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

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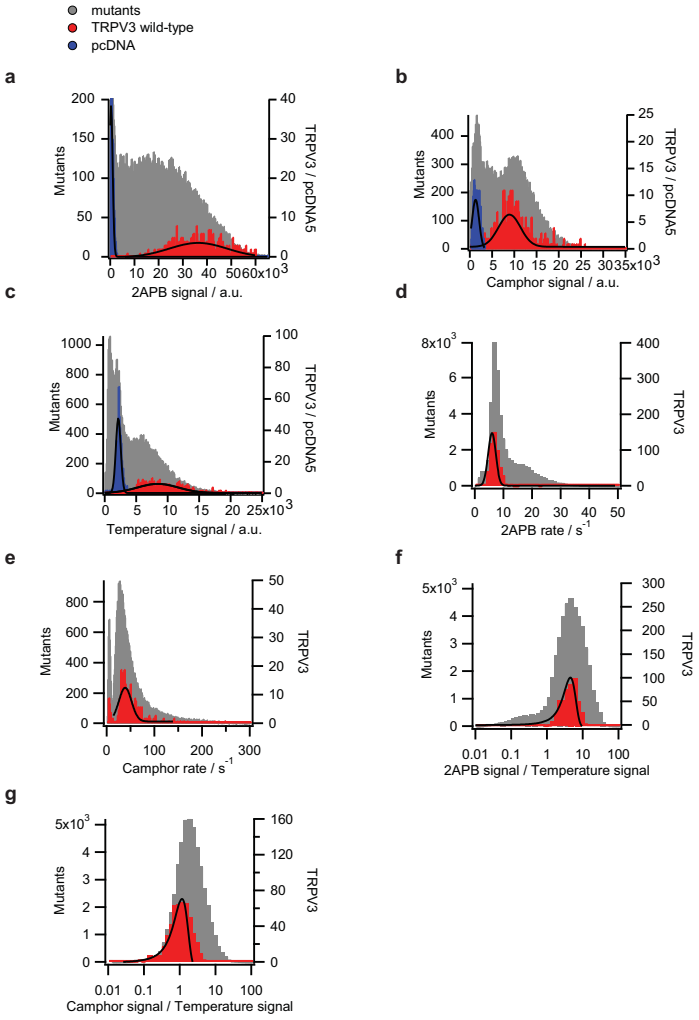
Supplementary Figure 1

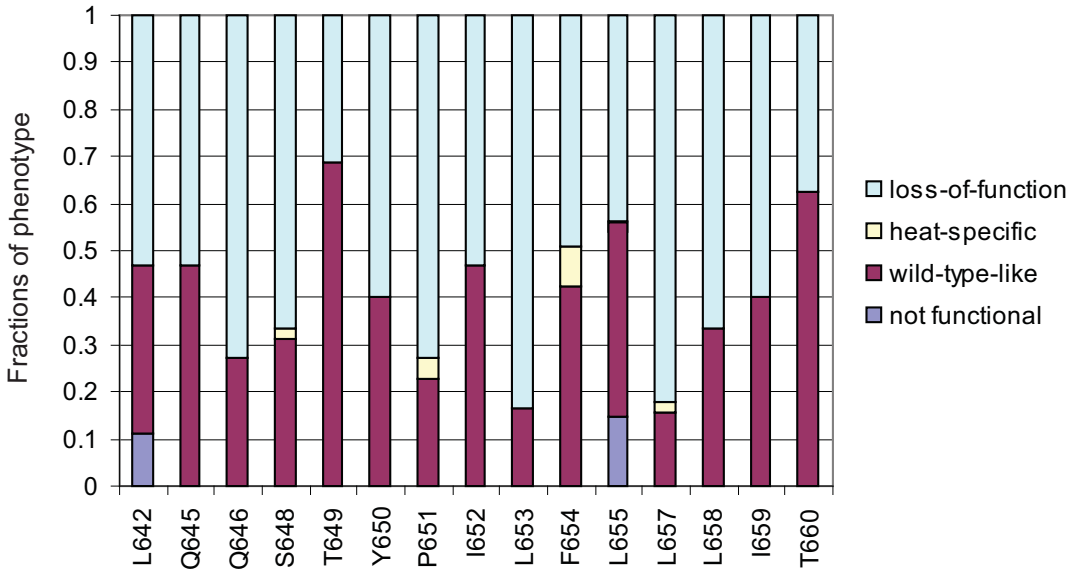


Supplementary Figure 1: Histograms of primary screen

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.

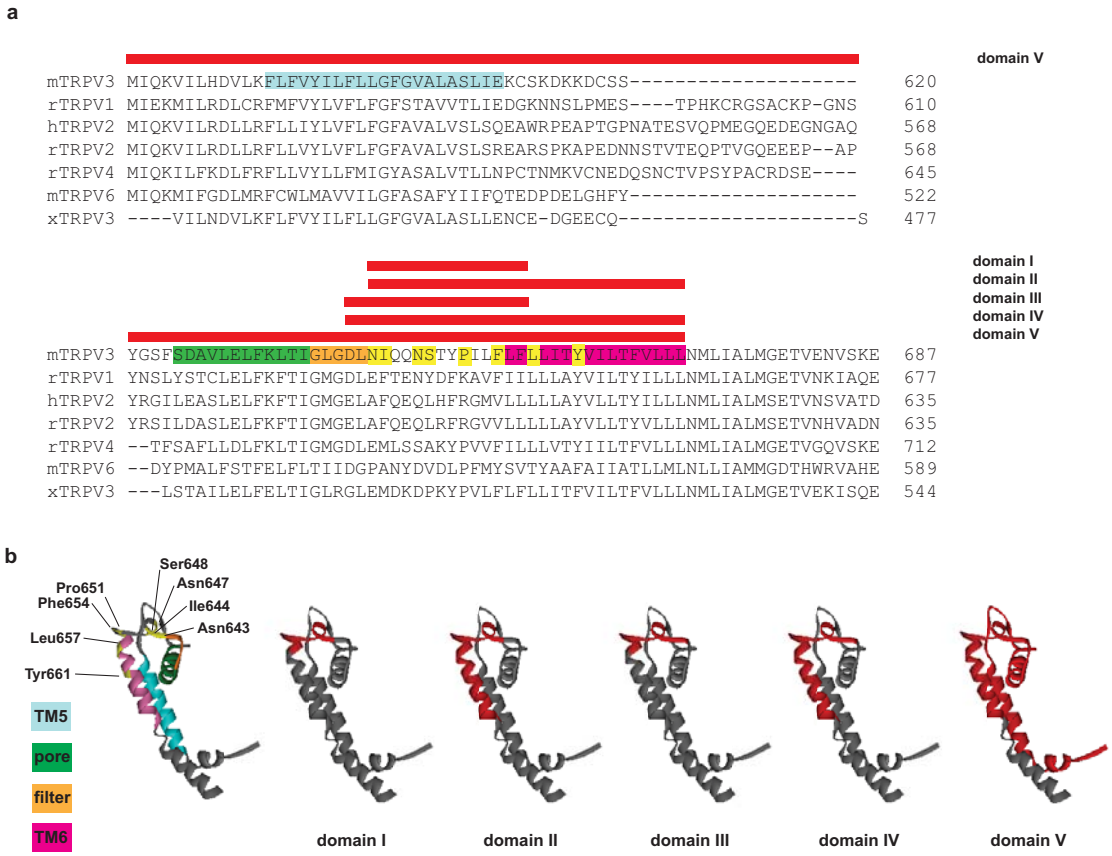
Supplementary Figure 2



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 µM 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



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 EC_{50} values of confirmed hits

Point mutations of 15 confirmed hits and their respective EC_{50}-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) / μM**	**EC_{50} (camphor) / mM**
TRPV3 wild-type	8.7±2.7	1.4±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