

Bitter taste receptors 38 and 46 regulate intestinal peristalsis

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Supplementary Figure S1

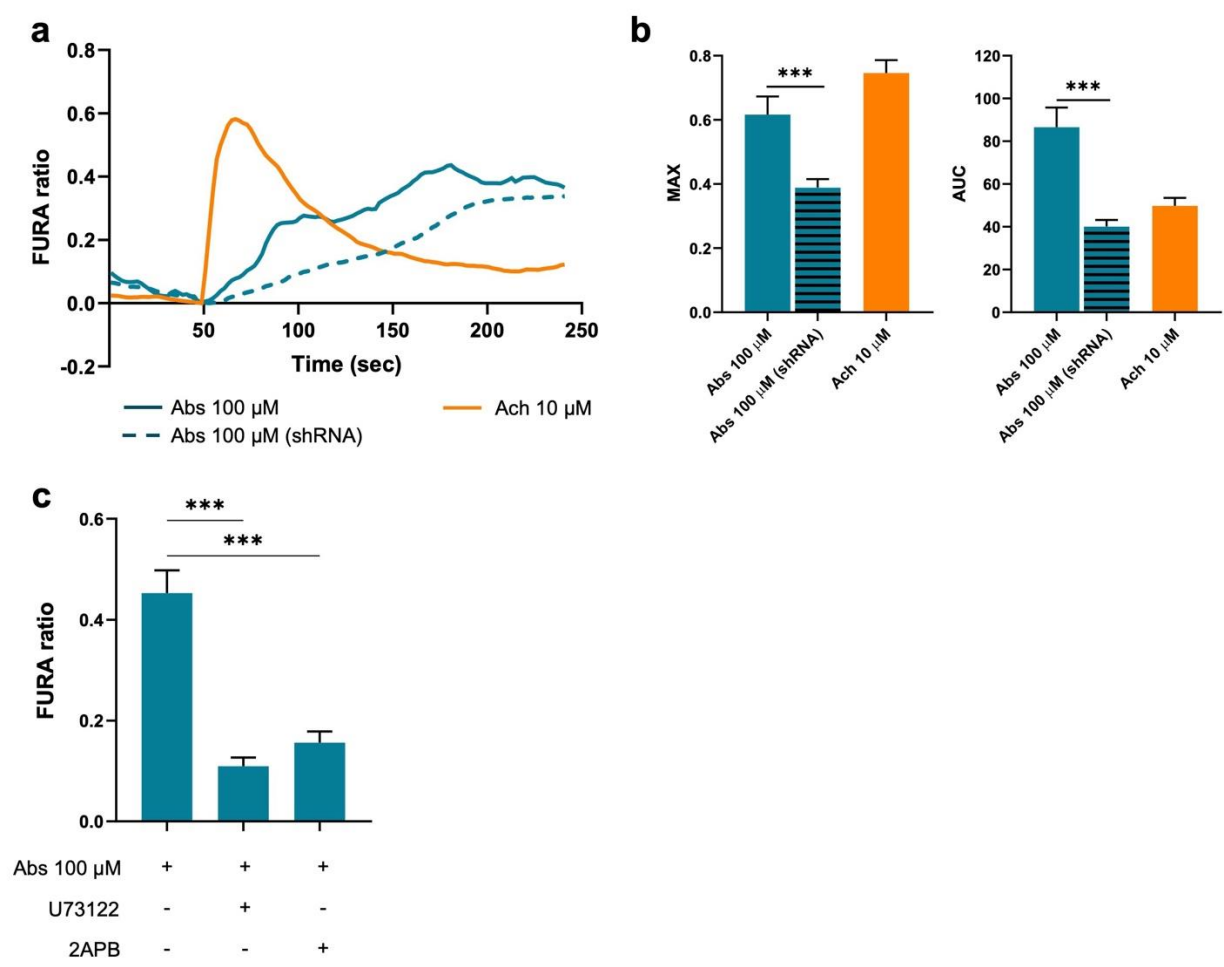


Figure S1. Cytosolic Ca^{2+} analysis after TAS2R46 activation with Abs 100 μ M. **a)** representative FURA 2-AM traces and cytosolic Ca^{2+} **b)** MAX and AUC of HISMIC or TAS2R46-silenced HISMIC (referred as shRNA) stimulated with Abs 100 μ M and Ach 10 μ M. Data are expressed as mean $\pm$ SEM of at least 40 cells acquired in four independent experiments. FURA 2-AM ratio of HISMICs stimulated with **c)** Abs 100 μ M in presence/absence of PLC inhibitor (U73122, 10 μ M) and IP₃R inhibitor (2APB, 10 μ M). Data are expressed as mean $\pm$ SEM of maximum peak of at least 40 cells acquired in four independent experiments. CTRL, no treated cells; Abs, absinthin; Ach, acetylcholine; MAX, maximum peak amplitude; AUC, area under the curve. *** p < 0.001 vs Abs 100 μ M.

Supplementary Figure S2

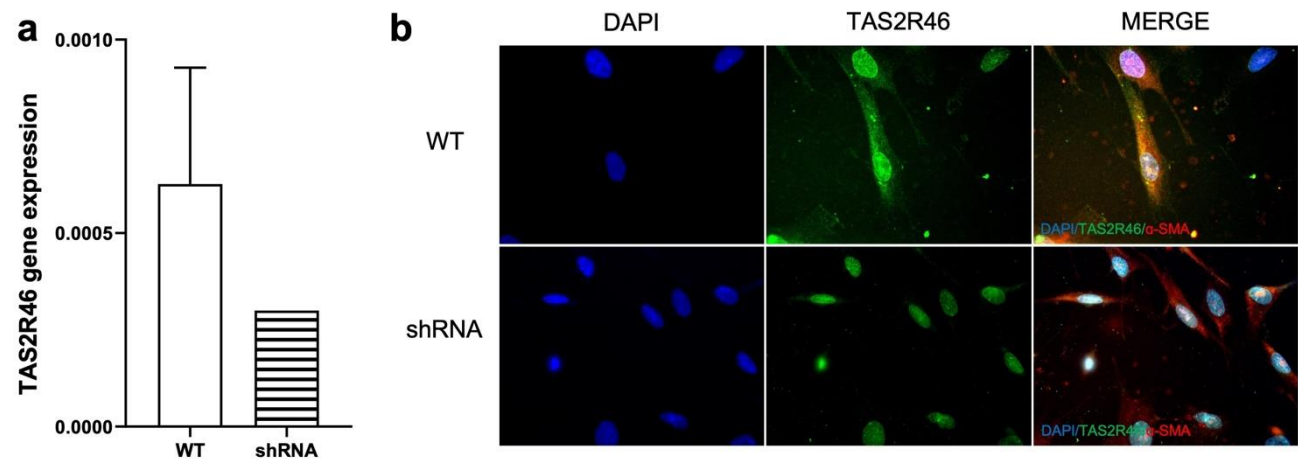


Figure S2. TAS2R46 silencing in HSMC. **a)** analysis of gene expression through qRT-PCR and **b)** indirect immunofluorescence analysis of TAS2R46 expression on HSMC (WT) and HSMC-silenced (shRNA).

Supplementary Figure S3

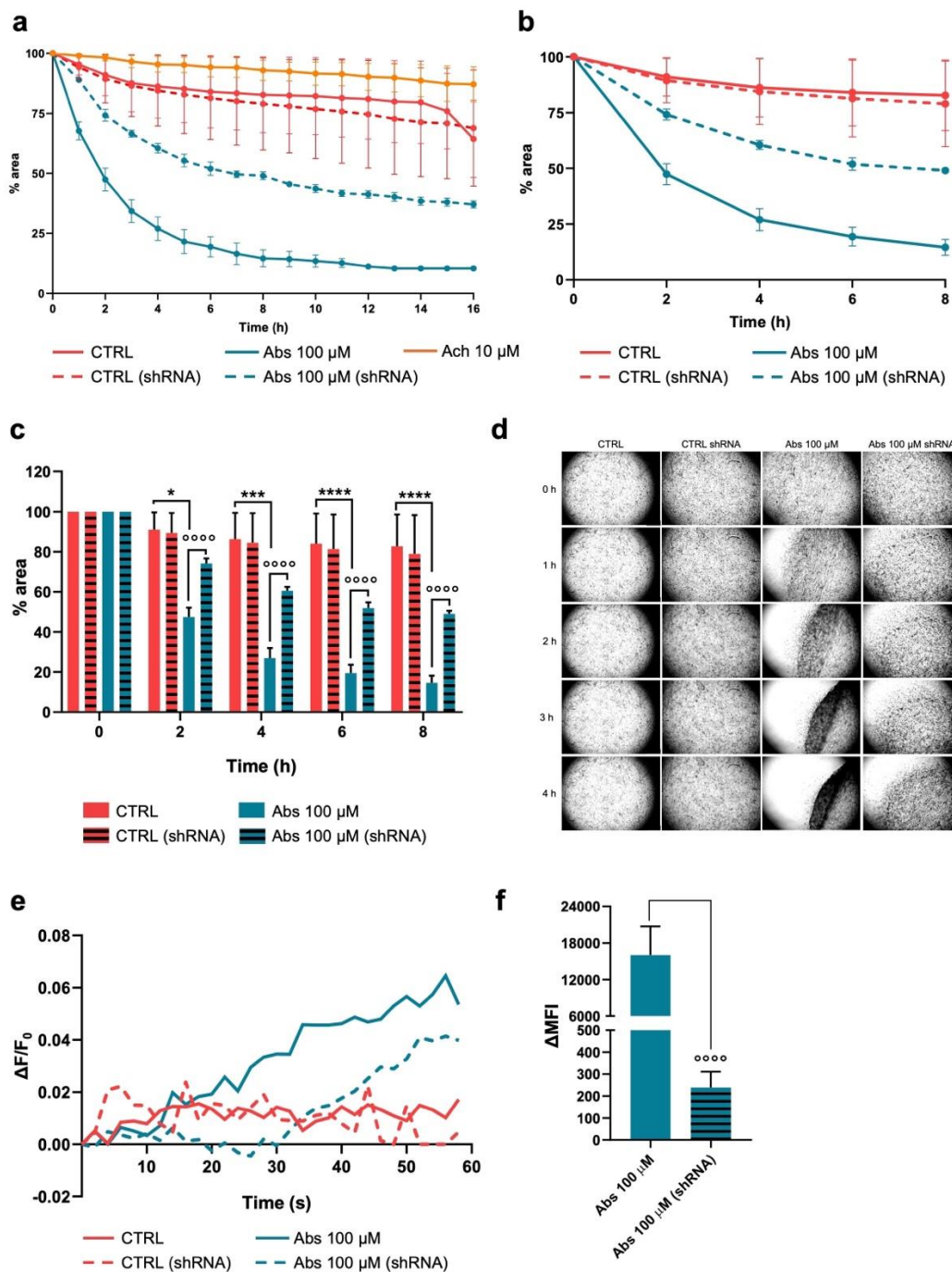


Figure S3. TAS2R46 activation induced cell contraction and change in membrane potential. **a-d)** HISM cells and TAS2R46-silenced HISM (referred as shRNA) were seeded on collagen disc and treated with Abs 100 μ M. **a)** Collagen disc areas were measured every hour starting immediately after stimulation (0h) until 16 hours later. **b)** zoom of collagen contraction between 0-8 hours after stimulation and **c)** histogram zoom representation. **d)** representative collagen contraction discs at 40x magnification. Data are expressed as mean $\pm$ SEM of three independent experiments. **e)** Membrane potential analysis indicated as $\Delta F/F_0$ of FluoVolt fluorescence acquired with micro-plate reader and **f)** FluoVolt delta mean fluorescence intensity (MFI) before and after stimulation with Abs 100 μ M measured with FACS. CTRL, no treated cells; Abs, absinthin; Ach, acetylcholine; Δ MFI, delta mean fluorescence intensity. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$ vs CTRL; °°°° $p < 0.0001$ vs Abs 100 μ M

Supplementary Table S1

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
<i>hTAS2R1</i>	TGTGGTGGTGAATGGCATTG	TAAGAGAATTGCACAATTCGCAG
<i>hTAS2R3</i>	ACACATGATTCAGGGATAATAATGC	TACCATCACCTAGAAACTCTC
<i>hTAS2R4</i>	TACAGTGGTCAATTGCAAAACTTGG	CTGACCTTTCCGTATTTGAAGAG
<i>hTAS2R5</i>	TGGTCCTCATATAACCTCATTATC	CCAGGACCCAGAAGATACTA
<i>hTAS2R7</i>	GTGCTATATCCAGATGTCTATGC	ATCCAGGAAATCACCTGTCTC
<i>hTAS2R8</i>	AGTTATCGCCAGAATTTGTTTGATC	GAAGACATTAAGGCAGGTGG
<i>hTAS2R9</i>	TGAATTGACCATAGGGATTTGGG	AAGAGCAGCATAAAGAAGCCATC
<i>hTAS2R10</i>	GACTTGTAAACTGCATTGACTGTG	GCTGGTGGCAAACACATAC
<i>hTAS2R13</i>	TCAGTAAAAGAGAGCTGTCCTC	CATAATTCTTAATCCTGTTCCAGAC
<i>hTAS2R14</i>	GCTTTGGCAATCTCTCGAATTAG	TGTCCAGATATTAGTAAGCATTCTG
<i>hTAS2R16</i>	CCAGGCTCATAACAGTTGCAT	GAAGCGCGCTTTCATGCTT
<i>hTAS2R19</i>	GGTTTACTCTGGGTCATGTTATTC	GCAAACAAAATATGCTGAGGCTAG
<i>hTAS2R20</i>	GCACTGATAAATTTCAATTGCCTGG	GAAGTTGGATTCAACACAGTTGAA
<i>hTAS2R30</i>	GTTATTACTACATTGGTATGCAACTC	GAGGCTAGTAGCAAGCCAGCT
<i>hTAS2R31</i>	GCATTGGTAAATTCCATTGAGCG	AGCTGGATTAAACACAGTTGAATAC
<i>hTAS2R38</i>	ACAGTGATTGTGTGCTGCTG	TGGCTTGGTAGCTGTGGTTC
<i>hTAS2R39</i>	TGTCGCCATTTCTCATCACCTTA	TGCCACTTGTGGAAACTGCC
<i>hTAS2R40</i>	GAGTGCATCACTGGCATCCTT	CAGCATCATCCAAATCTGTAGC
<i>hTAS2R41</i>	GGTTGCTGCCCTTGGATATGA	TACTCGACCTTCTGGGCAGA
<i>hTAS2R42</i>	CATTTTTTTTCCTTACAAGTGGCC	GCTTGCTGTTTCCCAGAATGAG
<i>hTAS2R43</i>	GCTAATGGCTTCATAGCACTGG	TGCTGAAATGGTTGATCACTGC
<i>hTAS2R45</i>	CTCCTTTGCTGACCAAATTGTC	AGTTGCTGAAATGGCCGGTTAC
<i>hTAS2R46</i>	GAGTTGAATCCAGCTTTTAACAG	GGCAATCTTGAGCAAATAAAATATGC
<i>hTAS2R50</i>	GGTAAATTTCAATTGACTGGGTGAAGAG	GCTGGATTCAACACAGTTAAATACCAA
<i>hTAS2R60</i>	CAGGCAATGGCTTCATCACTG	TGGTCTTACCCATTACCACTG
<i>GAPDH</i>	AACGTGTCAGTGGTGGACCTG	AGTGGGTGTCGCTGTTGAAGT