Supplementary methods

Human tissue

Eighteen human fungiform samples were obtained through word-of-mouth and flyer recruitment on the University of Colorado – Anschutz Medical Campus (CU AMC). After giving informed consent, each subject underwent fungiform papillary biopsies in which 3 fungiform papillae were removed without anesthesia using sterile iridectomy scissors (protocol 14-0439 approved by the Colorado Institutional Review Board). In addition, 5 human fungiform samples were obtained through the Smell & Taste Clinic, Department of Otorhinolaryngology, Technical University of Dresden under the approval of the Ethics Commission of the Technical Univ. of Dresden, #EK499112019. 12 human laryngeal samples were obtained through Children's Hospital Colorado via supraglottoplasties performed for obstructive sleep apnea in children (ages 3-25 months old) in which laryngeal tissue is removed during the normal course of surgery (protocol 14-0776 approved by the Colorado Institutional Review Board). Finally, for the human duodenal tissue, the Pathology Shared Resource has a Colorado Multiple-Institutional Review Board approved protocol (COMIRB 15-1641) to store de-identified tissue from clinical procedures. The provided tissue was remnant archival tissue from a surgical procedure. This research is considered non-human subjects research as the material was provided in a de-identified manner to the study with the Pathology Shared Resource acting as the Honest Broker.

Reagent validation

The P2X2 antisera we employed are directed against a peptide in the C-terminal region of rat P2X2 (457-472 STSTDPKGLAQL). This region of the c-terminus is conserved even in c-terminal variants [36-38] so the antisera that we employed is expected to react with all P2X2 variants described as being functionally present in human tissues. The relevant c-terminal region is lacking in variants f and g which are not of functional relevance and have only been described in rats [36, 37]. The P2X2 antiserum was validated in a heterologous expression system employing human P2X2 expressed in HEK cells (hP2X2- HEK). hP2X2-HEK cells and non-transfected HEK cells were stained with antisera against P2X2 both in the absence and presence of P2X2 blocking peptide (**Table 2, S1**). hP2X2- HEK cells show P2X2 immunoreactivity which is absent in the presence of P2X2 blocking peptide (S1). Non-transfected HEK cells also do not show P2X2 immunoreactivity (**S1**).

P2X2 and P2X3 antisera were also tested in human, monkey, and mouse intestinal tissues, which all express P2X2 and P2X3 in submucosal plexus neurons (**S2**), consistent with previous work [27]. Intestinal tissues were stained with antisera against Tuj1 to identify both ganglion cells and nerve fibers of the submucosal plexus as well as with P2X2 or P2X3 to validate immunoreactivity within fixed, frozen human tissues. Submucosal neurons from humans, mice, and monkeys all demonstrated immunoreactivity for P2X2, P2X3, and Tuj1.

PLCβ2 antiserum, generated against EPLVSKADTQESRL (C-terminal amino acids 1168 to 1181 of mouse sequence (85% identical to human sequence) was validated on mouse taste tissue both by Western blot and by blocking of staining with cognate peptide (0.1µg/ml for antiserum used at 1:1000).

Finally, to account for different fixation conditions (German human samples and rhesus macaque were fixed in 10% formalin while and other human tissues were fixed in 4% PFA or PLP), mouse tissues were fixed under these different conditions. All demonstrated reactivity for both P2X3 and P2X2 regardless of primary fixative employed (data not shown).

Immunohistochemistry

The tissues were sectioned at 14µm-16µm on a cryostat and mounted directly on Superfrost Plus slides (Fisher Scientific) in a 1:3 or 1:4 series. Since taste buds are 40-50 um across, this section thickness allowed a single taste bud to be present in adjacent slides. After drying, slides were rinsed in deionized water, and then underwent antigen retrieval in buffer (1X Tris-EDTA, pH 9.0 for all human samples) at a temperature of 85°C for 10 minutes. After being allowed to cool, the slides were rinsed three times for 5 minutes each in 0.1M PBS, non-specific binding was blocked for at least 1hr at room temperature in blocking solution (2% normal goat serum, 1% bovine serum albumin, 0.3% Triton in PBS). Next, the slides were incubated for at least two nights at 4°C with primary antibodies in blocking solution (**Table 2**). After incubation with primary antibodies, the tissue samples were rinsed with 0.1M PBS three times for 10 minutes per rinse. They were then incubated for 2 hours with fluorescent secondary antibodies at 1:800 dilution (**Table 2**). Finally, the slides were washed twice for 10 minutes each in 0.1M PBS and one time for 10 minutes in 0.05M PB before being coverslipped with DAPI Fluormount (SouthernBiotech – Birmingham, AL, USA).

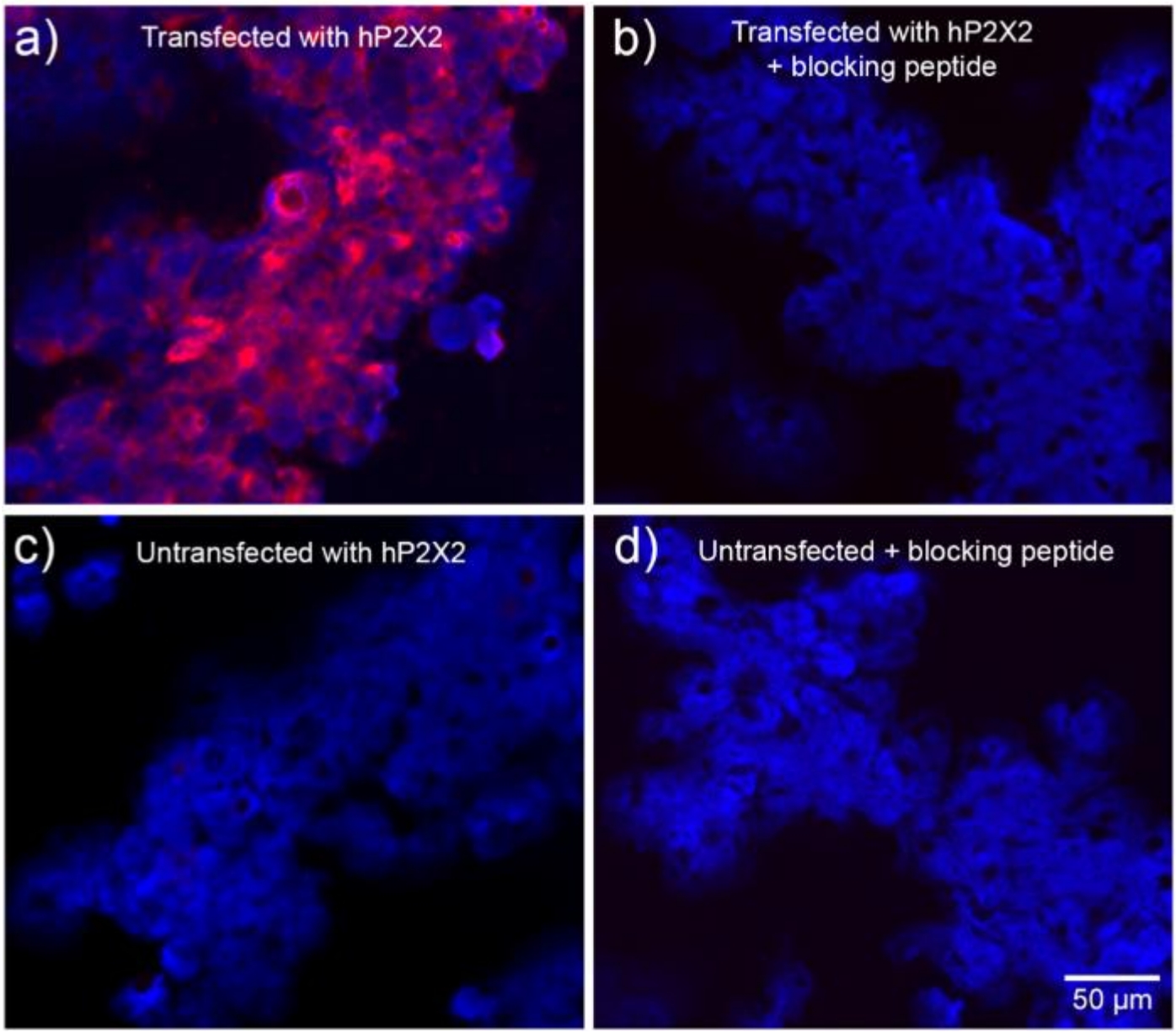
Data analysis

Images of human fungiform and laryngeal taste buds were scored for both P2X3 and P2X2-immunoreactivity by five experienced, independent scorers blinded to condition (0=not present, 1=unclassified, 2=present). When possible, adjacent sections were stained so that P2X2 and P2X3 could be scored across the same set of taste buds per sample. Up to five taste buds were scored per sample per staining condition (i.e. P2X2 vs. P2X3 staining) by these five independent scorers. Staining for the type II taste cell markers GNAT3 or PLCβ2 enabled selection of sections to include the main part of the taste bud. Only sections with taste buds containing two or more GNAT3- or PLCβ2-immunoreactive cells as well as a visible basal plexus stained by Tuj1 were scored to ensure that sections being scored included the central part of the taste bud and were not located at the edge of a taste bud, where nerve fiber density is lower. Any taste bud images receiving average scores between 0.6 and 1.4 or any images with a set of ratings including both a 0 and a 2 were designated as unclassified and further evaluated using confocal microscopy to determine the presence or absence of P2X2 or P2X3. These unclassified samples were imaged at 40x on a Leica SP8 confocal microscope, and scorers were

then asked to rescore these confocal images to make their final determinations. Samples with average above 1.5 were positive and those below 0.5 were considered to be negative. Any taste buds that were still unclassified after their confocal images were re-evaluated underwent quantitative colocalization analysis between the Tuj1 and P2X2/P2X3 channels using Coloc2, a Fiji plugin (**S3**) [29, 30]. A set of known positive and known negative taste buds also underwent colocalization analysis as a means of establishing parameters for Coloc2's Pearson correlation outputs (**S3**). These known positive and negative taste buds were used to generate a two-independent-groups mean difference plot to establish a cutoff value for re-categorizing unclassified samples as being positive or negative for P2X2 staining (**S4**) in combination with their original average scores [31]. Using this cut-off value, unclassified taste buds could then be classified into negative or positive categories. All previously unclassified taste buds were able to be classified as positive or negative using this cut-off value (r = 0.1).

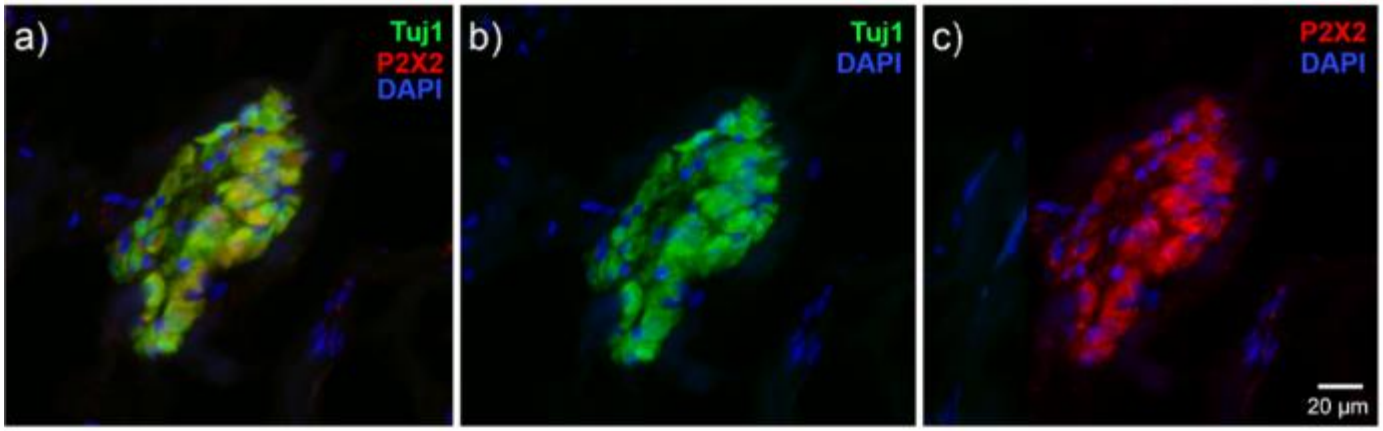
To validate the Pearson's values generated from Coloc2, images of taste buds undergoing this analysis were cropped to a tight square surrounding the taste bud but excluding much of the surrounding epithelium. Two r values were generated using Coloc2 – one with the channels (Tuj1 and P2X2/P2X3) as they were imaged and another with the P2X channel rotated 90° to decorrelate positional information. For all taste buds, when one channel was rotated 90 degrees, the Pearson's above threshold value was near 0, i.e. no correlation. This helped establish that the Coloc2 above-threshold Pearson's value was sufficient to determine colocalization between Tuj and P2X channels. These Pearson's values were used to determine a threshold for P2X immunoreactivity.

Supplementary 1 - figure



Supplementary figure 1. P2X2 antiserum from Alomone labs was validated in HEK cells in both untransfected cells and cells transfected with human P2X2 (a-d). This is the P2X2 antibody used in the majority of the samples. DAPI is shown in blue while P2X2 staining is shown in red. In HEK cells expressing human P2X2, P2X2 immunoreactivity appears red (a). However, with the addition of blocking peptide, no staining is observed (b). Untransfected HEK cells do not demonstrate immunoreactivity for P2X2 with or without peptide (c, d).

Supplementary 2 - figure



Supplementary figure 2. P2X2 antisera were validated in duodenal tissue (a-c). Tuj1 was used to visualize the nerve cells of the submucosal plexus (b), which demonstrated immunoreactivity to two separate antibodies against P2X2 (ThermoFisher, Alomone – **Table 2**) (c).

Supplementary 3 – table. Pearson's above threshold values for unclassified taste buds are bolded.

Subject number	X3 or X2	Taste Bud #	Average panel rating	Pearson's (above threshold)
1	X2	1	0.2	
1	X2	2	0	
1	X2	3	0.4	**0.01**
1	X3	1	2	
1	X3	2	2	
1	X3	3	1.8	0.27
2	X2	1	2	0.67
2	X2	2	2	0.72
2	X3	1	2	0.39
2	X3	2	2	0.38
2	X3	3	2	
2	X3	4	2	
3	X2	1	0	-0.07
3	X2	2	0.2	-0.2
3	X3	1	2	
3	X3	2	2	
3	X3	3	2	
4	X2	1	0.2	
4	X3	1	1.8	
4	X3	2	2	
4	X3	3	2	
5	X2	1	0.2	
5	X2	2	0.2	
5	X2	3	0	
5	X2	4	0	
5	X2	5	0	
5	X3	1	2	
5	X3	2	2	
5	X3	3	2	
5	X3	4	2	
6	X2	2	0.2	
6	X2	3	0	

6	X2	4	0.2	
6	X2	5	0.2	
6	X3	1	2	
6	X3	2	2	
6	X3	3	2	
6	X3	4	2	
6	X3	5	2	
7	X2	1	0	
7	X2	2	0	
7	X3	1	1.8	
7	X3	2	1.8	
7	X3	3	2	

Subject number	X3 or X2	Taste Bud #	Average panel rating	Pearson's (above threshold)
8	X2	1	0.2	
8	X2	2	0.2	
8	X2	3	0.4	
8	X3	1	2	
8	X3	2	2	
8	X3	3	2	
9	X2	1	0	
9	X2	2	0	
9	X2	3	0	
9	X3	2	2	
9	X3	3	2	
10	X2	1	0.2	
10	X2	2	0.2	
10	X2	3	0.2	
10	X3	1	2	
10	X3	2	2	
10	X3	3	2	
11	X2	1	0.4	
11	X2	2	0	
11	X2	3	0.2	-0.06
11	X2	4	0.2	
11	X2	5	0.2	
11	X3	1	1.6	
11	X3	2	2	
11	X3	3	2	
11	X3	4	2	
11	X3	5	2	
12	X2	1	0	0.04
12	X2	2	0	
12	X3	1	1.2	0.24
12	X3	2	1.6	
13	X2	1	0.2	
13	X3	1	2	

14	X2	1	2	0.16
14	X3	1	2	0.28
15	X2	1	0	
15	X3	1	2	
16	X2	1	0.8	**0.05**
16	X3	1	2	0.31
17	X2	2	0	
17	X3	1	2	
17	X3	2	2	
18	X2	1	0	
18	X2	2	0	-0.23
18	X2	3	0	
18	X3	1	2	
18	X3	2	2	
Subject number	**X3 or X2**	**Taste Bud #**	**Average panel rating**	**Pearson's (above threshold)**
19	X2	3	1	**-0.05**
19	X3	1	2	0.43
20	X2	1	0.2	-0.13
20	X3	1	2	
20	X3	2	2	
21	X2	1	0.4	
21	X2	2	1	**0.02**
21	X2	3	0.2	
21	X2	4	0.6	**-0.08**
21	X2	5	0	
21	X3	1	2	
21	X3	2	2	0.25
21	X3	3	2	
21	X3	4	2	0.28
21	X3	5	2	
22	X2	1	0	
22	X2	2	0	
22	X2	3	0	
22	X2	4	0.2	

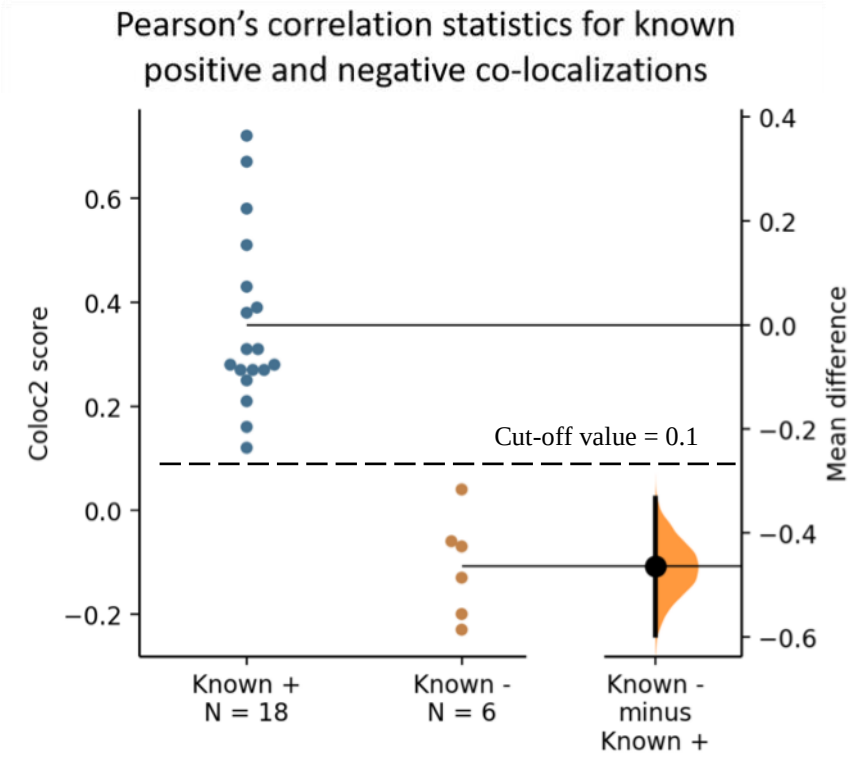
22	X2	5	0	
22	X3	1	2	
22	X3	2	2	
22	X3	3	1.8	
22	X3	4	2	
22	X3	5	2	
23	X2	1	0.2	
23	X2	2	0.2	
23	X2	3	0	
23	X2	4	0	
23	X2	5	0	
23	X3	1	2	
23	X3	2	2	
23	X3	3	2	
23	X3	4	2	
23	X3	5	2	
24	X2	1	0	
24	X2	2	0	
24	X2	3	0.2	
24	X2	4	0.2	
24	X3	1	2	
24	X3	2	2	
24	X3	3	2	
25	X2	1	1.6	**0.2**
25	X2	2	1.6	**0.16**
25	X3	1	2	0.58
25	X3	2	2	0.51

Subject number	X3 or X2	Taste Bud #	Average panel rating	Pearson's (above threshold)
26	X2	1	0.8	**0.17**
26	X2	2	1.4	**0.3**
26	X2	3	1.6	
26	X2	4	1.6	
26	X2	5	2	0.12

26	X3	1	2	0.27
26	X3	2	2	0.27
26	X3	3	2	
26	X3	4	2	
26	X3	5	2	0.31
27	X2	1	0.2	
27	X3	1	2	
28	X2	1	0	
28	X3	1	2	
29	X2	1	0	
29	X2	2	0	
29	X3	1	2	
29	X3	2	2	
29	X3	3	2	
29	X3	4	2	
29	X3	5	2	
30	X2	1	0.8	**-0.05**
30	X3	1	2	0.21
30	X3	2	2	
31	X2	1	0.2	
31	X2	2	0	
31	X2	3	0.2	
31	X2	4	0.2	
31	X2	5	0	
31	X3	1	2	
31	X3	2	1.8	
31	X3	3	2	
31	X3	4	1.8	
31	X3	5	1.8	
32	X2	1	0	
32	X2	2	0	
32	X3	1	1.6	
32	X3	2	1.8	
32	X3	3	1.6	
33	X2	1	0	

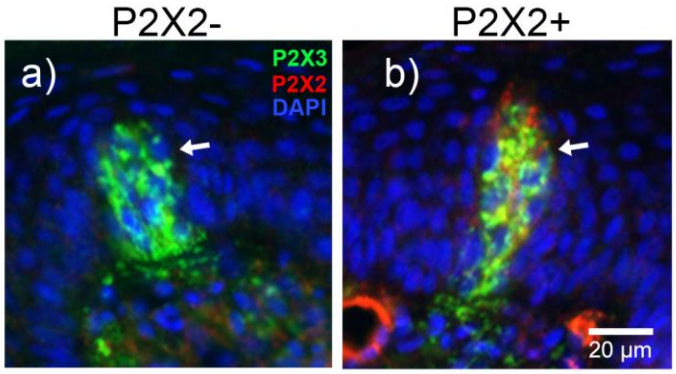
33	X2	2	0	
33	X2	3	0.2	
33	X3	1	1.4	0.26
34	X2	1	0	
34	X2	2	0	
34	X2	3	0	
34	X2	4	0	
34	X3	1	1.6	
34	X3	2	1.8	
34	X3	3	2	
35	X2	1	0.2	
35	X2	2	0	
35	X2	3	0	
35	X3	1	2	
35	X3	2	2	
35	X3	3	2	

Supplementary 4 - figure



Supplementary figure 4. 18 positive and 6 negative taste buds, as determined by the expert panel, were confocaled and analyzed using Coloc2 for correlation between Tuj1 and P2X2 or P2X3 channels. A two-independent-groups mean difference plot shows a significant difference of -0.464 between Pearson's correlation co-efficients for colocalized and non-colocalized samples, p=0.0. We utilized a value of 0.1 as a cut-off for determining the colocalization status for the taste buds in the indeterminate groups following expert panel review.

Supplementary 5 – figure.



Supplementary figure 5. Taste buds from subjects classified as P2X2- (a) and P2X2+ (b), were stained with P2X2 and P2X3 antibodies using a Vectra multispectral imaging system using Akoya's HRP-conjugated secondary polymer and TSA-based Opal fluorophores. Four subjects' taste buds (2 P2X2-, 2 P2X2+) were stained with examples shown in (a) and (b). Note the near absence of red immunolabel denoting P2X2 in panel (a).