no	
821	G/aliphatic	nonpolar	neutral	D/acid	acidic polar	negative	Non-conservative	neutral	-57	78%	no	
825	A/aliphatic	nonpolar	neutral	G/aliphatic	nonpolar	neutral	Conervative	neutral	-71	87%	no	
826	P/cyclic	nonpolar	neutral	F/aromatic	nonpolar	neutral	Non-conservative	neutral	-16	57%	no	

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**Supplementary Fig. 1**

Detection of heterologously expressed *Drosophila* TRPC channels in HEK293 cells. Shown are exemplary Western blot analyzes of the protein extracts from transfected HEK293 cells. Demonstration of expression by means of a commercial GFP (A, left) and TRPL antibody (A, right). Detection of expression by means of a new n-terminal TRP $\gamma$ -AK (Pineda) (B, left) and by means of a new internal TRP $\gamma$ -AK (Pineda) (C, left), the specificity was confirmed by the immunogenic peptide (B and C, right).



**Supplementary Fig. 2**

Validation of the approach for functional characterization of TRPL multiple and single mutants. Changes in intracellular calcium concentration over time in response to application of 40  $\mu$ M ETYA in Fura-2-loaded HEK293 expressing TRPL-WT compared to TRPL mutants. Statistical analyses of data obtained during at least three independent experiments are presented as bar graphs. Data are means of the normalized (WT = 100%) signal amplitudes  $\pm$  SEM representing the characterized mutants, in total, **DDFE631VDFD**,  $n = 46$ ; **D631V**,  $n = 113$ . Substituted amino acid residues are marked in bold.