

Supplementary Figure 1

Transcriptome profiling of vampire bat and fruit bat sensory ganglia

Number of mRNA-seq reads from sensory ganglia of vampire bat (*Desmodus rotundus*, top) and fruit bat (*Carollia brevicauda*, bottom) ganglia that align to the cow proteome. Blue line indicates expected number of sequencing reads for genes with similar expression levels in the two samples based on the total number of aligned reads from each.

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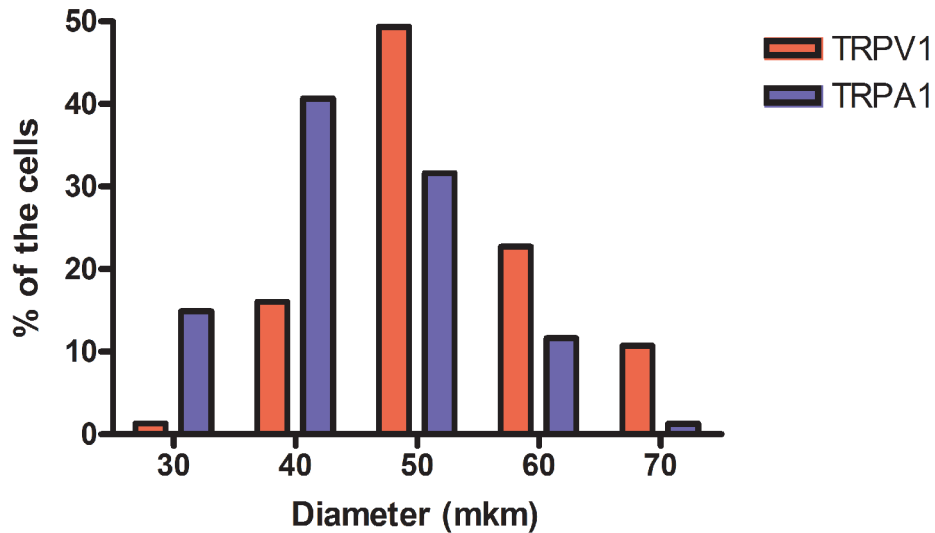
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Top: Vampire bat (*Desmodus rotundus*) TRPV1-L
 Bottom: Fruit bat (*Carollia brevicauda*) TRPV1-L

Supplementary Figure 2

Protein sequence alignment for vampire bat (*Desmodus rotundus*, top) and fruit bat (*Carollia brevicauda*, bottom) TRPV1-L isoforms.



Supplementary Figure 3

Neuronal size distribution of TRPV1-positive and TRPA1-positive neurons in trigeminal ganglia of vampire bats detected by *in situ* hybridization histochemistry of frozen tissue sections.

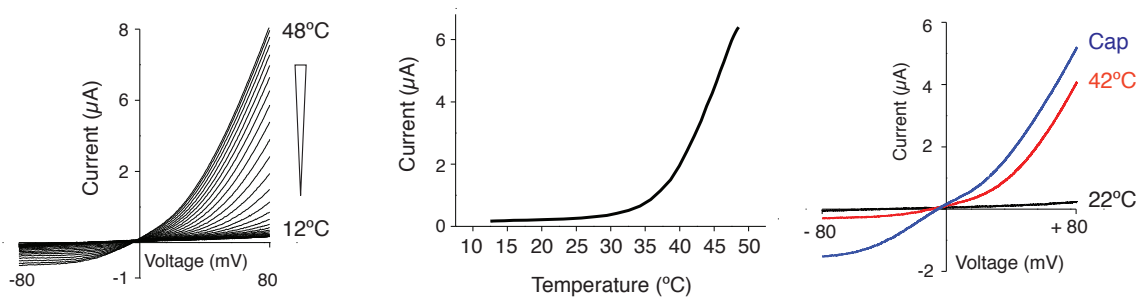
TRPV1	Q_{10}	Q_{10}	Temperature threshold (°C)
Vampire bat (Short)	5.1 ± 0.8	1.4 ± 0.1	31.2 ± 1.5
Vampire bat (Long)	11.5 ± 1.2	1.6 ± 0.1	40.2 ± 0.7
Short:Long (1:1)	5.7 ± 1.3	1.4 ± 0.1	33.9 ± 1.2
Zebrafish	4.6 ± 0.5	1.4 ± 0.1	32.9 ± 1.2
Fruit bat (Short)	5.1 ± 1.2	1.4 ± 0.3	30.8 ± 2
Fruit bat (Long)	9.9 ± 1.4	1.5 ± 0.2	40 ± 0.5
Cow (Long)	16 ± 1.7	1.6 ± 0.3	40.5 ± 0.6
Mole (Short)	4.4 ± 0.5	1.5 ± 0.3	31.3 ± 1.1
Mole (Long)	7.5 ± 0.6	1.7 ± 0.2	38.3 ± 0.9
Rat	21.7 ± 1.1	1.3 ± 0.1	42.3 ± 1.2

Supplementary Figure 4

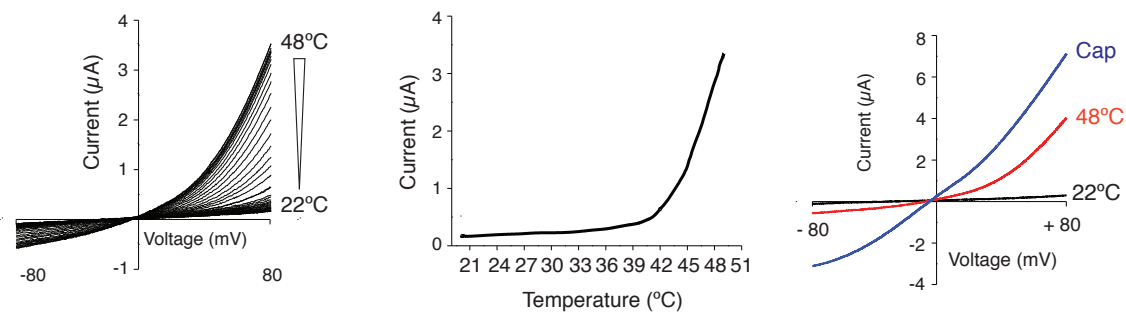
Thermal properties of cloned TRPV1 channels

TRPV1 cDNAs expressing short or long channel isoforms from various organisms (as indicated) were expressed in oocytes and subjected to temperature ramps while recording membrane currents in two-electrode voltage-clamp mode ($V_h = -80$ mV). Temperature coefficient (Q_{10}) for baseline and steepest component of the heat-evoked current and activation thresholds were derived from Arrhenius plots, as described in Methods.

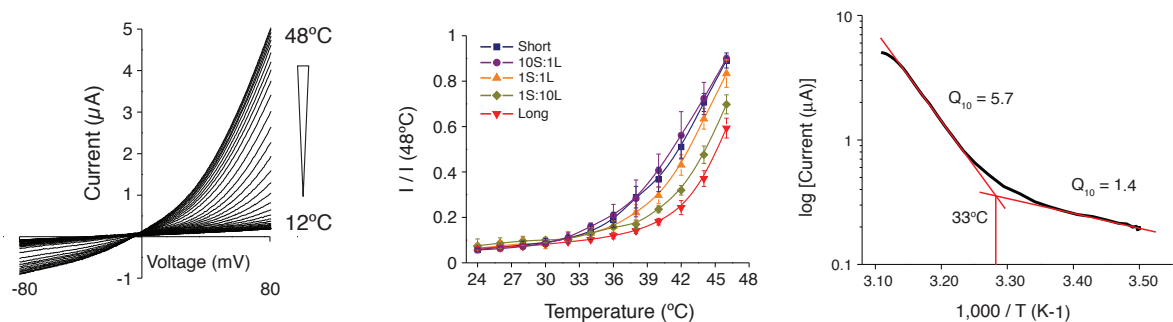
Vampire bat TRPV1-S isoform



Vampire bat TRPV1-L isoform



Vampire bat TRPV1-S:TRPV1-L = 1:1

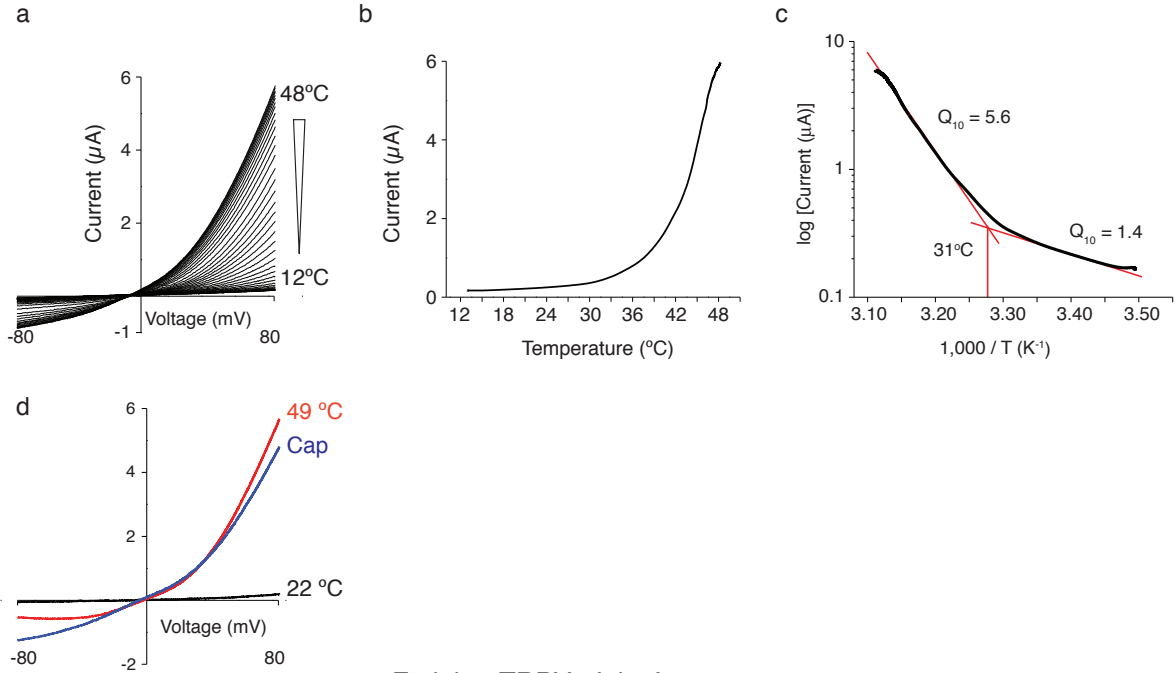


Supplementary Figure 5

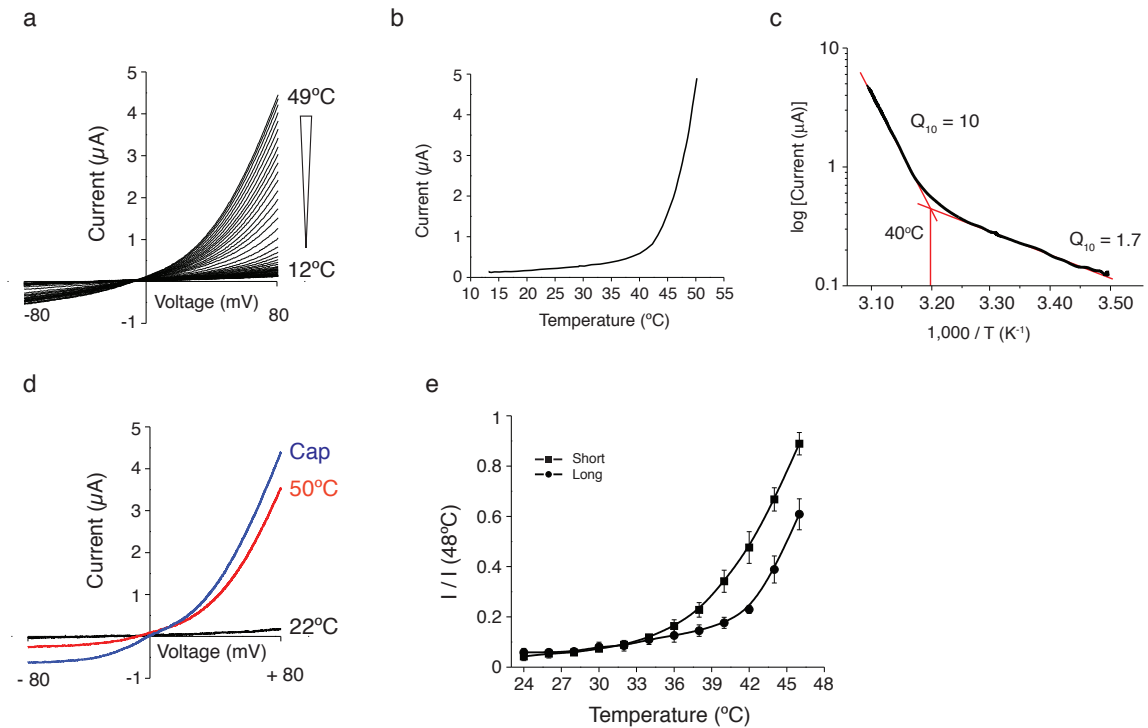
Functional analysis of vampire bat TRPV1 channel isoforms

Data are shown for oocytes expressing vampire bat (*D. rotundus*) TRPV1-S, TRPV1-L, or both, as indicated. In each case, left panel shows representative current-voltage relationships for heat-evoked responses during temperature ramp; middle panel shows temperature-response relationship; right panel shows representative current-voltage relationships for heat and capsaicin (10 μ M)-evoked responses, or Arrhenius plot showing thermal threshold and Q_{10} values for baseline and evoked responses. All data were collected in two-electrode voltage-clamp mode ($V_h = -80$ mV).

Fruit bat TRPV1-S isoform



Fruit bat TRPV1-L isoform



Supplementary Figure 6

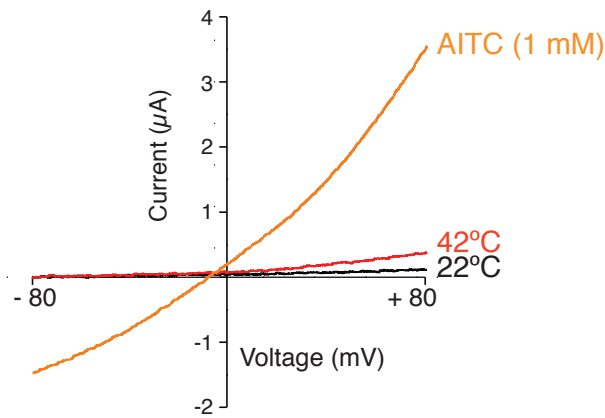
Functional analysis of fruit bat TRPV1 channel isoforms

Data are shown for oocytes expressing fruit bat (*C. brevicauda*) TRPV1-S, TRPV1-L, or both, as indicated. In each case, panel a shows representative current-voltage

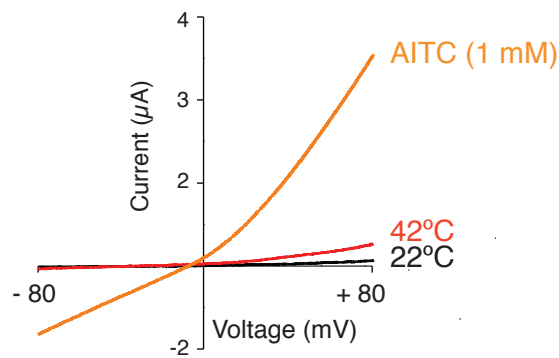
relationships for heat-evoked responses during temperature ramp; panel **b** shows temperature-response relationship; panel **c** shows Arrhenius plot showing thermal threshold and Q_{10} values for baseline and evoked responses; panel **d** shows representative current-voltage relationships for heat and capsaicin ($10 \mu\text{M}$)-evoked responses; panel **e** compares average temperature-response relationships for short and long TRPV1 isoforms.

All data were collected in two-electrode voltage-clamp mode ($V_h = -80 \text{ mV}$).

Vampire bat TRPA1



Fruit bat TRPA1



Supplementary Figure 7

Functional analysis of vampire and fruit bat TRPA1 channels

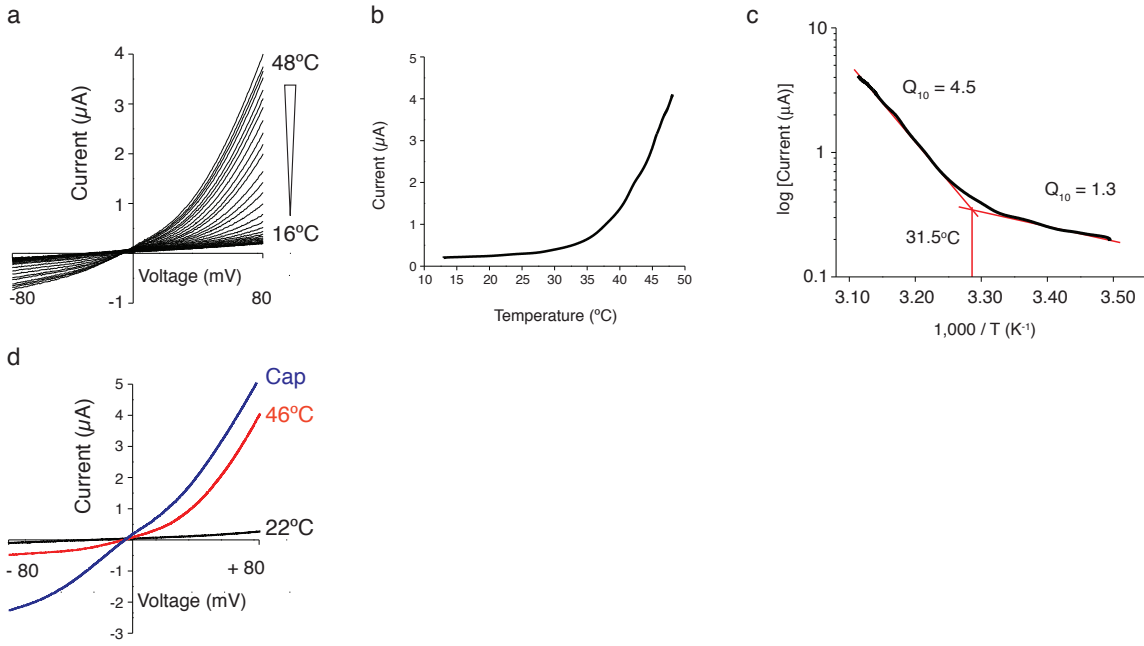
Thermal and chemical sensitivities of cloned vampire (*D. rotundus*) and fruit (*C. brevicauda*) bat TRPA1 channels, as indicated. Current-voltage relationships were determined by two-electrode voltage clamp recording of TRPA1-expressing oocytes challenged with heat (42°C) or AITC (1 mM).

Desmodus rotundus	TCTTTTCATT--TTTGTTTTTCAGCAGTTTGACTAAGATGTA	2154
Carollia brevicauda	TCTTTTCATT--TTTGTTTTTCAGCAGTTTGACTAAGATGTGCTTAGGTA	2398
Pteropus vampyrus	-----ATTGATA-----TTAGG--	1039
Pteropus rodricensis	-----ATTGATA-----TTAGG--	1039
Rousettus aegyptiacus	-----ATTGATA-----TTAGG--	1037
Bos taurus	TCTCTTCATCTTTTTGGTTTTTCAGCATTGACTAAAACGTGCTTAGGTA	3101
Equus caballus	-----TTCAGTAGTTTACTGAGATGTGCTCAGGTA	1186
Sus scrofa	TCTCTTCATC--TTTGTTTTTCAGCAGTTTACTAAGA-AACTTAGGGG	2300
Canis familiaris	TCTCTTCATT--TTTGTTTTTCAGCAGT----CTAAAATGTACTTAGGTT	2958
Scapanus orarius	-----	1465

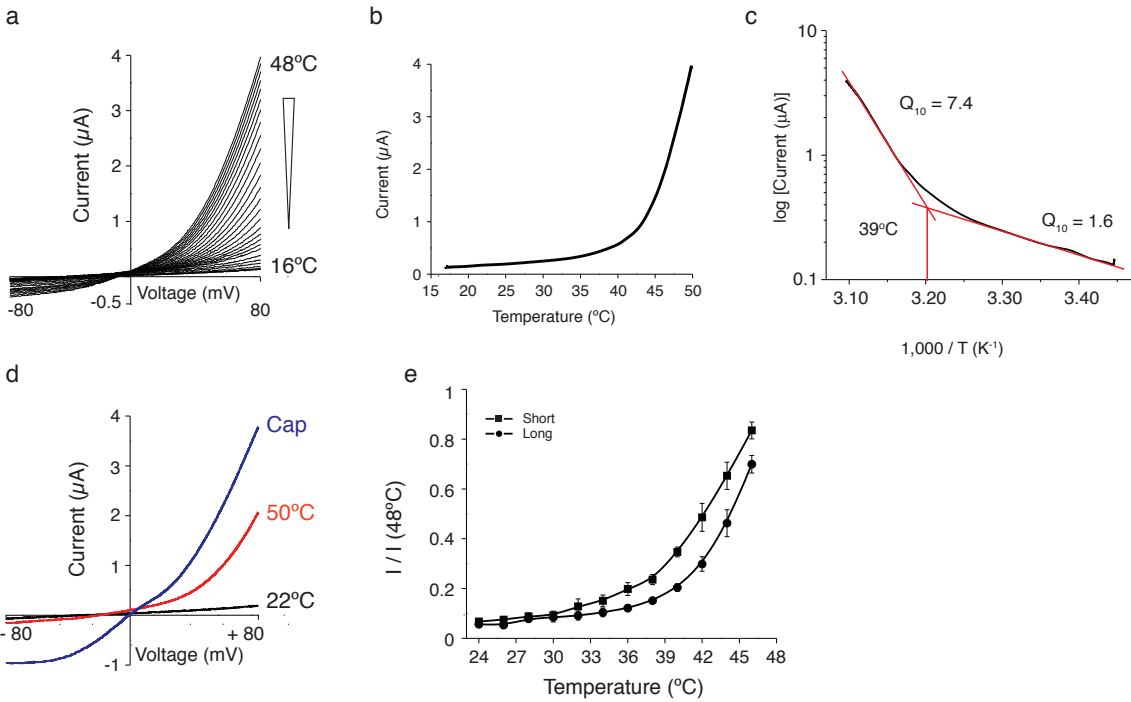
Supplementary Figure 9

Alignment of exon 14a from microbats with intronic region of other mammals

The region encoding exon 14a in vampire (*D. rotundus*) and fruit (*C. brevicauda*) bats is highlighted. Splice acceptor (-AG-) and donor (-GT-) sites are highlighted in green and grey, respectively. Numbers at right indicate relative distance from exon 14 (position 1 corresponds to 12 nucleotides from the end of exon 14 in all species shown). Dashes indicate regions of non-identity.



Coast mole TRPV1-L isoform



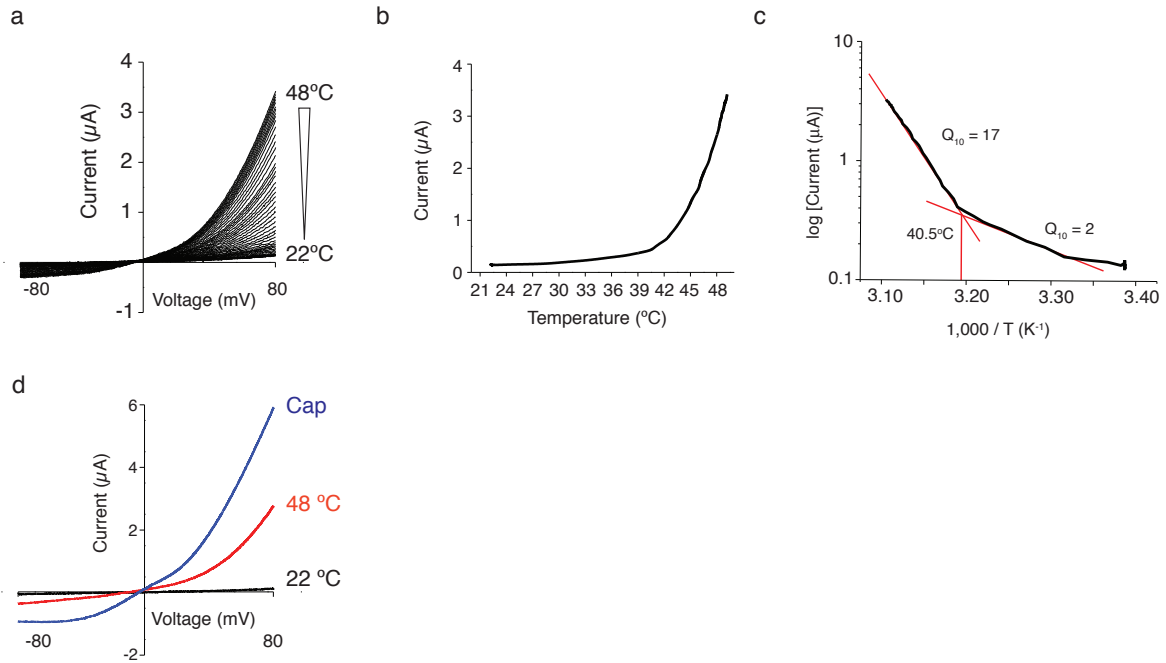
Supplementary Figure 10

Functional analysis of Coast mole TRPV1 channel isoforms

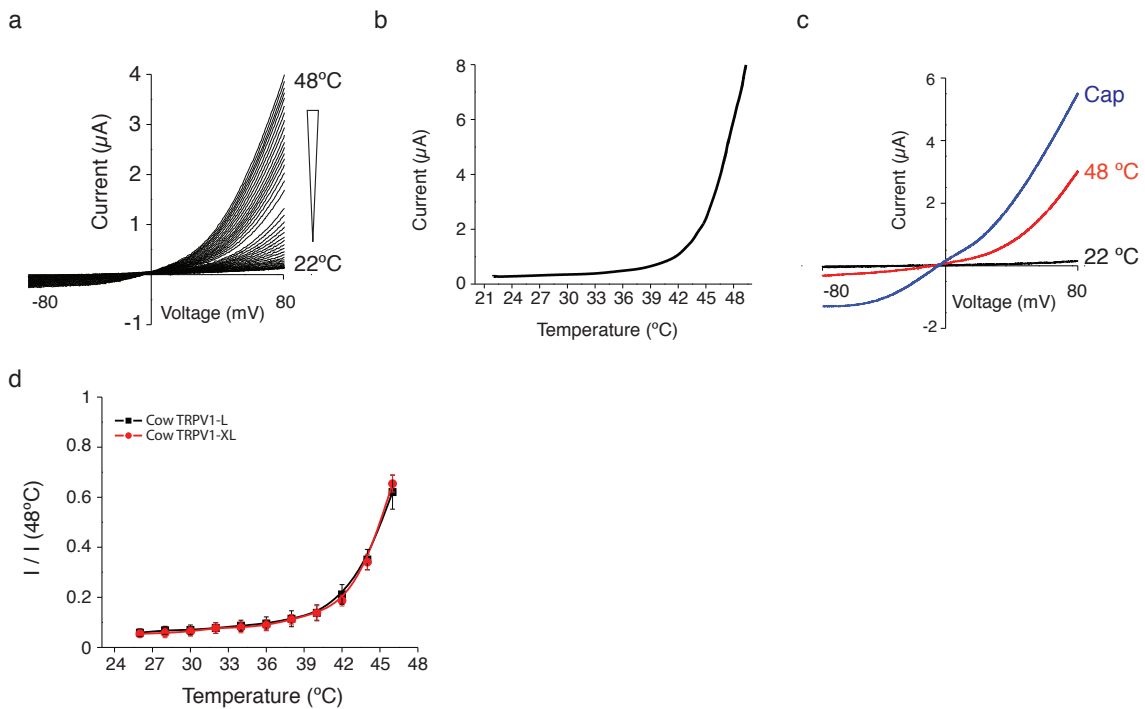
Data are shown for oocytes expressing Coast mole (*Scapanus orarius*) TRPV1-S or TRPV1-L isoforms, as indicated. In each case, panel a shows representative current-

voltage relationships for heat-evoked responses during temperature ramp; panel **b** shows temperature-response relationship; panel **c** shows Arrhenius plot showing thermal threshold and Q_{10} values for baseline and evoked responses; panel **d** shows representative current-voltage relationships for heat and capsaicin (10 μM)-evoked responses. Panel **e** in bottom group compares average temperature-response relationships for short and long TRPV1 isoforms. All data were collected in two-electrode voltage-clamp mode ($V_h = -80$ mV).

Cow TRPV1-L isoform



Cow TRPV1-XL isoform



Supplementary Figure 11

Functional analysis of bovine TRPV1 channel isoforms

Data are shown for oocytes expressing cow (*Bos taurus*) TRPV1-L or TRPV1-XL isoforms, as indicated. For TRPV1-L, panel a shows representative current-voltage

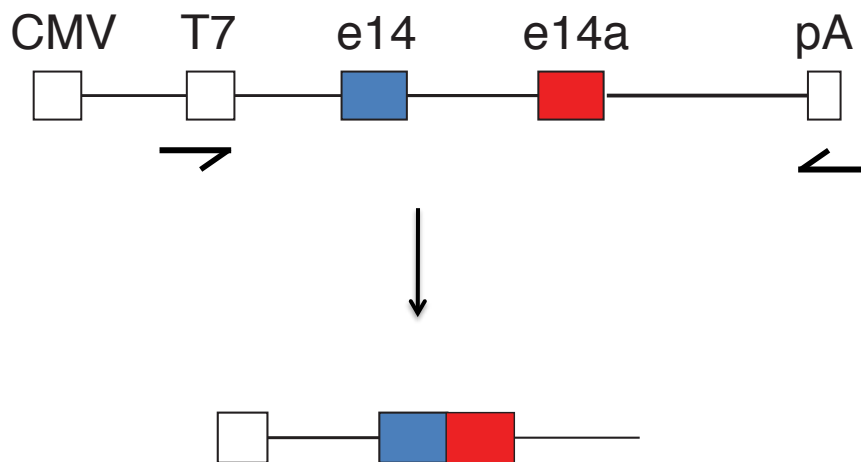
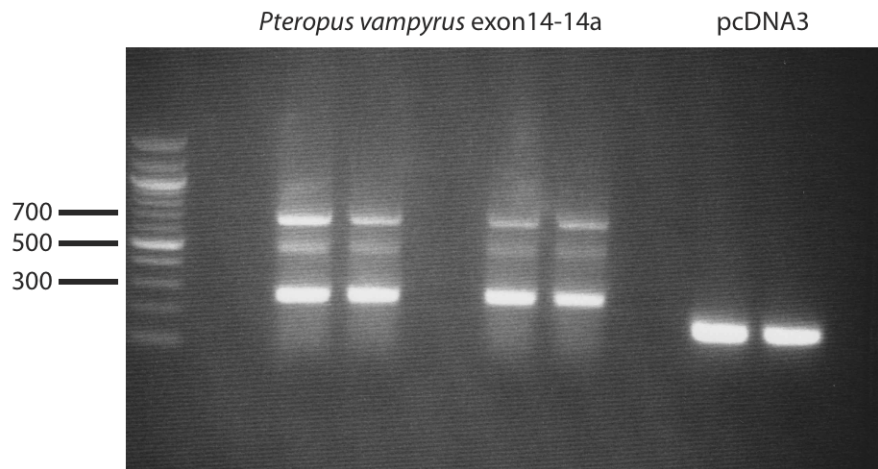
relationships for heat-evoked responses during temperature ramp; panel **b** shows temperature-response relationship; panel **c** shows Arrhenius plot showing thermal threshold and Q_{10} values for baseline and evoked responses; panel **d** shows representative current-voltage relationships for heat and capsaicin (10 μM)-evoked responses. For TRPV1-XL panel panel **a** shows representative current-voltage relationships for heat-evoked responses during temperature ramp; panel **b** shows temperature-response relationship; panel **c** shows representative current-voltage relationships for heat and capsaicin (10 μM)-evoked responses; panel **d** compares average temperature-response relationships for short and long TRPV1 isoforms. All data were collected in two-electrode voltage-clamp mode ($V_h = -80 \text{ mV}$).

Desmodus rotundus	----GGCAGGCACAGGAGGGAAGGACAGGAAGAGTGAAGGGTACACCTT	452
Carollia brevicauda	----GGCAGGTACAGGAGGTAAGAACGAGAAGAGTAAAGGGTACACCTT	454
Pteropus vampyrus	--CCAGGTAGGCAGAGGAGGGGAATGGGA-CAGTAAAGGGTACACCTT	466
Pteropus rodricensis	--CCAGGTAGGCAGAGGAGGGGAATGGGA-CAGTAAAGGGTAAACCTT	466
Rousettus aegyptiacus	--CCAGGTAGGCAGAGGAGGCAATGGGA-GAGTAAAGGGTGACCTT	465
Bos taurus	-----TTCACACGCAGGCAGGC--AGAGGAGGGAAGGT----GG	354
Equus caballus	AGCCAGGCAGGCAGAGGAGGGAAGATGGGACGAGTAAAGGGTACATCTT	477
Sus scrofa	-----GTGCAGAGGAGGGAAGGGTGGGAGGGGGCCAGGGTACACCTT	374
Canis familiaris	----ATCCAGCTGGCAGA-GGAAGAAATGGGAAGAGTAAACGAGTATATCAT	473
Scapanus orarius	-GTCTGTAGGGAGAGGAGGGAAGAGTGGGAGGAGGAGGGTACATCTG	469
Desmodus rotundus	-GAGCAGAAAAGCCCTGCAGAAAATCACTACCAGAAAA-AAGTCCTTTAA	500
Carollia brevicauda	-GAGCAGAAAAGCCCTGCAGAAAATCACTACCAGAAAA-AAGTTGTTTAA	502
Pteropus vampyrus	-GAGTGGAAAAGTCTCGCGAAAATCACTACCAGAAAAAAGTCCCTTTAC	515
Pteropus rodricensis	-GAGTGGAAAAGTCTCGCGAAAATCACTACCAGAAAAAAGTCCCTTTAC	515
Rousettus aegyptians	-GAGTGGAAAAGTCTCGCGAAAATCACTACCAGAAAAAAGTCCCTTTAC	514
Bos taurus	-GAGCAGAGGAGCCCTGCGGAGAACCACTACCAGAAACCAAGTCTTTAAA	403
Equus caballus	-GGACAGAACAGCCCTGCGGAAAAGTCACTGCCAGAAAACAAAAGCCTTGT-	525
Sus scrofa	-GAACCGAAGCCCTGCGGAAAATCACTACCAGAAA-A-A-GTCCTTTAA	420
Canis familiaris	AAAGCAGAAATGCTCTGCAGAAAATCACTACCAGAAAAAAGTCCCTTTAA	523
Scapanus orarius	-GAGCAGAAACAGTCTGAGGAAAATCATTAGTGGAAAG-AAGTCCTTTGAA	517
Desmodus_rotundus	TTTTAAG-CTT-TATT-----ATGGTAGATCTG-TGGACACTCC	3015
Carollia_brevicauda	TTTTAAG-CTT--TT-----ATGGTAGATCTG-TGGACACTCC	3257
Pteropus_vampyrus	TTTTAAC-CTT-TATT-----ATGGTAGATCTG-TAGACACTAC	1745
Pteropus_rodricensis	TTTTAAC-CTT-TATT-----ATGGTAGATCTG-TAGACACTAC	1745
Rousettus_aegyptiacus	TTTTAAG-CTT-TATT-----ATTGTAGATCTG-TGGACACTAC	1747
Bos_taurus	CTTTAGC-ATTTAATT-----ATGGTAGATCTG-TGGAAATGTC	3918
Equus_caballus	TTTTAAA-CTT-TATT-----ATGATAGATCTGT-TGGAAATGCC	1921
Sus_scrofa	TTTTAAA-GTT-TATT-----TTAGTAGATATGT-TGGAAATGCC	3008
Canis_familiaris	TTTTAAA-CTT-TATT-----ATGGTAGATATG-TG-AAATGCC	3887
Scapanus_orarius	TTTTTATATT-CATCAACTGATTTTATTTTATTTTTTTTGG-AGAACAG	2137
Desmodus_rotundus	AAGGT---ATTTGCTAATCCCATAAAACCTTGT--GGGACTCAGCC---	3055
Carollia_brevicauda	AAGGT---ATTTGCTGATCCCATAAAACCTTTT--GGGACTCAGCC---	3297
Pteropus_vampyrus	AAGAT---ATTTGCTAATCTCATCAAACCTTGT--GGGATTCAGCC---	1785
Pteropus_rodricensis	AAGAT---ATTTGCTAATCTCATCAAACCTTGT--GGGATTCAGCC---	1785
Rousettus_aegyptiacus	AAGAT---ATTTGCTAATCTCATCAAGCTTGT--GGGATTCAGCC---	1787
Bos_taurus	AAGAT---GTTTCAATCCCATCAAACCTTGT--GGGACTCAACC---	3958
Equus_caballus	GAGGT---GTTGCGAGTCCCATCAAGCTGTT--GGGATTCAAC---	1961
Sus_scrofa	AAGGT---ATTAGCCAATCCCATCAAAC-----TCAACC---	3039
Canis_familiaris	AAAGT---ATTTGCCAATCCCATAAAACCTTTT--GGGACTCAACC---	3927
Scapanus_orarius	AAAAGGCGGATTAT---TGAAATACACTGCTAAGAGAAGCAGCGGGCC	2183
Desmodus_rotundus	GTGAGTCTTGAG-T-AGTCTTACTCTA-GG----TTATGTAGAGTA--	3147
Carollia_brevicauda	GTGAGTCTTGAG-T-AGCCCTTACTCTA-GG----TTATGTAGAGTA--	3389
Pteropus_vampyrus	GTGGGTCTTAAG-TTAAACCTTACCCTA-GG----GCATGCAAAGTA--	1878
Pteropus_rodricensis	GTGGGTCTTAAG-TTAAACCTTACCCTA-GG----GCATGCAAAGTA--	1878
Rousettus_aegyptiacus	GTGGGTCTTAAG-TTAAACCTTACCCTA-GG----GCATGCAAAGTA--	1880
Bos_taurus	GTGGGTCTTGAG-T-AGACCTTATTCTAG-T----GTGTATAGAGTA--	4189
Equus_caballus	GTGGGTCTTGAG-T-AGAACTTACTCTAG-G----GC-TGTAGAGTA--	2055
Sus_scrofa	GTGGATCTTGAG-T-AGCTCTTGTCTAG-T----GCTTATAGAGTA--	3134
Canis_familiaris	GTGGGTCCCTCA-T-AGACCTTACTATA-GG----TACATAGAGTA--	4021
Scapanus_orarius	AACCTTTATTAAGAT-ATACTTACTACAAAATACACACATTAAATGTA	2293
Desmodus_rotundus	-CGTGGGGGC-ATTTCTGGTAT-T-TTA-----GCTTAAT----	3178
Carollia_brevicauda	-CGTGG-GGC-ATTTCTGGTAT-T-TTA-----GCTTAAT----	3419
Pteropus_vampyrus	-AGTAG-GGC-ATTTTTAGTAT-T-TTG-----TCTAGATCC---	1910
Pteropus_rodricensis	-AGTAG-GGC-ATTTTTAGTAT-T-TTG-----TCTAGATCC---	1910
Rousettus_aegyptiacus	-AGTAG-GGC-ATTTTTAGTAT-T-TTA-----GCTAGATCC---	1912
Bos_taurus	-AGCAG-GGC-ATTTCTGGTAT-T-TTA-----GCTTGATCC---	4221
Equus_caballus	-AGGAG-GGC-ATTTCTGGCGT-C-TCA-----GTTTGATCC---	2087
Sus_scrofa	-AGTAG-TG--ATTTCTGATGT-TTTTA-----ACTTGGTTC---	3166
Canis_familiaris	-AGTAG-GGC-ATTTCTGGTAT-TTTTA-----GCCTGATC---C	4054
Scapanus_orarius	TAGTTT-GGTAAGTTTCAGCAAATATACACTCAGGTAACCTCCATCTCAC	2342

Supplementary Figure 12

Alignment of exon 14a from cows and moles with intronic region of other mammals

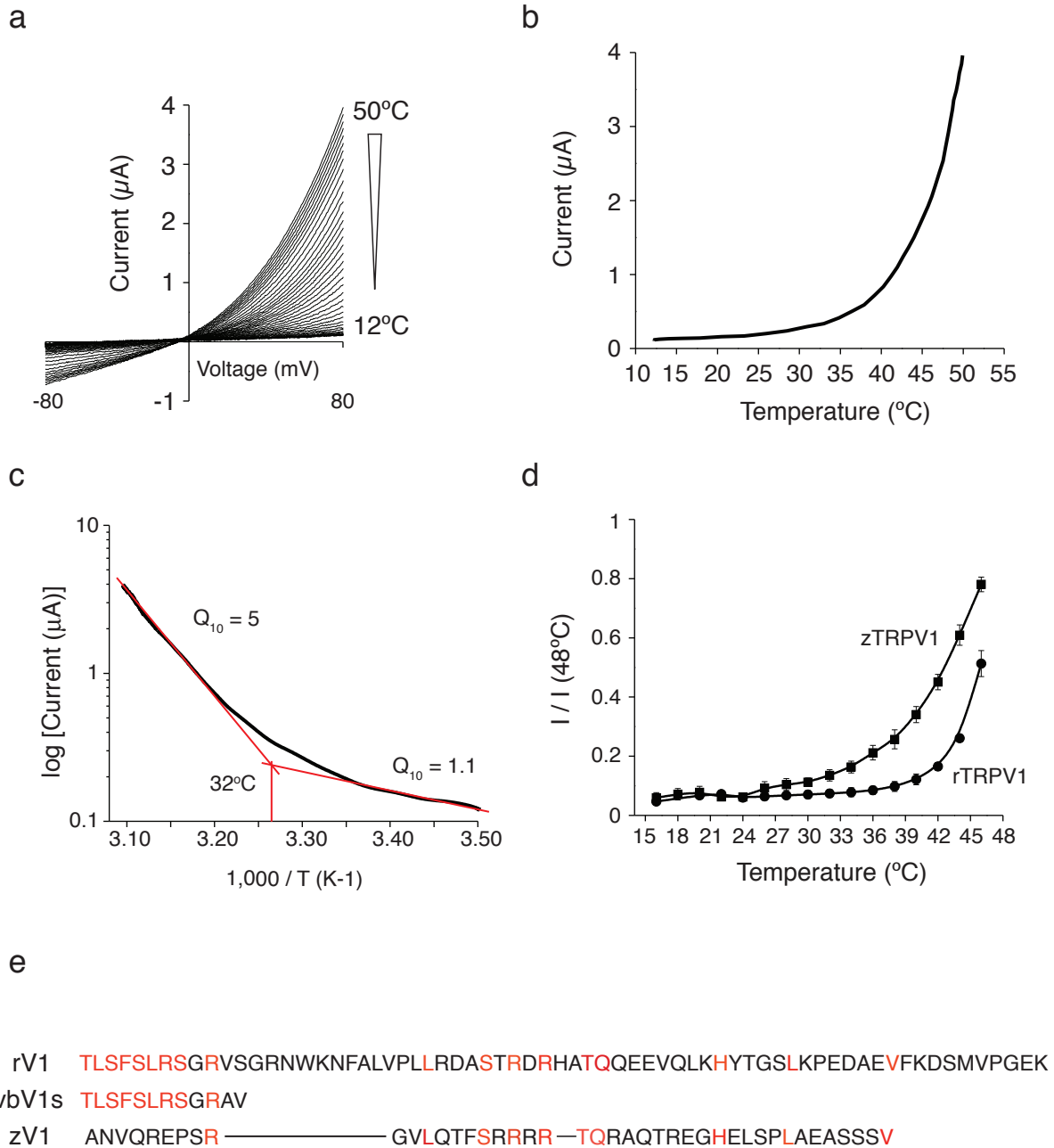
The region encoding exon 14a in cow (*Bos taurus*; panel a) and Coast moles (*Scapanus orarius*; panel b) is highlighted. Splice acceptor (-AG-) and donor (-GT-) sites are highlighted in green and grey, respectively. Numbers at right indicate relative distance from exon 14 (position 1 corresponds to 12 nucleotides from the end of exon 14 in all species shown). Dashes indicate regions of non-identity.



Supplementary Figure 13

In vivo splicing assay suggests presence of TRPV1 exon 14a in megabats

Diagram shows structure of mini-gene used for in vivo splicing assay in HEK293 cells, indicating location of CMV promoter and polyadenylation site (pA), defining the start and end of the transcribed unit. Transcripts were reverse transcribed using oligo-dT in combination with a primer annealing to T7 promoter to amplify reverse transcripts. Reaction products were resolved on 1.5% agarose gel (shown above with samples run in quadruplicate), and major bands collected for sequencing. Control reaction with vector (pcDNA3) is shown at right. Schematic of major splice product (~300 bp) is shown at bottom.



Supplementary Figure 14

Functional characterization and sequence analysis of zebrafish TRPV1

a-b, Representative current-voltage and temperature-response relationships for zebrafish TRPV1 channels expressed in oocytes. **c**, Arrhenius plot showing thermal threshold and Q_{10} values for baseline and evoked responses. **d**, Relative heat response profiles of zebrafish and rat TRPV1 channels expressed in oocytes (holding potential (V_h) = -80 mV; $n \geq 5$; mean \pm s.d.). **e**, Alignment of deduced amino acid sequences for C-terminal domain of rat TRPV1, vampire bat TRPV1-S, and zebrafish TRPV1 channels.

Global coordinates for bat collection

Sampling Point	Latitude (N)	Longitude (E)	Meridian
1	987682	600729	19
2	990981	601590	19
3	989922	605086	19
4	986630	604268	19
5	983530	605045	19

Summary of species examined

Species	Transcriptome	Genomic (experimental)	Genomic (database)
<i>Desmodus rotundus</i> (vampire bat) (n = 4)	X	X	
<i>Carollia brevicauda</i> (microbat) (n = 6)	X	X	
<i>Anoura cultrata</i> (microbat) (n = 1)	X		
<i>Uroderma bilobatum</i> (microbat) (n = 1)	X		
<i>Sturnira lilium</i> (microbat) (n = 1)	X	X	
<i>Pteropus vampyrus</i> (megabat) (n = 1)		X	
<i>Pteropus rodricensis</i> (megabat) (n = 1)		X	
<i>Rousettus aegyptiacus</i> (megabat) (n = 1)		X	
<i>Scapanus orarius</i> (coast mole) (n = 1)	X	X	
<i>Bos taurus</i> (cow) (n = 1)	X		X
<i>Canis lupus</i> (dog)			X
<i>Sus scrofa</i> (pig)			X
<i>Mus musculus</i> (mouse) (n ≥ 6)	X		X
<i>Rattus norvegicus</i> (rat)			X
<i>Oryctolagus cuniculus</i> (rabbit)			X
<i>Loxodonta africana</i> (elephant)			X
<i>Homo sapiens</i> (human)			X
<i>Macaca mulatta</i> (macaque)			X

Supplementary Figure 15

Compendium of animals examined

Top table indicates global coordinates at which bat species were collected. Bottom table summarizes all species examined, either through analysis of tissue samples and/or bioinformatically. Where relevant, the number of individual animals examined for each species is indicated in parenthesis.