non-taster alleles of T1R3 used contructs of complementary DNA coding for T1R3 from C57BL/6 and 129/Sv mice, respectively[7–11,21].

Immunoprecipitation

Antibodies against T1R3 were generated using a peptide corresponding to residues 824–845 of the mouse receptor. PEAK^{rapid} cells (Edge Biosciences) were transfected with HA–T1R1, HA–T1R2 and T1R3 in various combinations and were gathered and disrupted in buffer containing 50 mM Tris-HCl at pH 7.5, 300 mM NaCl, 1% NP-40, 0.5% w/v sodium deoxycholate, and protease inhibitors (Roche). Lysates were incubated overnight at 4 °C with mouse monoclonal anti-HA antibody (Santa Cruz) and immune complexes were collected with protein A/G–agarose beads. Samples were fractionated by SDS–PAGE, transferred to nitrocellulose membrane and probed with anti-T1R3 antibody. As a control for the specificity of the interactions, we have shown that artificially mixing extracts from cells expressing tagged T1R1 or T1R2 with extracts from cells expressing T1R3 does not produce complexes. Similarly, co-transfection of a Rho-tagged mGluR1 receptor[15] did not produce T1R–GluR1 complexes.

Nerve recording

Lingual stimulation and recording procedures were performed as previously described[27]. Neural signals were amplified (2,000 ×) with a Grass P511 AC amplifier (Astro-Med), digitized with a Digidata 1200B A/D convertor (Axon Instruments), and integrated (r.m.s. voltage) with a time constant of 0.5 s. Taste stimuli were presented at a constant flow rate of 4 ml min^{-1} for 20-s intervals interspersed by 2-min rinses between presentations. All data analyses used the integrated response over a 25-s period immediately after the application of the stimulus. Each experimental series consisted of the application of six tastants bracketed by presentations of 0.1 M citric acid to ensure the stability of the recording. The mean response to 0.1 M citric acid was used to normalize responses to each experimental series.

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Competing interests statement

The authors declare that they have no competing financial interests.

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addendum

Virus-mediated killing of cells that lack p53 activity

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Some background information to our work on adeno-associated virus (AAV)-induced apoptosis in cells lacking p53 activity was omitted owing to space constraints. The oncosuppressive activity of parvoviruses has been reviewed[1,2]. AAV inhibits cell cycle progression[3], even when ultraviolet-inactivated[4], as do AAV-coded Rep proteins[5]. p53-dependent cytopathic effects of parvovirus H1 have been reported[6]. H1 is an autonomous virus that can replicate in cells and lyse them. This is different from AAV, which is defective and does not replicate in the conditions we used. H1 and AAV share little sequence homology and the structures of the DNA termini are not the same. □

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