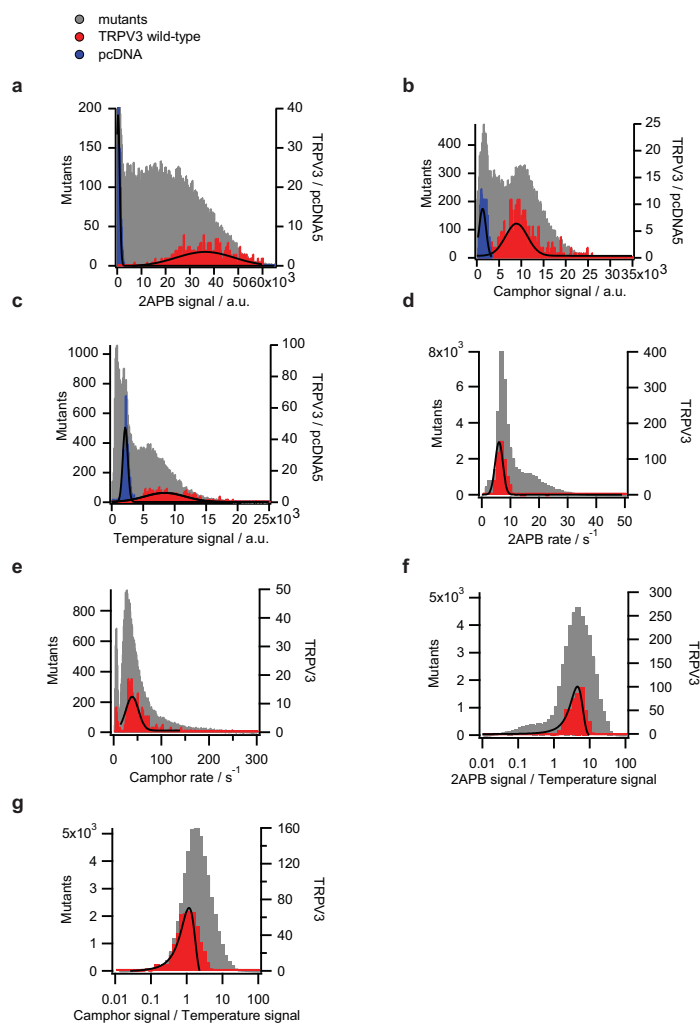


Pore region of TRPV3 ion channel is specifically required for heat-activation

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Supplementary Figure 1

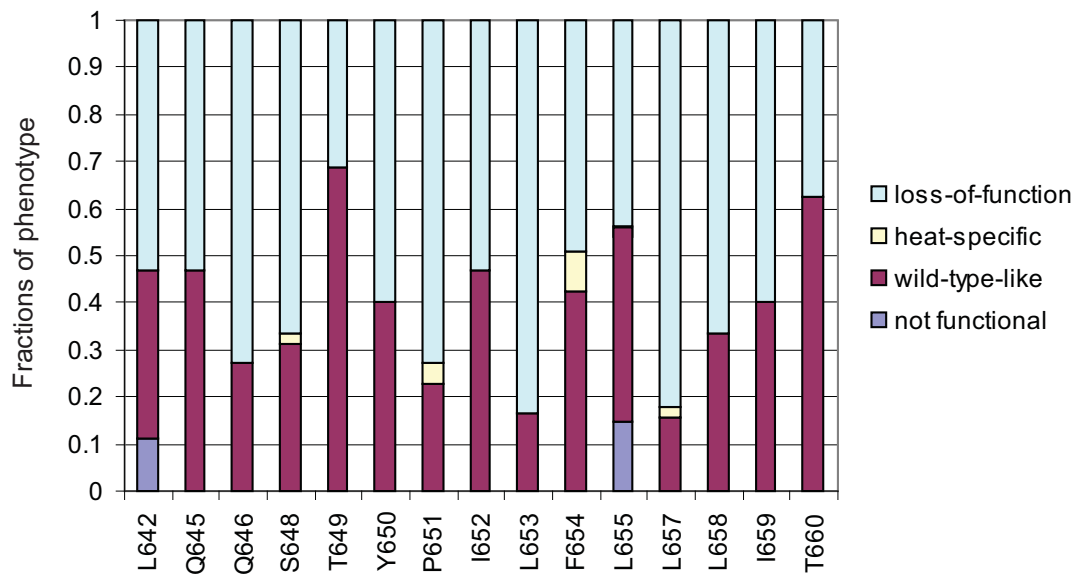


Supplementary Figure 1: Histograms of primary screen

a-c, Histograms of collected data for maximal responses to 2APB (a), camphor (b) and temperature (c) for all clones (grey), TRPV3 (red) and pcDNA (blue).

d-g, Histograms of collected data for rates of signal increase for 2APB (d), camphor (e) and ratios of maximal responses for 2APB/temperature (f) and camphor/temperature (g). Black lines are Gaussian fits to histograms.

Supplementary Figure 2



Supplementary Figure 2: Detailed screen in mouse TRPV3

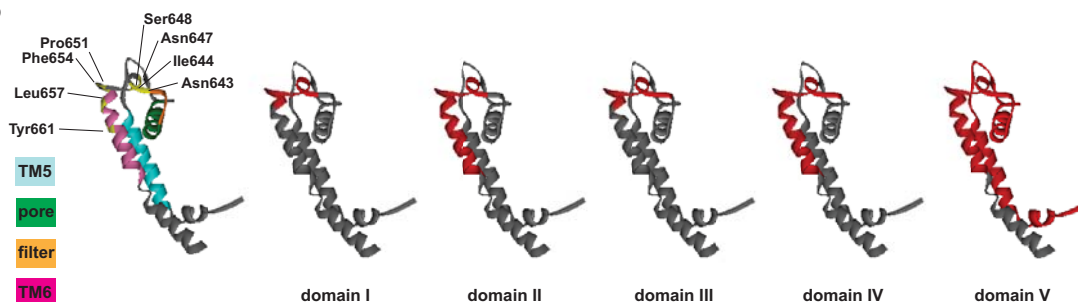
Fractions of phenotypes observed in single point-mutation screen for mouse TRPV3 resulting from responses to heat, 30 μ M 2APB and 1.75 mM camphor. The total number of tested clones was 45 for each residue. Note, that the numbers are biased by the degeneration of the genetic code, e.g changes into serine are more frequent than for example mutations to methionine.

Supplementary Figure 3

a

		domain V
mTRPV3	MIQKVI LDV LK FLFVYILFLLGFGVALASLIE KCSKDKKDCSS-----	620
rTRPV1	MIEKMILRDLCRFMFVYL VFLFGFSTAVVT LIEDGKNSLPMES---TPHKCRGSACKP-GNS	610
hTRPV2	MIQKVI LDL LRFLLIYLVFLFGFAVALVSLSQEAWRPEAPTGNATESVQPMEGQEDEGNGAQ	568
rTRPV2	MIQKVI LDL LRFLLIYLVFLFGFAVALVSLSREARSPKAPEDNNSTVTEQPTVGQEEEP--AP	568
rTRPV4	MIQKILFKDLRFLRLLVYLLFMIGYASALVTLNPNCTNMKVCNEDQSNCTVPSYPACRDSE----	645
mTRPV6	MIQKMIFGDL MRFCW LMAVVILGFASAFYIIFQTE DPDEL GHFY-----	522
xTRPV3	----VILNDVLKFLFVYILFLLGFGVALASLLENCE-DGEECQ-----S	477
		domain I domain II domain III domain IV domain V
mTRPV3	YGSE SDAVLELFKLLTGLGDLNIQQNSTYPIILFLRLITTYVILTFVLLI NMLIALMGETVENVSKE	687
rTRPV1	YNSLYSTCLELFKFTIGMGDLEFTENYDFKAVFIILLLAYVILTYILLLNMLIALMGETVNKIAQE	677
hTRPV2	YRGILEASLELFKFTIGMGELAFQEQLHFRGMVLLLLLAYVLLTYILLLNMLIALMSETVNSVATD	635
rTRPV2	YRSILDASLELFKFTIGMGELAFQEQLRFRGVLLLLLAYVLLTYVLLLNMLIALMSETVNHVADN	635
rTRPV4	--TFSAFLLDLFKLTIGMGDLEMLSSAKYPVVFILLLVTYIILTFVLLLNMLIALMGETVGVQSKE	712
mTRPV6	--DYPMALFSTFELFLTIIDGPANYD VDL PFMYSVTYAAFAIATLLMLNLLIAMMGDTHWRVAHE	589
xTRPV3	---LSTALELFELTIGLRGLEMDKPKYPVLFLLITFVILTFVLLLNMLIALMGETVEKISQE	544

b



Supplementary Figure 3: Chimeric constructs

a, Alignment of amino-acid sequences of TRPV channels used for constructing chimeric channels (Supplementary Table 3). Red bars indicate positions of domains (I-V) that were exchanged for different constructs. Abbreviations are h: human, m: mouse, r: rat, x: xenopus. Colours in the sequence of TRPV3 indicate locations of TM5 (blue), pore region (green), selectivity filter (orange), TM6 (pink) and identified point-mutations (yellow).

b, Structural models of one subunit with colours corresponding to the alignment.

Supplementary Table 1: mutations and EC₅₀ values of confirmed hits

Point mutations of 15 confirmed hits and their respective EC₅₀-values for activation by 2APB and camphor. Mutations that were individually engineered are shown in bold. Errors are s.d., n=15 for wild-type TRPV3 and n=3 for mutants.

Mutations	EC ₅₀ (2APB) / μ M	EC ₅₀ (camphor) / mM
TRPV3 wild-type	8.7 \pm 2.7	1.4 \pm 0.2
Thr17Ser, Ala302Val, Tyr461Phe	5.3	1.1
Asn79His, Ala245Val, Met440Val	12.4	1.6
Asn647Tyr	13.4	1.4
Ile595Phe, Leu657Ile	6.8	1.0
Ile289Thr, Lys434Met , Leu670Phe , Phe780Leu	14.0	1.9
Ala32Glu, Ser77Pro, Ile204Asn, Thr660Ser , Met717Val	12.3	1.8
Tyr409His, Asn643Ser , Leu720Pro	10.8	1.5
Ile186Glu, Asn220Ser, Tyr461Phe , Phe489Ser	6.1	1.4
Ile123Phe, Ile453Asn , Ile765Thr	11.0	1.4
Gln114Arg, Ile289Val, Thr397Ser	11.3	1.6
Lys358Glu	10.6	1.3
Asn71Thr, Asn197Ser, Lys774Asn	8.3	1.3
Ile644Ser	5.1	1.1
Tyr451Asn , Tyr661Cys	5.6	1.3
Phe445Ile , Trp481Arg	6.6	1.6

Supplementary Table 2: additional point mutations in mouse TRPV3

Sequencing results for single-point mutations that specifically affect heat-activation in mouse TRPV3.

Position	Mutation
Ser648	Ile
Pro651	Ala
Phe654	Ser, Lys
Leu657	Glu

Supplementary Table 3: point mutations in xenopus TRPV3

Sequencing results for single-point mutations that specifically affect heat-activation in xenopus TRPV3.

Position	Mutation
Glu638	His, Lys, Phe
Met639	Ala, Arg, Glu, Gly, Lys, Ser, Thr, Trp, Val
Asp640	Leu, Lys, Tyr, Val
Lys641	Pro
Asp642	Met, Pro, Tyr
Pro643	Leu

Supplementary Table 4: chimeras

Chimeras and their tested responses to stimulation by compounds (2APB, camphor, THC or capsaicin) and heat. Chimeras are ordered for constructs that were aimed to investigate necessity (1) or sufficiency (2) of the replaced domain and named with the insert first and the background second. Species are abbreviated h: human, m: mouse, r: rat, x: xenopus. Numbers indicate inserted fragment as illustrated in Supplementary Figure 3.

Chimera	Compound tested	Compound response	Heat response
1. rV1mV3-II	2APB, camphor	EC50 shifted	reduced
hV2mV3-I	2APB, camphor	EC50 shifted	no
hV2mV3-II	2APB, camphor	like mV3 wild-type	no
rV2mV3-II	2APB, camphor, THC	EC50 shifted	no
rV4mV3-II	2APB, camphor	EC50 shifted	no
mV6mV3-I	2APB, camphor	no response	no
mV6mV3-II	2APB, camphor	no response	no
mV6mV3-III	2APB, camphor	no response	no
mV6mV3-IV	2APB, camphor	no response	no
mC3mV3-II	2APB, camphor	no response	no
xV3mV3-II	2APB, camphor	no response	no
2. mV3hV2-I	2APB, THC	no response	no
mV3hV2-II	2APB, THC	no response	no
mV3hV2-V	2APB, camphor	no response	no