Electronic Supplementary Material (ESI) for Food & Function.

Supplementary material

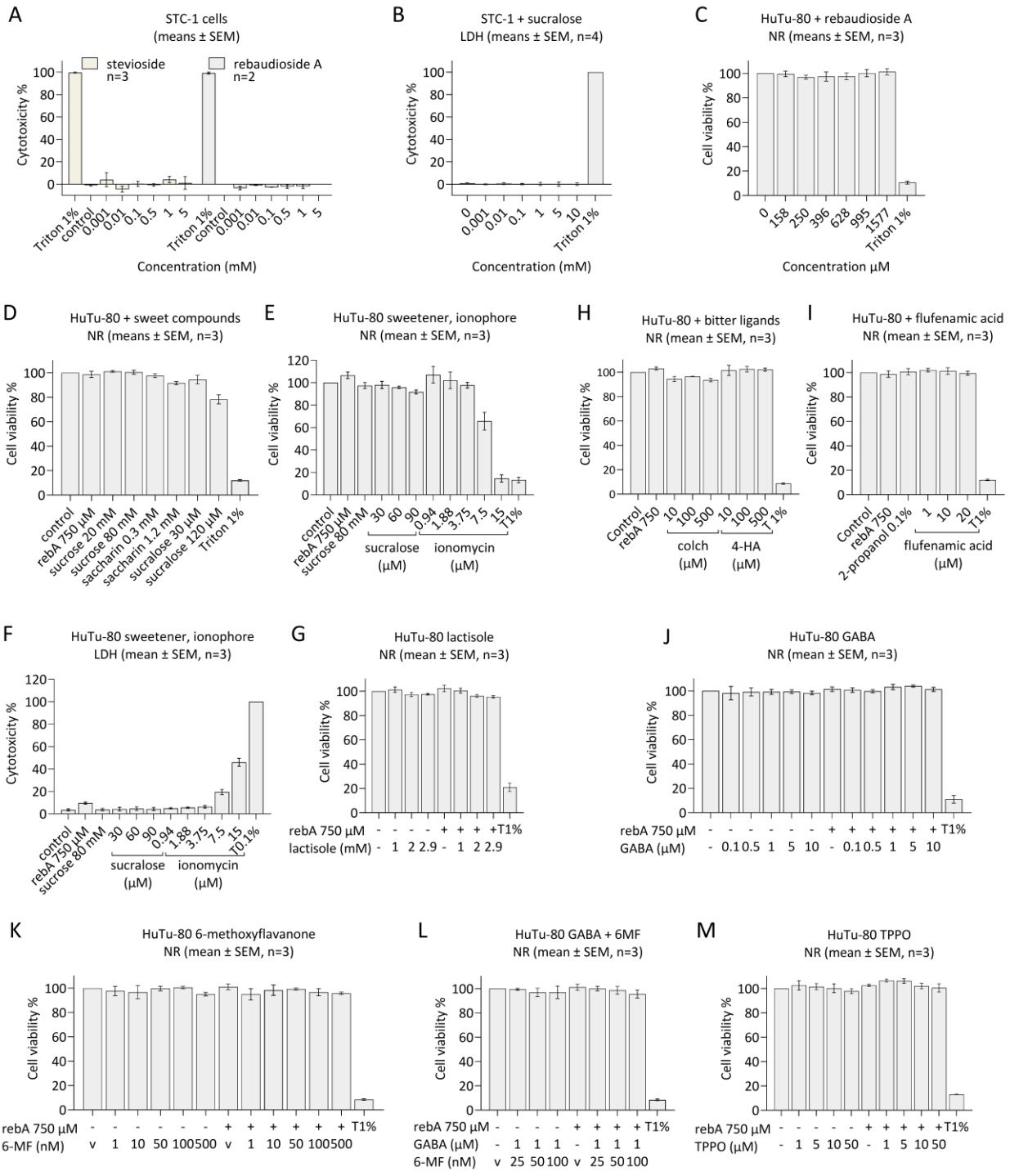

Figure S1. Cytotoxicity by neutral red test (NR) and/or lactate dehydrogenase test (LDH) tests. (A-B) Sweet compounds in STC-1 cells. (C) Curve of rebA in HuTu-80 cells. (D-F) Sweet compounds in HuTu-80 cells. (G) Sweet taste receptor blocker lactisole. (H-I) Bitter compounds in HuTu-80 cells. (J-L) Bitter taste receptor blockers. (M) TRPM5 blocker. OD values are expressed relatively to the control assay buffer (100%). Triton bars correspond to total cell-death. v: solvent vehicle.

Table S1. Statistics. Confidence intervals (CI) and *P*-values of every comparison.

A. Gurmarin

Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted *P* Value
rebA 1 mM vs. gurmarin 0.03 µg/ml + rebA 1 mM	-0.1409	-2.833 to 2.551	ns	>0.9999
rebA 1 mM vs. gurmarin 0.1 µg/ml + rebA 1 mM	-0.4927	-3.185 to 2.200	ns	>0.9999
rebA 1 mM vs. gurmarin 0.3 µg/ml + rebA 1 mM	-0.05482	-2.747 to 2.637	ns	>0.9999
rebA 1 mM vs. gurmarin 3 µg/ml + rebA 1 mM	-1.153	-3.845 to 1.539	ns	>0.9999

B. MDL

Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted *P* Value
control vs. MDL 10 µM	0.3427	-0.4376 to 1.123	ns	0.5229
rebA 1 mM vs. rebA 1 mM + MDL 10 µM	1.217	0.4368 to 1.997	**	0.0053

C. Sweet compounds

Uncorrected Fisher's LSD	Mean Diff.	95.00% CI of diff.	Summary	Individual *P* Value
control vs. rebA 750 µM	-1.013	-1.234 to -0.7931	****	<0.0001
control vs. sucrose 20 mM	-0.01933	-0.2395 to 0.2009	ns	0.8515
control vs. sucrose 80 mM	-0.2897	-0.5099 to -0.06946	*	0.0142
control vs. saccharin 0.3 mM	-0.02267	-0.2429 to 0.1975	ns	0.8263
control vs. saccharin 1.2 mM	-0.006667	-0.2269 to 0.2135	ns	0.9485

D. Sweetener and calcium ionophore

Uncorrected Fisher's LSD	Mean Diff.	95.00% CI of diff.	Summary	Individual *P* Value
control vs. rebA 750 µM	-2.328	-2.916 to -1.740	****	<0.0001
control vs. sucrose 80 mM	-0.6430	-1.169 to -0.1170	*	0.0195
control vs. sucralose 30 µM	-0.02200	-0.5480 to 0.5040	ns	0.9307
control vs. sucralose 60 µM	-0.5477	-1.074 to -0.02170	*	0.0422
control vs. sucralose 90 µM	-0.3770	-0.9030 to 0.1490	ns	0.1488
control vs. ionomycin 0.94 µM	-0.3813	-0.9073 to 0.1446	ns	0.1445
control vs. ionomycin 1.88 µM	-0.3643	-0.8903 to 0.1616	ns	0.1621
control vs. ionomycin 3.75 µM	-0.3837	-0.9096 to 0.1423	ns	0.1422

E. Lactisole

Bonferroni's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted *P* Value
control vs. lactisole 1 mM	-0.3900	-14.63 to 13.85	ns	>0.9999
control vs. lactisole 2 mM	-2.907	-17.15 to 11.33	ns	>0.9999
rebA 750 µM vs. lactisole 1 mM	-7.740	-21.98 to 6.501	ns	0.5469
rebA 750 µM vs. lactisole 2 mM	-16.98	-31.22 to -2.742	*	0.0175

F. Bitter compounds

Uncorrected Fisher's LSD	Mean Diff.	95.00% CI of diff.	Summary	Individual *P* Value
control vs. rebA 750 µM	-1.478	-1.811 to -1.144	****	<0.0001
control vs. colchicine 1 µM	-0.08979	-0.4983 to 0.3187	ns	0.6574
control vs. colchicine 10 µM	-0.06805	-0.4766 to 0.3405	ns	0.7366
control vs. colchicine 100 µM	-0.1446	-0.5531 to 0.2640	ns	0.4762
control vs. colchicine 500 µM	0.02641	-0.3821 to 0.4349	ns	0.8961
control vs. 4-HA 1 µM	0.3097	-0.09878 to 0.7183	ns	0.1323
control vs. 4-HA 10 µM	0.1258	-0.2828 to 0.5343	ns	0.5351
control vs. 4-HA 100 µM	0.1200	-0.2886 to 0.5285	ns	0.5539
control vs. 4-HA 500 µM	-0.02895	-0.4375 to 0.3796	ns	0.8861
control vs. fluf. acid 1 µM	0.03100	-0.3775 to 0.4395	ns	0.8781
control vs. fluf. acid 10 µM	-0.03600	-0.4445 to 0.3725	ns	0.8587
control vs. fluf. acid 20 µM	0.06067	-0.3479 to 0.4692	ns	0.7642

G. GABA

Uncorrected Fisher's LSD	Mean Diff.	95.00% CI of diff.	Summary	Individual *P* Value
rebA 750 µM vs. GABA 0.1 µM	11.98	-4.585 to 28.55	ns	0.1486
rebA 750 µM vs. GABA 0.5 µM	16.59	0.02789 to 33.16	*	0.0496
rebA 750 µM vs. GABA 1 µM	20.61	4.048 to 37.18	*	0.0169
rebA 750 µM vs. GABA 5 µM	20.12	3.551 to 36.68	*	0.0194
rebA 750 µM vs. GABA 10 µM	12.95	-3.619 to 29.51	ns	0.1198
control vs. GABA 0.1 µM	-1.483	-18.05 to 15.08	ns	0.8549
control vs. GABA 0.5 µM	-0.1200	-16.69 to 16.45	ns	0.9882
control vs. GABA 1 µM	-0.6733	-17.24 to 15.89	ns	0.9338
control vs. GABA 5 µM	2.327	-14.24 to 18.89	ns	0.7744
control vs. GABA 10 µM	1.340	-15.23 to 17.91	ns	0.8688

H. 6-Methoxyflavanone

Uncorrected Fisher's LSD	Mean Diff.	95.00% CI of diff.	Summary	Individual *P* Value
vehicle vs. 6-MF 50 nM	-15.27	-35.21 to 4.659	ns	0.1238
vehicle vs. 6-MF 100 nM	-11.51	-31.44 to 8.425	ns	0.2387
vehicle vs. 6-MF 500 nM	-12.04	-31.97 to 7.895	ns	0.2187
rebA 750 µM vs. 6-MF 50 nM	-56.72	-76.65 to -36.79	****	<0.0001
rebA 750 µM vs. 6-MF 100 nM	-50.63	-70.56 to -30.70	****	<0.0001
rebA 750 µM vs. 6-MF 500 nM	-25.89	-45.82 to -5.958	*	0.0141

I. GABA 1 µM + increasing 6-MF (*combination*)

Uncorrected Fisher's LSD	Mean Diff.	95.00% CI of diff.	Summary	Individual *P* Value
vehicle vs. combination 25 nM	-10.51	-29.64 to 8.618	ns	0.2611
vehicle vs. combination 50 nM	-14.40	-33.53 to 4.734	ns	0.1302
vehicle vs. combination 100 nM	-18.04	-37.17 to 1.088	ns	0.0628
rebA 750 µM vs. combination 25 nM	-28.69	-47.82 to -9.562	**	0.0058
rebA 750 µM + vs. combination 50 nM	-36.74	-55.87 to -17.61	***	0.0009
rebA 750 µM + vs. combination 100 nM	-34.37	-53.50 to -15.24	**	0.0015

J. TPPO

Uncorrected Fisher's LSD	Mean Diff.	95.00% CI of diff.	Summary	Individual *P* Value
vehicle vs. TPPO 5 µM	10.74	-8.021 to 29.50	ns	0.2361
vehicle vs. TPPO 50 µM	7.970	-10.79 to 26.73	ns	0.3729
rebA 750 µM vs. TPPO 5 µM	4.983	-13.78 to 23.74	ns	0.5735
rebA 750 µM vs. TPPO 50 µM	27.44	8.675 to 46.20	**	0.0078

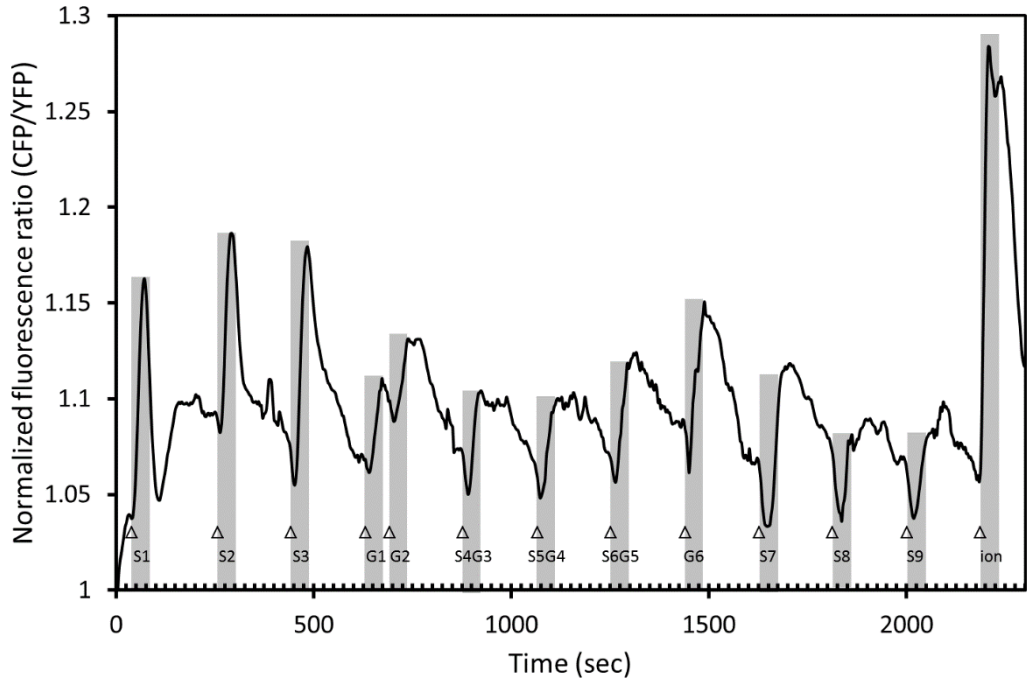

Figure S3. Intracellular [Ca^{2+}] changes of the injections series of Sucrose (S), Gurmarin (G) and the combinations of both. Effect of gurmarin on sucrose-induced calcium responses in STC-1 cells stably expressing the Cameleon YC3.6 calcium reporter protein. Shown are the global averages of all imaged cells (1315 ROIs). A moving average (16.25 seconds, 5 values) was used to suppress effects of high frequency calcium oscillations (~3-6 sec, ~0.1 amplitude) and cell synchronization.

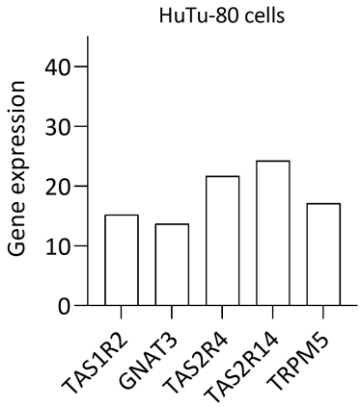

Figure S4. Gene expression level in unstimulated HuTu-80 cells of the TRPM5 calcium-dependent channel, together with the taste receptors for rebA TAS1R2, TAS2R4, TAS2R14, and the G protein α-gustducin (GNAT3). Values are normalized intensity obtained from GSM887155.[49]