SUPPLEMENTARY FIGURE LEGENDS

FIGURE 1:

A; MG activated human TRPA1 receptors expressed in HEK 293t cells. All cells responding to 1 μM or 3 mM MG also showed an increase in intracellular calcium upon stimulation with carvacrol (250 μM, 30 s) which was used as an index of successful transfection. B; when applied to untransfected HEK 293t cells 10 mM MG did not evoke any responses (n=502), but the same concentration led to biphasic calcium transients in cells expressing the hTRPA1-3C mutant (n=668). The first phase of the response was absent in the hTRPA1-K710Q mutant (n = 161) and resembles calcium transients evoked by 1 mM MG in the hTRPA1-3C mutant (lower panel n = 110). The hatched area (upper pannel) indicates the different activation kinetic due to the lysine at position 710 of hTRPA1, especially at high MG concentration. C; summary of 10 mM MG effects on hTRPA1 mutants. The K710R (light grey) is more sensitive to MG compared to the K710Q mutant (light blue). The same applies to the hTRPA1-3C/K710R (black column) mutation compared to the hTRPA1-3C/K710Q mutant (dark blue) in which responsiveness to 10 mM MG is largely lost.

FIGURE 2:

ESI-TOF mass spectrometry analysis of the MG-induced changes in the course of the reaction.

A; experimentally obtained isotope distribution for the synthetic peptide (black) compared with calculated isotope distribution (red). B; deconvoluted spectrum of the peptide after the treatment with MG 500 μM for 15 min. The native peptide remains the most intense peak (monisotopic *m/z* 7661.50). For selected *m/z* regions with peak assignments see Figure 3B, a detailed list is provided in suppl. Table 1. C; spectrum of the *m/z* 3300-3500 region. The peptide fragment 17-45, present in the control spectrum of untreated peptide is shifted by 2 mass units in the MG treated sample (black: native, monoistopic *m/z* 3421.42, red: MG-treated, monoistopic *m/z* 3419.40) signifying the loss of 2H and the formation of a disulfide bond. The treated sample also shows the peak at *m/z* 3380.42 (green) corresponding to addition of MG to the fragment 18-45. D; following the nucleophilic attack of neighboring thiol to hemithioacetal, disulfide is formed with elimination of hydroxyacetone (see Figure 8). As a proof-of-concept we incubated 1 M DTT with 0.3 M MG and detected the resulting ketoalcohol as shown by obtained (black) and predicted (red) spectra of $[CH_2(OH)C(O)CH_3 + Na]^+$.

FIGURE 3:

DTT does not influence MG binding to lysine but reduces MG-evoked inward currents in the 3C hTRPA1 mutant:

Mass spectra of untreated A) lysine (1mM) and lysine following treatment with MG (10 mM) B; reaction of MG with lysine forms $[K\text{-}MG + NH4]^+$ (the chemical structure shown in D) as a main product. C; the same product is detected as dominant peak following DTT application after reaction of lysine and MG. D; reaction scheme depicting the formation of the detected product in the reaction of MG with lysine. E; DTT (5 mM) repeatedly and transiently reduced 3mM MG-induced currents in the hTRPA1-3C mutant indicating that further cysteine residues in the vicinity could by modified by MG and contribute to the responsiveness of hTRPA1-3C at higher MG concentrations.

SUPPLEMENTARY TABLES:

TABLE 1

Peptide fragments detected by ESI-TOF mass spectrometry after the exposure of 64 amino acids synthetic peptide to MG (aa 607-670, intracellular N-terminus).

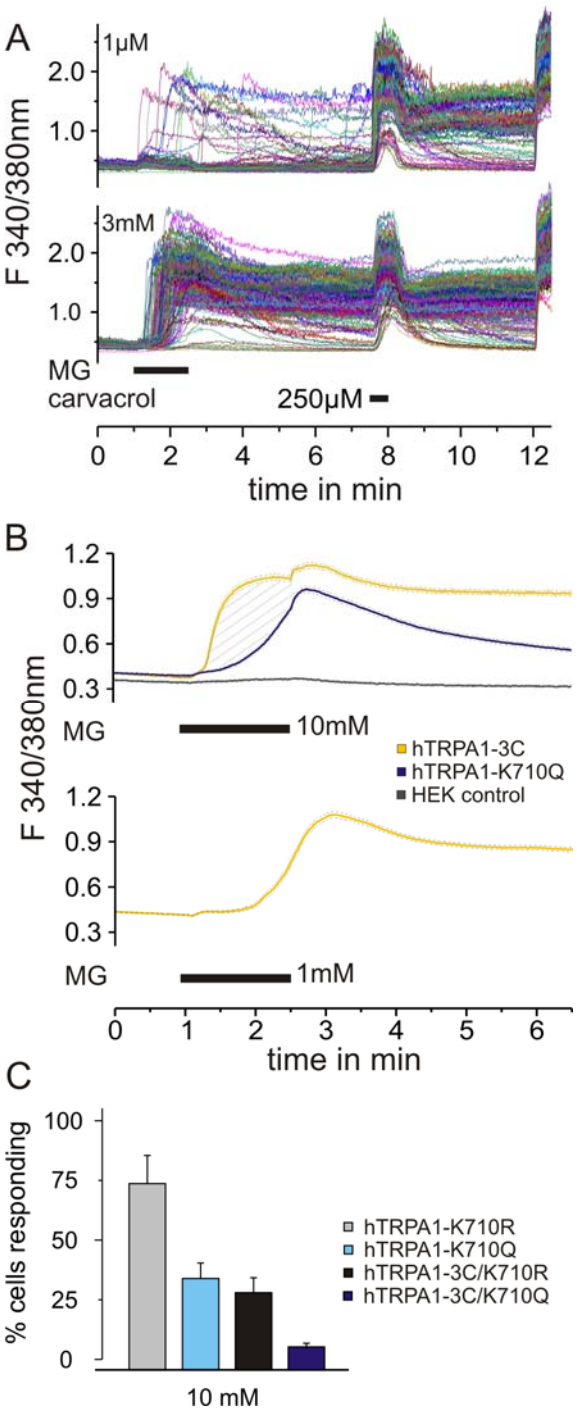
Unmodified	1 S-S	2 S-S	1MG	2MG	3MG
27-33	29-45	12-45	15-34	12-42	1-64
1-13	28-45	13-45	51-62	36-60	
17-21	23-53	17-45	18-45	18-47	
7-40	33-63	1-64	26-35		
1-64	24-50		23-40		
60-64			20-49		
26-31					

TABLE 2
Table of fibre populations and sensory properties, together with response to methylglyoxal stimulation

					pre MG application		MG 10mM response			post MG application	
strain	fiber type	n	cv (m/s)	median v. Frey (mN)	heat threshold (°C)	cold threshold (°C)	no. of spikes per 10 min	response latency (s)	responsive fibers n / n	heat threshold (°C)	cold threshold (°C)
C57BL/6	C-MH	11	0.37	5.7	40.0 ± 1.1		19.4 ± 5.4	45.1 ± 5.4	9 / 11	39.0 ± 2.4	
	C-MHC	9	0.43	8	40.8 ± 1.1	20.1 ± 2.4	63.0 ± 12.5	31.7 ± 10.2	9 / 9	41.6 0 ± 1.6	24.9 ± 1.0
	C-MC	2	0.37	6.85		25.6 ± 6.4	15.0 ± 2	14.4 ± 6.9	2 / 2		12.6 (n=1)
	C-M	2	0.4	3.35			171.5 ± 96.5	8.3 ± 1.7	2 / 2		
	A δ – RA non-responder	8	8.2	1			1.3 ± 0.6		0 / 8		
	A δ – RA responder	4	9.6	<1			1082.5 ± 528.5	54.0 ± 14.2	4 / 4		
	A δ - HTM non-responder	3	9.0	5.7					0/3		
TRP A1 -/-	C-MH	3	0.44	8	36.1 ± 2.2		11.3 ± 5.2	153.5 ± 122.3	2 / 3	33.9 ± 1.5	
	C-MHC	4	0.46	6.85	39.6 ± 1.8	18.22 ± 3.1	33.2 ± 3.3	82.1 ± 31.7	4 / 4	34.3 ± 4.3	n.t.
	A δ – HTM non-responder	1	2.1	8					0/8		

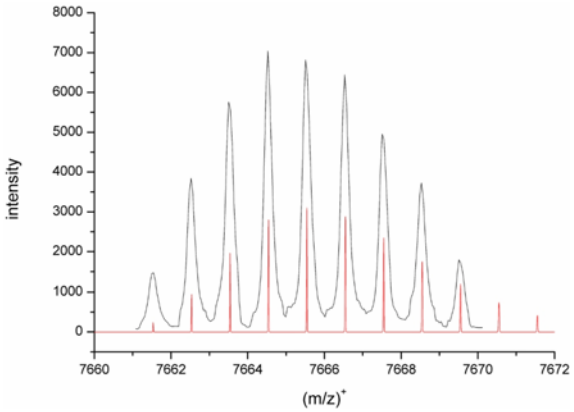
C-MH, mechano-, heat-sensitive; C-MHC, mechano-, heat-, cold-sensitive, C-MC, mechano-cold sensitive; C-M, mechano-sensitive; HTM, high threshold mechano-sensitive; n.t. not tested

SUPPLEMENTARY FIGURE 1

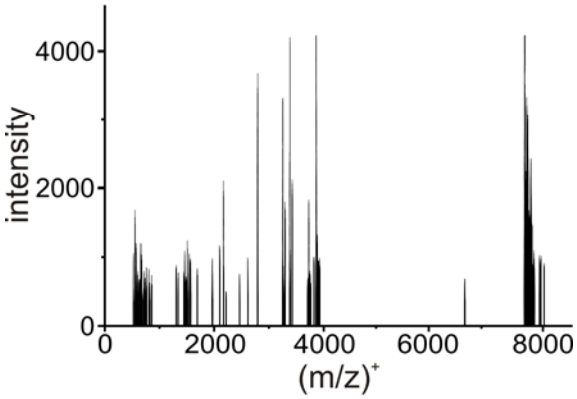


SUPPLEMENTARY FIGURE 2

A


intensity
8000
7000
6000
5000
4000
3000
2000
1000
0
7660
7662
7664
7666
7668
7670
7672
(m/z)+

B


intensity
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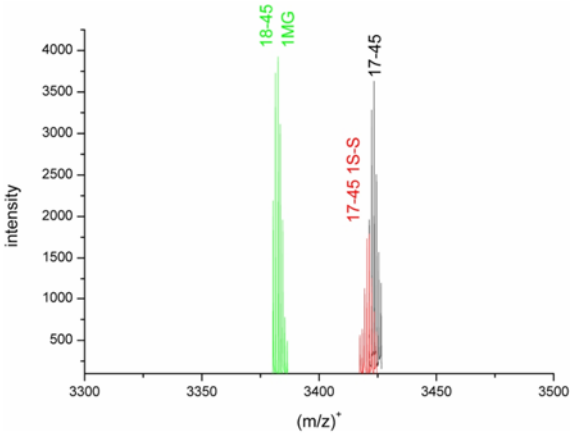
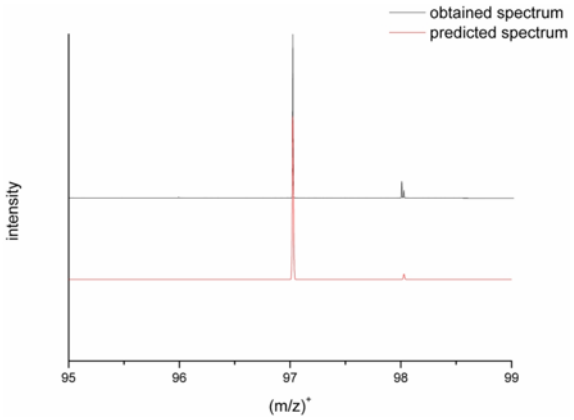
2000
0
0
2000
4000
6000
8000
(m/z)+

C


18-45
1MG
17-45
17-45 1S-S
intensity
4000
3500
3000
2500
2000
1500
1000
500
3300
3350
3400
3450
3500
(m/z)+

D


obtained spectrum
predicted spectrum
intensity
95
96
97
98
99
(m/z)+

SUPPLEMENTARY FIGURE 3