Title:

Diverse sensitivities of TRPA1 from different mosquito species to thermal and chemical stimuli

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Figure S1. DNA sequence alignment and amino acid sequence alignment of mosquito TRPA1s

(a) DNA sequence alignment showing the beginning of the *TrpA1A* gene from 4 mosquito species. Nucleotides are aligned in triplets, and the codons from the exon region are translated into amino acids listed below. Three critical methionine codons are highlighted red, and the upstream stop codons are highlighted green.

(b) Amino acid sequence alignment of TRPA1 splicing variants from 4 mosquito species. Ankyrin repeats are labeled with black and grey bars, a linker region with a red bar, transmembrane domains with blue bars, and a pore loop with a green bar. Asterisks note the reported conserved residues for electrophile detection.

(c) Cartoons indicate specific mosquito TRPA1 transcripts analyzed in different experiments.

Figure S2. Effects of the rate of temperature change on the heat-evoked activation of mosquito TRPA1

(a) An example of a linear 10_oC temperature rise during TRPA1 activation (left). The adopted temperature trace is highlighted with red color. An Arrhenius plot of the current evoked by heat stimulation is shown based on the left trace (right).

(b) There was a positive correlation between the rate of temperature increase and the heatevoked current density (left), whereas there was no correlation between the rate of temperature increase and the temperature threshold for heat activation. All the data were recorded and measured from HEK293 cells expressing AgTRPA1A(S). R-squared values are shown in both scatter diagrams.

Figure S3. AaTRPA1A and AaTRPA1B possess similar thermosensitivities

(a) Changes in cytosolic calcium concentrations (upper) in response to temperature elevation

(lower) or AITC application in HEK293 cells expressing AaTRPA1A (left) or AaTRPA1B (right).

(b) Density comparison of the currents activated by increasing bath solution temperature from 15_{0} C to near 42_{0} C using HEK293 cells expressing AaTRPA1A or AaTRPA1B in wholecell patch-clamp recording. All values are means \pm S.E.M, n = 9 for AaTRPA1A, n = 21 for AaTRPA1B, unpaired Student's *t*-test.

Figure S4. Phylogenic tree and distribution map of the four mosquito species investigated

(a) A phylogenic tree and the estimated divergence dates of the four mosquito species investigated. Figure is a simplified version based on the publication of Kamali et al. Nodes are at mean divergence time (in millions of years with standard errors).

(b) Distribution map of the four mosquito species investigated. Different colors of the areas of distribution correspond to different mosquito species, red for *Anopheles gambiae*, brown for *Anopheles stephensi*, purple for *Aedes aegypti*, blue for *Culex pipiens pallens*. Blue lines indicate the habitats of *Culex* mosquitoes. The habitats of mosquitoes overlap with each other.

Figure S5. Expression levels and plasma membrane localization of variants of AgTRPA1 in HEK293 cells analyzed with Western blotting

(a) Full-length blots of representative protein expression of Myc-tagged AgTRPA1 in whole cell lysate (whole), cytoplasm and membrane. 1/5000 diluted anti-Myc and 1/2000 diluted anti- β -actin primary antibodies were used to incubate the upper blot and the lower blot, respectively. (b) Quantified analysis of Myc-tagged AgTRPA1 in whole cell lysate (whole), cytoplasm and membrane. All values normalized to β -actin are means \pm S.E.M, n = 3. No significant difference, one-way ANOVA with Bonferroni's *post hoc* analysis. (c-e) Comparison of the 3 mM citronellal-evoked (left) and heat-evoked (right) currents in HEK293 cells expressing AgTRPA1D, AgTRPA1A(L) and AgTRPA1A(S) with or without Myc tags.

All values are means \pm S.E.M; $5 \le n \le 20$ each; **, P < 0.01, unpaired Student's t-test. (d) Representative biotinylated AgTRPA1 membrane protein (pull) and the eluate of cell lysate (input), and comparison by the quantitative analysis (e).

Figure S6.

Representative calcium imaging traces for screening the effects of the 22 chemicals on AgTRPA1A(S).

Each trace represents one DsRed positive HEK239 cell, indicating the transfection was successful.

Table S1. Primer sequences used in this research

(a) Primer sequences designed for the 5' and 3' RACE of TrpA1 of *Anopheles stephensi*, *Aedes aegypti* and *Culex pipiens pallens*.

(b) Primer sequences designed for the amplification of TrpA1 of *Anopheles stephensi*, *Aedes aegypti* and *Culex pipiens pallens*. And primer sequences designed for subcloning TrpA1(A) and TrpA1(A) of *Anopheles gambiae*.

(c) Primer sequences designed for the amplification of two Myc-tagged *Anopheles gambiae TrpA1* variants with a double gly-gly-ser linker.

Anopheles gambiae	ATT	GAA	ccc	• • •	ATC	ATC	СТА	TAC	TAC	ACT	TCA	AGT	• • •	• • •	GCA	GCT
Anopheles stephensi	TGA	GCC	CAA	• • •	TCA	CAA	TAC	CCA	AAC	CCA	TCA	AGT	• • •	• • •	GCA	ccc
Aedes aegypti	AAC	CGT	CCA	• • •	TCG	TTA	GCA	GAA	ттт	ACA	GCG	тсс	TGA *	ATC	GAT	CAT
Culex pipiens pallens	CGG	ΤΑΑ	CCA	TCG	тсс	ΑΤΑ	TCA	GGT	GTT	ATC	тсg	AGC		ΑΤΤ	GAA	ΑΑΤ
				First m	ethio	nine		S	econd	methi	ionine					
Anopheles gambiae	ΑΑΤ		стg			TTA										СТА
					м	L	S	v	Α	м	Ŷ	т	R	к	S	L
Anopheles stephensi	ΑΑΤ		ссс		ATG	ΤΤΑ	тсс	GTT	GCT	ATG	ΤΑΤ	ACA	CGA	AAA	тсс	стт
					М	L	5	v	Α	М	Ŷ	т	R	к	5	L
Aedes aegypti	ACT	CGA	СТА		ATG	ΤΤΑ	CCA	ATT	ATG	ATG	TAC	AGC	CGG	AAA	TCA	стс
					M	L	Ρ	1	м	М	Ŷ	5	R	к	5	L
Culex pipiens pallens	AAC	AGA	ΑΤΤ	TGA	TAT	СТА	сст	GTT	ACA	ATG	ATT	AAC	CGG	AAA	TCA	ATC
										м	1	N	R	κ	5	1
		hird me														
Anopheles gambiae	CGA	САТ	ATG	TGG	CGC	AAC	TGT	GCA	CAA	CGG	AAC	GGG			ттс	ACG
	R	н		w	R		С	Α		R	N	G			F	т
Anopheles stephensi	CGA	CAC	ATG	TGG	CGC	AAC	TGT	TCG	CAA	CGA	AGC	GGA			ттс	ACG
	R	н	м	w	R	N	с	5	Q		5	G			F	т
Aedes aegypti	AGT	CAA	ATG	СТА	CGA	AAC	GGC	ТАС	CGA	GGG	AAC	ACA			стс	TCG
571	5	Q	м	L	R	N	G	Ŷ	R	G	N	т			L	5
Culex pipiens pallens	AGA	CAG	ATG	TTT	CGT	AAC	TAT	CGC	ACA	AAT	CCA	AAT	САТ	CCG	TTG	AGT
	R	Q	м	F	R	N	Y	R	т	N	Ρ	N	н	Ρ	L	5

а

AgTRPA1D		-MPTPLYLIHSPRSVRSDTDHNHPTCEVNHEEEDLQQTQAFKNW SLL LPTGHG Q
AsTRPA1C	1	MMPTPLYLIHSPRSVRSDTEQNLPTCDANQEEDDLQQTQAFKNW 11SL/LPTGHGQ
AaTRPA1C	1	-MPTPLYLIHSARSVRSETGRPLGVCGTPPEEEELQHAPPLGNW DIS LS: KGN B
CpTRPA1C	1	-MPTPLYLCHSARTTATSDEPDRTLGVCDSPAEEDDLHSAPHFGNW MAILERIGGN
AgTRPA1A	1	MLS AMYT
AsTRPAIA	1	MLS AMYT
AaTRPA1A	1	MLPINMYS RX SLSQ. D :
AaTRPA1B	1	MLPINMYS <mark>SK</mark> SLSQI D :
CpTRPA1A	1	

AgTRPA1D		N KVN INAHDNNELQA LTOPAEAEVCLLSDSEY IIRAAEAGNLEEFIRLYEGDNNRL
AsTRPA1C		G KVN INAHDINELQA LTOPAEAEVCLLSDSEY IIRAAEAGNLEEFIRLYEGDNNRL
AaTRPA1C		A KGSNMHQ EHSELQA LT QGDAEVCLLSESEY IIRAAESGNL EFIRLYESDNGRL
CpTRPA1C		G KADNMDQ E SELQS LSQPAEHEVCLLSESEY IIRAAESGNLEEFVRLYEGDNGRL
AgTRPA1A		NCAQRNGF FATELKIF NWORVLREQG9ARINAEE FIQAAEAGNLEEFIRLYEGDNNRL
Astrpala	18	NCSORSGF LATPLKIF NWSRVLRLOGSARINTEE LOGAAEAGNLEEFIRLYEGDNNRL
AaTRPA1A		NGYRGNTLSTTPLKIFANW RVLRLHPSARINFALILQAAESGNLFEFIRLYESDNGRL
AaTRPA1B		NGYRGNTL TTPLKIEANW RVLRLHFSARINEALIL QAAESGNL EFIRLYESDNGRL
CpTRPA1A	15	RUNPNHPLSIATPLKIPATWERVLRLOPSTRINEEIILEAAESGNLEEFTRLYEGDNGRL

ANK1

ANK2

AgTRPA1D	118	svkdskgrtaahqaaarnrvniltfihgqggnlnaqdmugntplhtavendsldalefll
AsTRPA1C	119	svkdskgrtaahqaaarnrvniltfih <mark>s</mark> qgg <mark>n</mark> lnaqdmygntplhtavendsldalefll
AaTRPA1C	118	svkdskgrtaahqaaarnr <mark>i</mark> niltfih <mark>e</mark> qggdlnaqd <mark>si</mark> gntplhlavendsldalefll
CpTRPA1C	120	ALKDS GRTATHQAAARNRVNIINNIYTGRGDLNEQDMFGNTPLHLAVENDSLDALEFLL
AgTRPA1A	78	svkdskgrtaahqaaarnrvniltfih <mark>s</mark> qgg <mark>nlnaqdmy</mark> gntplh <mark>t</mark> avendsldalefll
Astrpala	78	svkdskgrtaahqaaarnrvniltfih <mark>s</mark> ogg <mark>nlnaqdmu</mark> gntplh <mark>t</mark> avendsldalefll
AaTRPA1A	78	svkdskgrtaahqaaarnr <mark>i</mark> niltfih <mark>e</mark> qggdlnaqd <mark>su</mark> gntplhlavendsldalefll
AaTRPA1B	78	svkdskgrtaahqaaarnr <mark>i</mark> niltfih <mark>e</mark> qggdlnaqd <mark>si</mark> gntplhlavendsldalefll
CpTRPA1A	75	A KDS: GRTATHQAAARNRVNILN: IYTGRGDLNEQDNFGNTPLHLAVENDSLDALEFLL

		ANK3	ANK
gTRPA1D 1	78	KIPVATNVLN=KKLAPVHLATEQNKVH2LQVMGKYREVIDIQQGGE	HGRTALHLAAIYD
STRPA1C 1	79	KIPVATNILN ^E KKLAPVHLATE <mark>Q</mark> NKV <mark>NE</mark> LQVMGKYREVIDIQQGGE	HGRTALHLAAIYD
aTRPA1C 1	78	KIPVATNILNDKKLAPVHLATELNKVKGLQVMGKYRE <mark>T</mark> IDIQQGGE	HGRTALHLAAIYD
pTRPA1C 1	80	KIPVATNILNDKKLAPVHLATELNKVKGLQVMGKYRDVFDIQQGGE	HGRTALHLAAIYD
AgTRPA1A 1	38	KIPVATN <mark>ULN KKLAPVHLATE</mark> ONKU <mark>H LOVMGKYREVIDIQOGGE</mark>	HGRTALHLAAIYD
STRPA1A 1	38	KIPVATNILN [,] KKLAPVHLATE <mark>ONKVN</mark> ,LQVMGKYREVIDIQQGGE	HGRTALHLAAIYD
aTRPA1A 1	38	KIPVATNILNDKKLAPVHLATELNKVKGLQVMGKYRE <mark>T</mark> IDIQQGGE	HGRTALHLAAIYD
AaTRPA1B 1	38	KIPVATNILNDKKLAPVHLATELNKVKGLQVMGKYRE <mark>T</mark> IDIQQGGE	HGRTALHLAAIYD
pTRPA1A 1	35	KIPVATNILNDKKLAPVHLATELNKVKGLQVMGKYR ^{DV} FDIQQGGE	HGRTALHLAAIYD

ANK5

AgTRPA1D	238	EECARILISEFGACPRKPCNNGYYPIHEAAKNASSKTMEVFFQWGESKGCTREEMISFYI
AsTRPA1C	239	EECARILISEFGACPRKPCNNGYYPIHEAAKNASSKTMEVFFQWGESKGCTREEMISFYI
AaTRPA1C	238	EECARILISEFGACPR ^P CNNGYYPIHEAAKNASSKTMEVFFQWGESKGCTREEMISFYI
CpTRPA1C	240	EECARILISEFGAS <mark>PRKPCNNGYYPIHEAAKNASSKTMEVFFQWGESKGCTREEMI</mark> SFYI
AgTRPA1A	198	EECARILISEFGACPRKPCNNGYYPIHEAAKNASSKTMEVFFQWGESKGCTREEMISFYD
AsTRPAIA	198	EBCARILISEFGACPRKPCNNGYYPIHEAAKNASSKTMEVFFQWGESKGCTREEMISFYL
AaTRPA1A	198	EECARILISEFGACPREPCNNGYYPIHEAAKNASSKTMEVFFQWGESKGCTREEMISFYI
AaTRPA1B	198	EECARILISEFGACPR ^E PCNNGYYPIHEAAKNASSKTMEVFFQWGESKGCTREEMISFYI
CpTRPA1A	195	EECARILISEFGASPRKPCNNGYYPIHEAAKNASSKTMEVFFQWGESKGCTREEMISFY

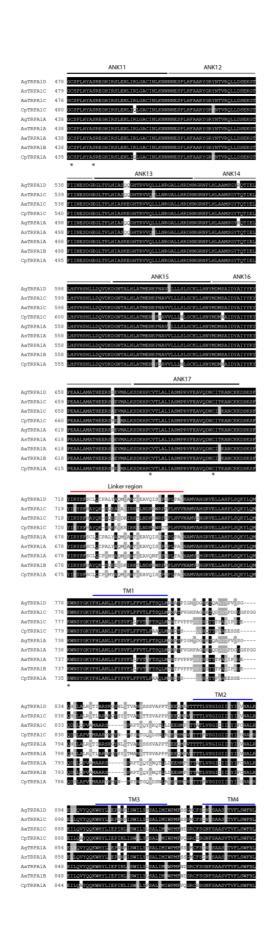
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AgTRPA1D	298	SEGNVPLHSAVHGGDIKAVELCLKSGAKISTQQH	DLSTPVHLAAAQGAIEIVKLMFRMQP
AsTRPA1C	299	SEGNVPLHSAVHGGDIKAVELCLKSGAKISTQQH	dlstpvhlaaaqgaieivklmf <mark>r</mark> mqp
AaTRPA1C	298	SEGNVPLHSAVHGGDIKAVELCLKSGAKISTQQH	DLSTPVHLAAAQGAIEIVKLMFLMQP
CpTRPA1C	300	SEGNVPLHSAVHGGDIKAVELCMKSGAKISTQQH	DLSTPVHLAAAQGAIDIVKLMFMMQP
AgTRPA1A	258	SEGNVPLHSAVHGGDIKAVELCLKSGAKISTQQH	dlstpvhlaaaqgaieivklmf <mark>r</mark> mqp
AsTRPA1A	258	SEGNVPLHSAVHGGDIKAVELCLKSGAKISTQQH	DLSTPVHLAAAQGAIEIVKLMFRMQP
AaTRPA1A	258	SEGNVPLHSAVHGGDIKAVELCLKSGAKISTQQH	DLSTPVHLAAAQGAIEIVKLMF
AaTRPA1B	258	SEGNVPLHSAVHGGDIKAVELCLKSGAKISTQQH	DLSTPVHLAAAQGAIEIVKLMFIMQP
CpTRPA1A	255	SEGNVPLHSAVHGGDIKAVELC <mark>M</mark> KSGAKISTQQH	dlstpvhlaaaqgai ivklmfmqp

ANK8

AgTRPA1D	358	LEKRISLNCTDIQKMTPLHCAAMFDHPEIVEYLV <mark>K</mark> EGADINA <mark>M</mark> DKE <mark>K</mark> RSPLLLS <mark>S</mark> SRGGW
AsTRPA1C	359	lekrislnctdiqkmtplhcaamfdhpeiveylv <mark>k</mark> egadinamdke <mark>k</mark> rspllls <mark>s</mark> srggw
AaTRPA1C	358	\mathbf{c} EKRISLNCTDIQKMTPLHCAAMFDHPEIVEYLVQEGADINALDKENRSPLLLSASRGGW
CpTRPA1C	360	${\tt LEKRISLNCTDIQKMTPLHCAAN}$ FOHPEIVEYLVQEGADINALDKENRSPLLLSASRAGW
AgTRPA1A	318	lekrislnctdiqkmtplhcaamfdhpeiveylv <mark>k</mark> egadinamdke <mark>k</mark> rspllls <mark>s</mark> srggw
Astrpala	318	LEKRISLNCTDIQKMTPLHCAAMFDHPEIVEYLV <mark>K</mark> EGADINAMDKE <mark>K</mark> RSPLLLS <mark>S</mark> SRGGW
AaTRPA1A	318	$\mathbf{c}_{EKRISLNCTDIQKMTPLHCAAMFDHPEIVEYLVQEGADINALDKENRSPLLLSASRGGW$
AaTRPA1B	318	$\mathbf{g}_{EKRISLNCTDIQKMTPLHCAAMFDHPEIVEYLVQEGADINALDKENRSPLLLSASRGGW$
CpTRPA1A	315	LEKRISLNCTDIQKMTPLHCAANFDHPEIVEYLVQEGADINALDKENRSPLLLSASRAGW

	ANK9	ANK10
AgTRPA1D 418	RTVMALIRLGANISLKDA	NSRNVLHLVIMNGG <mark>CLDEFAKEVCR</mark> TQSE <mark>I</mark> YLLQLLNEKDDA
AsTRPAIC 419	RTVMTLIRLGANISLKDA	NSRNVLHLVIMNGG <mark>CLDEFAKEV</mark> CHTQSE <mark>I</mark> YLLQLLNEKDD <mark>A</mark>
AaTRPA1C 418	RTVMVLIRLGANISLKDV	NSRNVLHLVIMNGGRLDEFAKEVS <mark>CTQSE</mark> TYLLLLNEKDET
CpTRPA1C 420	RTVMILIRLGANIELKDV	NSRNVLHLVIMNGGRLDEFAK <mark>O</mark> VS <mark>TTQSE</mark> KYLLQL <u>M</u> NEKDDT
AgTRPA1A 378	RTVMALIRLGANISLKDA	NSRNVLHLVIMNGG <mark>CLDEFAKEV</mark> CRTQSE <mark>I</mark> YLLQLLNEKDD <mark>A</mark>
AsTRPAIA 378	RTVMTLIRLGANISLKDA	NSRNVLHLVIMNGG <mark>CLDEFAKEV</mark> CHTQSE <mark>I</mark> YLLQLLNEKDD <mark>A</mark>
AaTRPA1A 378	RTVMVLIRLGANISLKDV	NSRNVLHLVIMNGGRLDEFAKEVS <mark>C</mark> TQSE <mark>T</mark> YLL <mark>L</mark> LLNEKDET
AaTRPA1B 378	RTVMVLIRLGANISLKDV	NSRNVLHLVIMNGGRLDEFAKEVS <mark>CTQSE</mark> TYLLLLNEKDST
CpTRPA1A 375	RTVMILIRLGANI <mark>E</mark> LKDV	NSRNVLHLVIMNGGRLDEFAK <mark>Q</mark> VS <mark>TTQSE</mark> KYLLQL ^A NEKDDT

b



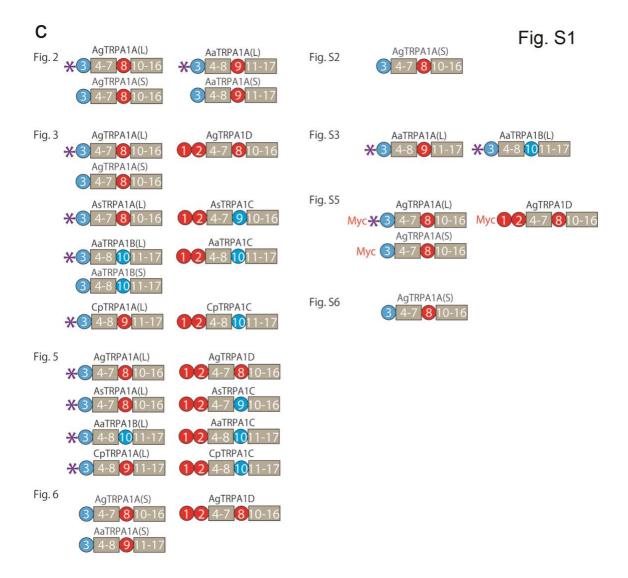
		<u></u>	TM5		
AgTRPA1D	954	LLELOREDO GIYVVMFLEI	LOTLIKVLIVESILI	IAFGLAFYILLS	KWSPOWNHL
AsTRPAIC	958	LLFLQRFDQ	LQTLIKVLIVFSILI	IAFGLAFYILLS	KVSPQVNHL
AaTRPAIC	948	LLFLQRFDQVGIYVVMFLEI	LQTLIKVLTVFSILI	IAFGLAFYILLS	KVSRMI TQVNHL
CpTRPAIC	948	LLFLQRFDQVG YVVMFLEI	LQTLIKVLTVFSILI	TAFGLSFYILLS	K KSHCANHQ
AgTRPAIA	914	LLFLQRFDQ	LQTLIKVLIVFSILI	IAFGLAFYILLS	KVSPQVNHL
Astrpala	918	LLFLQRFDQ	LQTLIKVLIVESILI	IAFGLAFYILLS	KVS PQVNHL
AaTRPAIA	908	LLFLQRFDQVGIYVVMFLEI	LQTLIKVLTVFSILI	IAFGLAFYILLS	KVSFMI TQVNHL
AaTRPA1B	908	LLFLORFDOVGIYVVMFLEI	LOTLIKVLTVFSILI	IAFGLAFYILLS	KVSRMI TQVNHL
CpTRPA1A	904	LLFLQRFDQVG	LQTLIKVLTVFSILI	IAFGLSFYILLS	K KSHQANHQ
CpTRPAIA	904	nasucestove www.sasa		IAFGLSEYILLS	KSHQANHQ TM6
CpTRPA1A AgTRPA1D	904		oop		
		pore-l	oop De styvoey	.P. P.P.P.SFV ILCI	TM6
AgTRPAID	1011	pore-	oop De styvoey	IF FFPSF ILCI	TM6
AgTRPA1D AsTRPA1C	1011 1015	pore-lo SFSSIPMSLERTFSMMLGEM SFSSIPMSLLRTFSMMLGEM SFSSIFMSLLRTFSMMLGEM	oop III SAANOSAHVG III SAANOSAHVG	LP PSPSF ILCI LP PSPSF ILCI LP PSPSF ILCI	TM6
AgTRPA1D AsTRPA1C AaTRPA1C	1011 1015 1008	pore-la SFSSIPMSLERTFSMMLGEN SFSSIPMSLLRTFSMMLGEN SFSSIPMSLLRTFSMMLGEN	OOP EDF (STYVQPYHVG) EDF (STYVQPYHVG) EDF (STYVQPYYQNH	LP PEPSF ILC LP PEPSF ILC LYPIPSF ILC LPYPIPSF ILS	TM6 FMILMPILLMNLL FMILMPILLMNLL FMILMPILLMNLL
AgTRPAID AsTRPAIC AaTRPAIC CpTRPAIC	1011 1015 1008 1005	pore-l SFSSIPMSLIRTFSMHLGEM SFSSIPMSLLRTFSMHLGEM SFSSIPMSLIRTFSMHLGEM	DOD DS STYVORYHVG DS STYVORYHVG DS STYVORY DS STYVORY BS STYVORY	LPEPEPSEVILCI LPEPEPSEVILCI LYPIPSEAILCI LPYPIPSETLS PEPEPSEVILCI	TM6 FMILMPILLMNLL FMILMPILLMNLL FMILMPILLMNLL
AgTRPAID ASTRPAIC AATRPAIC CPTRPAIC AgTRPAIA	1011 1015 1008 1005 971	pore-la SFSSIPMSLLRTFSMMLGEM SFSSIPMSLLRTFSMMLGEM SFSSIPMSLLRTFSMMLGEM SFSSIPMSLLRTFSMMLGEM	DOD DS STYVOPYHVG DS STYVOPYHVG DS STYVOPYHVG DS STYVOPYHVG DS STYVOPYHVG	LP PEPSF ILCI LP PEPSF ILCI TYPIPSP ILCI LPYPIPSF ILS PEPSF ILCL PEPSF ILCL	TM6 FMILMPILLMNLL FMILMPILLMNLL FMILMPILLMNLL FMILMPILLMNLL FMILMPILLMNLL
AgTRPAID AsTRPAIC AsTRPAIC CpTRPAIC AgTRPAIA AsTRPAIA	1011 1015 1008 1005 971 975	pore-1 SFSSI PHOLIARTSHMLGEN SFSSI PHOLIARTSHMLGEN SFSSI PHOLIARTSHMLGEN SFSSI PHOLIARTSHMLGEN SFSSI PHOLIARTSHMLGEN	DOD DS STYVOPYHVG DS STYVOPYHVG DS STYVOPYHVG DS STYVOPYHVG DS STYVOPYHVG DS STYVOPYHVG DS STYVOPYHVG DS STYVOPYHVG	AF 07 PSS ILCI TYPIPSF ILCI TYPIPSF ILCI PYPIPSF ILCI PFFFFF ILCI PFFFFFF ILCI TYPIPSF ILCI	TM6 FMILMPILLMNLL FMILMPILLMNLL FMILMPILLMNLL FMILMPILLMNLL FMILMPILLMNLL

AgTRPAID	1071	IGLAVGDIESVRRNAQLKRLAMQVVLHTELERKLPQMWLEMVDKMELIEYPNEKKCKLGF
Astrpaic	1075	IGLAVGDIESVRRNAQLKRLAMQVVLHTELERKLPQMWLEMVDKMELIEYPNEKKCKLGF
AaTRPAIC	1068	IGLAVGDIESVRRNAQLKRLAMQVVLHTELERKLPQMWLEMVDKMELIEYPNEKKCKLGF
CpTRPA1C	1065	IGLAVGDIESVRRNAQLKRLAMQVVLHTELERKLPQMWLEMVDNELIEYPNEKKCKLGF
AgTRPAIA	1031	IGLAVGDIESVRRNAQLKRLAMQVVLHTELERKLPQMWLEMVDKMELIEYPNEKKCKLGF
ASTRPAIA	1035	IGLAVGDIESVRRNAQLKRLAMQVVLHTELERKLPQMWLEMVDKMELIEYPNEKKCKLGF
AaTRPAIA	1028	IGLAVGDIESVRRNAQLKRLAMQVVLHTELERKLPQMWLEMVDKMELIEYPNEKKCKLGF
AaTRPA1B	1028	IGLAVGDIESVRRNAQLKRLAMQVVLHTELERKLPQMWLEMVDKMELIEYPNEKKCKLGF
CpTRPA1A	1021	IGLAVGDIESVRRNAQLKRLAMQVVLHTELERKLPQMWLEMVD

AgTRPAID	1131	LDSVLRKWFCNPFTDDYKGGID YVLEN<mark>B</mark>EDYV<mark>AVELEKQKRKLRDIG</mark>ALD<mark>AQHQLLRLI</mark>
ASTRPAIC	1135	LDSVLRKWFCNPFTDDYKGGIDYVL NTEDYVAVELEKQKRKLRDIG ALDTQHQLLRLI
AaTRPAIC	1128	LDSVLRKWFCNPFTDDYKGGIDFVLDNNEDYVVTELEKQKRKLREI <mark>S</mark> SALD <mark>N</mark> QHQLLRLI
CPTRPAIC	1125	IDS LRKWFCNPF DEAKGGIDFVLDSNEDY VTELEKOKRKLREIGSALDSOHOLLRLI
AgTRPAIA	1091	
ASTRPAIA	1095	LDSVLRKWFCNPFTDDYKGGID VLANTEDYVAVELEKQKRKLRDIG ALDTQHQLLRLI
Aatrpala	1088	LDSVLRKWFCNPFTDDYKGGIDFVLDNNEDYVVTELEKQKRKLREI <mark>S</mark> SALD <mark>N</mark> QHQLLRLI
AaTRPAIB	1088	LDSVLRKWFCNPFTDDYKGGIDFVLDNNEDYVVTELEKQKRKLREI <mark>S</mark> SALD <mark>N</mark> QHQLLRLI
CpTRPAIA	1081	DS LRKWFCNPF DEAKGGIDFVLDSNEDY VTELEKOKRKLREIGSALDSOHOLLRLI

AgTRPAID	1191	VOKMEIKTEADDVDEGVSTSD V SS LLTGTR SRWSSPRIRKKLGAT SFNKSIGK
ASTRPAIC	1195	VOKMEIKTEADDVDEGVPTSDIK SS LTGNR SRWSSPRIRKKLGAT SFNKSIGK
		VQKMEIKTEADDVDEGV <mark>ATSD</mark> KG <mark>IG LRGPNG- SRWSSPRIRKKLRAAMSFNKSISK</mark>
		VQKMEIKTEADDVDEGVSTGICKG LT GCERMM GSRWSSPRIRKKLRAAMSFNKS SK
AgTRPAIA	1151	VOKMEIKTEADDVDEGVSTSDIKKSS LLTGTRSRWSSPRIRKKLGAT SFNKSICK
ASTRPAIA	1155	VQKMEIKTEADDVDEGVPTSD KISS LTGNR SRWSSPRIRKKLGAT SFNKSIGK
AaTRPA1A	1148	VQKMEIKTEADDVDEGVATSD KGIG.LRGPNG- SRWSSPRIRKKLRAAMSFNKSISK
		VQKMEIKTEADDVDEGVATSD KG G LRGPNG- SRWSSPRIRKKLRAAMSFNKSISK
CpTRPA1A	1141	VOKMEIKTEADDVDEGVSTORG LT GOTEMM GSRWSSPRIRKKLRAAMSFNKS SK

Ag	TRPAID	1249	*
As	TRPAIC	1253	*
Aa	TRPAIC	1247	*
CF	TRPA1C	1245	*
Ag	TRPAIA	1209	*
As	TRPAIA	1213	•
Aa	TRPAIA	1207	*
Aa	TRPAIB	1207	*
Cp	TRPAIA	1201	*



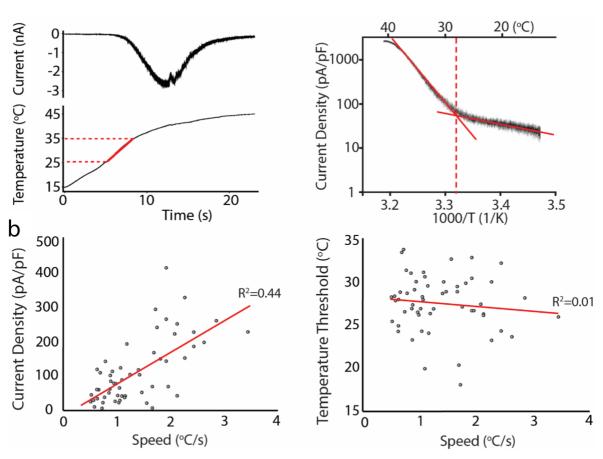
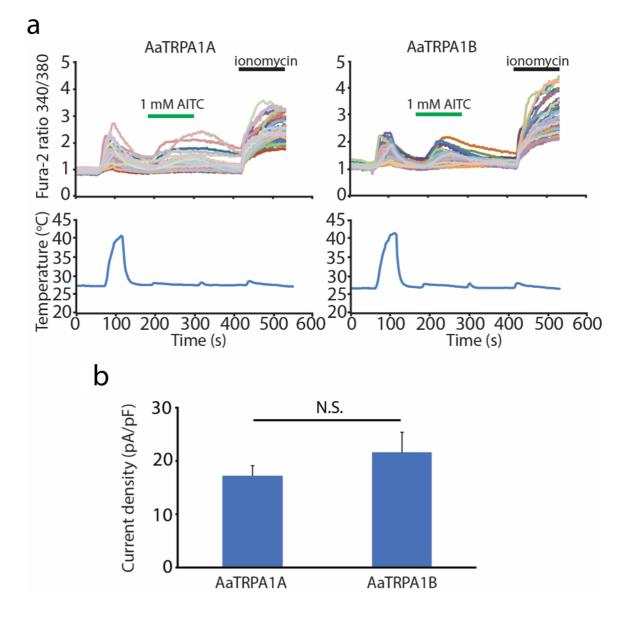
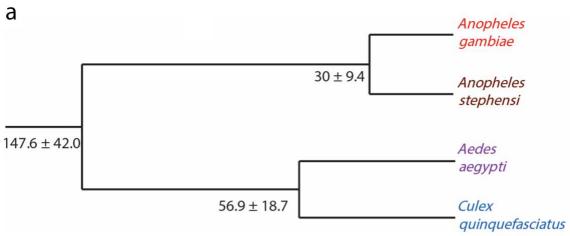
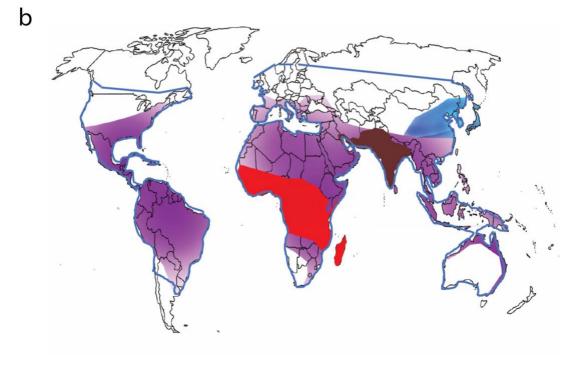


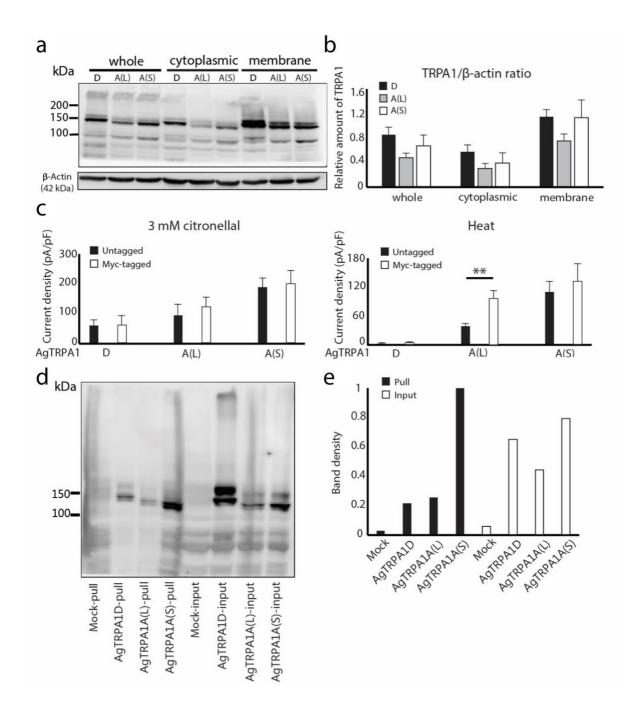
Fig. S2

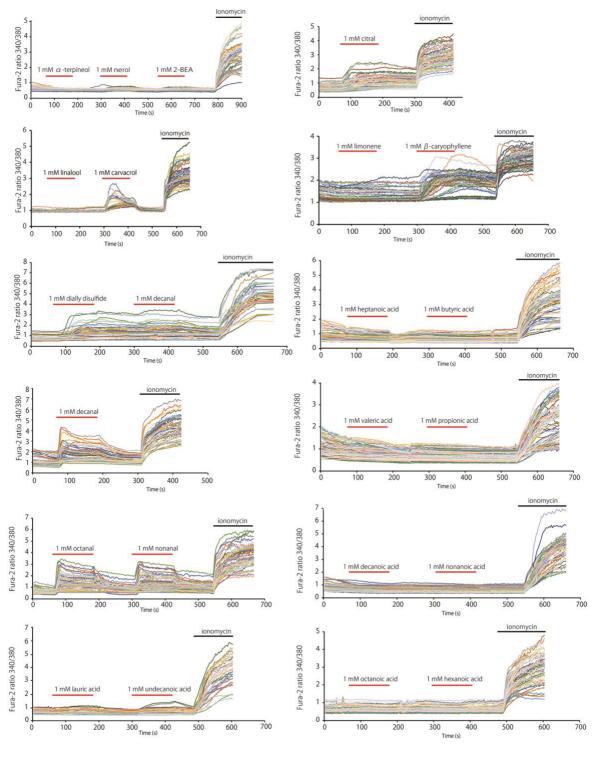












Supplementary Table S1

a			
Species	PCR step	5' RACE primer sequences	3' RACE primer sequences
Anopheles stephensi	1st step	5'-GTGTACGGCCGAGTGCAGTG-3' 5'-TTCACTCGCATCTGCTGGACC-3'	
	2nd step	5'-CTCCCGCGTGCATCCCTTCG-3'	5'-AAGCTGCTGCACAACTACATGG-3'
Aedes aegypti	1st step	5'-GGTGAGAATATTGATGCGGTTCC-3'	5'-TTCACTCGCATCTGCTGGACC-3'
	2nd step	5'-CGTCCTTTGCTATCCTTCACC-3'	5'-AAGCTGCTGCACAACTACATGG-3'
Culex pipiens pallens	1st step	5'-AGAAAGTCCATCTCGCCCAGCATC-3'	5'-GGAAAAGGGAACCTTCATCATCAACG-3'
	2nd step	5'-CATGACGACGTAGAGACCAACCTG-3'	5'-CGCAACGATGGAGAACAGACC-3'

b				
Species	PCR step for variant TrpA1C or TrpA1D	Primer sequences	PCR step for variant TrpA1A or TrpA1B	Primer sequences
Anopheles stephensi	1st step	F 5'-TCAGTCGTTCATCAACAGCATC-3'	1st step	F 5'-CATCGAAGGTACAGCAGTAGCAG-3'
		R 5'-ACACACGTACACGTACAAGCTC-3'		R 5'-ACACACGTACACGTACAAGCTC-3'
	2nd step	F 5'-TCAGTCGTTCATCAACAGCATC-3'	2nd step	F 5'-CCCAAACCCATCAAGTGCAC-3'
		R 5'-TAAGCGGCCGCGTGGCTCCGCTACAGTGC-3'		R 5'-TAAGCGGCCGCGTGGCTCCGCTACAGTGC-3'
	3rd step	F 5'-CTTGTGTCCTCTGAACGATGC-3'		
		R 5'-TAAGCGGCCGCACAGTGCCATTCATTTTCC-3'		
Aedes aegypti	1st step	F 5'-CAATCAGACGAAATCCACCACTAGCC-3'	1st step	F 5'-CTACTGTAAACCGTCCATCG-3'
		R 5'-CAAAATTCGTTGTCCTGCTG-3'		R 5'-CAAAATTCGTTGTCCTGCTG-3'
	2nd step	F 5'-CTCGGATCCACCATGCCAACTCCGCTC-3'	2nd step	F 5'-CTCGGATCCACCATGTTACCAATTATGATG-3'
		R 5'-TAAGCGGCCGCCTACTTGCTAATCGACTTG-3'		R 5'-TAAGCGGCCGCCTACTTGCTAATCGACTTG-3'
Culex pipiens pallens	1st step	F 5'-CAACCCCCTACGGAAGAATCGCTAC-3'	1st step	F 5'-GTCCACGGTAACCATCGTCCATATCAG-3'
		R 5'-TCTCTCGCATTACTTGCTCATGGACTTG-3'		R 5'-TCTCTCGCATTACTTGCTCATGGACTTG-3'
	2nd step	F 5'-CTTGGATCCACCATGCCAACTCCGCTG-3'	2nd step	F 5'-CTCGGATCCACCATGATTAACCGGAAATCAATC-3'
		R 5'-TAAGCGGCCGCTTACTTGCTCATGGACTTG-3'		R 5'-TAAGCGGCCGCTTACTTGCTCATGGACTTG-3'
Anopheles gambiae	TrpA1(A)	R 5'-TTTGGATCCACCATGCCTACTCCGCTG-3'	TrpA1(B)	R 5'-TTAGGATCCACCATGTGGCGCAACTG-3'
	subcloning	R 5'-TAAGCGGCCGCTCATTTGCCAATAGATTTG-3'	subcloning	R 5'-TAAGCGGCCGCTCATTTGCCAATAGATTTG-3'

c		
AgTrpA1 variants		Primer sequences
D	L	F 5'-CATGCCTACTCCGCTGTACTTAATTCATAG-3'
	inker	R 5'-GAGCCGCCTCCGCTTCCGCCTCCCAAGTCCTCTTCA
	vith er l	GAAATGAGCTTTTGCTCCATATCAAGCTTCAAGTCC-3'
A(L)	v sgi s-Vis	F 5'-CATGTTATCTGTTGCTATGTATACACGC-3'
	yc ta gly-f	R 5'-GAGCCGCCTCCGCTTCCGCCTCCCAAGTCCTCTTCA
	2 M gly-	GAAATGAGCTTTTGCTCCATATCAAGCTTCAAGTCC-3'
A(S)	Add	F 5'-CATGTGGCGCAACTGTGCACAAC-3'
	nop	R 5'-GAGCCGCCTCCGCTTCCGCCTCCCAAGTCCTCTTCA
		GAAATGAGCTTTTGCTCCATATCAAGCTTCAAGTCC-3'