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Purification and characterization of fat taste receptor-positive cells from mouse tongue papillae

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Corresponding Author:	Aziz Hichami FRANCE
Corresponding Author's Institution:	
Corresponding Author E-Mail:	aziz.hichami@u-bourgogne.fr
Order of Authors:	Aziz Hichami
	Amira Sayed Khan
	Hameed Ullah
	Naim Akhtar Khan
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TITLE:

Purification and Characterization of Fat Taste Receptor-positive Cells from Mouse Tongue Papillae

AUTHORS AND AFFILIATIONS:

Aziz Hichami[1], Amira Sayed Khan[1], Hameedullah Khan[1], Naim Akhtar Khan[1]

[1]Physiologie de la Nutrition & Toxicologie (NUTox), UMR INSERM U1231 Lipides, Nutrition & Cancer, Université de Bourgogne Franche-Comté (UBFC), 21000 Dijon, France

Email addresses of co-authors:

Amira Sayed Khan (Amira.Khan@u-bourgogne.fr)
Naim Akhtar Khan (Naim.khan@u-bourgogne.fr)
Hameedullah Khan (Hameed_Ullah@etu.u-bourgogne.fr)

Corresponding author:

Aziz Hichami (Aziz.hichami@u-bourgogne.fr)

SUMMARY:

We describe the isolation and purification of lipid gustatory cells that express functional CD36 receptor in mouse tongue papillae.

ABSTRACT:

Sweet, umami, bitter, salt, and sour are the five taste modalities; however, there is increasing evidence of a sixth taste modality related to the oro-sensory perception of dietary fatty acids. Fat taste is principally detected by cluster of differentiation 36 (CD36), G-protein-coupled receptor 120 (GPR120), and GPR40. Despite the high level of interest, it is very difficult to obtain ethical approval to isolate human taste bud cells (TBCs). Therefore, mouse TBCs are much sought after for *in vitro* studies. This study aimed to develop a method for the purification of CD36-expressing TBCs from mouse fungiform and circumvallate papillae.

After cervical dislocation, the tongue was removed, and an elastase/dispase enzyme mixture was injected under the epithelium and around the circumvallate papillae. The epithelium-containing taste buds were picked off and subjected to enzymatic digestion with the elastase and dispase mixture. The cells were isolated by using an anti-CD36 antibody coupled to phycoerythrin (PE) and anti-PE-antibodies coupled to magnetic beads. The mixture was then passed through a magnetic column in which the CD36-positive cells were retained.

The isolated cells were cultured for up to 5 days, and western blotting and quantitative reverse-transcription polymerase chain reaction (RT-qPCR) techniques revealed that purified cells expressed the receptors for CD36 and GPR120 as well as α-gustducin and phospholipase C (PLC) involved in downstream signal transduction. Using Fura-2-acetoxymethyl ester (Fura-2/AM), the selected positive cells were found to respond to dietary fatty acids via a CD36-induced increase in free intracellular Ca^{2+} concentrations. In conclusion, purified CD36-positive taste bud cells can

be of great help for *in vitro* investigation of taste bud physiology and for studying the mechanisms of fat taste perception.

INTRODUCTION:

Fat taste represents the sixth taste quality in addition to the five basic taste qualities, i.e., sweet, sour, bitter, salt, and umami[1-2]. Taste buds, which are responsible for the gustatory perception of tastants, are mainly present in three lingual papillae, i.e., fungiform, foliate, and circumvallate. Taste buds consist of 4 types of TBCs with distinct functions: Type I (glial-like) cells, Type II (taste receptor) cells, Type III (neuronal-like) cells, and Type IV (progenitor) cells. Type II cells express the taste receptors for sweet, umami, fat, and bitter. Bitter, umami, and sweet tastes are detected by the type 2 taste receptor (T2R) and the heterodimers, T1R1/T1R3 and T1R2/T1R3, respectively. T1R and T2R are coupled to a G-protein called gustducin. Cluster of differentiation 36 (CD36) and two G-protein-coupled receptors (GPCRs), i.e., GPR120 and GPR40, are implicated in the gustatory perception of dietary fats in rodents[3].

It is noteworthy that CD36 exhibits high affinity (in the order of nanomolar) for fatty acids[4]. Several reports have documented the expression of CD36 in lingual gustatory cells in humans[5] and other mammals[6-9]. As it is very difficult to obtain human TBCs, mouse TBCs must be isolated for *in vitro* studies. Hence, this study aimed to purify CD36-positive TBCs from enzymatically digested papillae by a positive selection approach using anti-CD36-PE and anti-PE-antibodies coupled to magnetic beads. This method gave greater purity of the selected cells with respect to their calcium signaling response when CD36 was activated by fatty acids. Thus, CD36-positive TBCs can be of great help to study the physiological aspects of fat taste signaling.

PROTOCOL:

NOTE: Male, 10–12-week-old C57BL/6J mice were used in this study. The general guidelines for the care and use of laboratory animals recommended by the Council of European Economic Communities were followed, and the protocol was approved by the Regional Ethical Committees "protocol number 16158". See **Table 1** for recipes of media and buffers used in this protocol.

1. Tongue isolation

1.1. Sacrifice male C57BL/6J mice (n=10) by cervical dislocation.

1.2. Cut the side layer of the mouth up to the ears with scissors on both the sides. Be careful not to cut the tongue. Hold the tongue with forceps, and cut the jaw ligament under the tongue and the bottom of the tongue.

NOTE: Do not damage the circumvallate area; cut out additional tissue to avoid damage to the tongue.

1.3. Place the isolated tongue in a Petri dish with Iscove's modified Dulbecco medium (IMDM)/MCDB complete medium until the dissection of all the mice (~50 min). To get rid of blood and hair, wash the tongues in the same dish with IMDM/MCDB complete medium.

2. Isolation of lingual epithelium

2.1. Transfer the tongues to cold (4 °C) Tyrode solution with calcium and incubate for 5 min. Using a syringe with 26 G needle, inject ~200 µL of elastase/dispase enzyme mixture under the epithelium and around the circumvallate papillae. Incubate the injected tongues for 15 min at 37 °C in Ca^{2+}-free Tyrode solution (in the CO_2 incubator).

2.2. Using scissors and forceps, peel off the epithelium containing fungiform papillae, remove the circumvallate papillae under a microscope, and place them in a microcentrifuge tube containing cold IMDM/MCDB complete medium (4 °C).

3. Isolation of taste bud cells

NOTE: Perform cell isolation in a laminar flow hood under sterile conditions.

3.1. Centrifuge the epithelium at 600 × *g* for 10 min at 4 °C, and discard the supernatant. Dissolve the pellet (epithelium) with 1 mL of the above enzyme mixture (step 2.1), cut the epithelium with scissors to facilitate enzyme action, and then incubate for ~10 min at 37 °C in the CO_2 incubator.

3.2. Transfer the supernatant into a new microcentrifuge tube, and perform a second round of digestion on the undigested tissue (debris) in the centrifuged tube from step 3.1. Centrifuge the tube containing the digested tissues at 600 × *g* (10 min, room temperature (RT)), remove the supernatant, suspend the pellet containing dissociated cells in the IMDM/MCDB complete medium, and keep the tube in the CO_2 incubator (37 °C, 5% CO_2, and 95% humidity) until the end of the isolation.

3.3. Repeat steps 3.1–3.3 three times with 1 mL of the enzyme mix to dissociate the undigested tissue. Pool all the dissociated cells into a 15 mL tube.

4. Purification of CD36-positive cells

NOTE: Magnetic separation of CD36-positive cells was performed according to the kit manufacturer's instructions (see the **Table of Materials** and **Figure 1**).

4.1. Remove cell clumps by passing the cells through 70 µm pre-separation filters to avoid clogging the column; count the number of cells. After centrifugation (300 × *g*/10 min), suspend the pellet in 1x magnetic-activated cell sorting (MACS) bovine serum albumin (BSA) buffer to obtain a concentration of 10^7 cells/80 µL of the MACS buffer

4.2. Add anti-CD36-PE (20 µL/10^7 cells), mix gently, and incubate for 10 min in the refrigerator (2–8 °C). Wash the cells by adding 2 mL (per 10^7 cells) of MACS buffer, centrifuge (300 × *g* for 10 min) the cell suspension.

4.3. Suspend the pellet in 80 µL of MACS buffer (per 10^7 total cells), and add 20 µL of anti-PE-coupled microBeads (per 10^7 total cells). Mix gently and incubate for 15 min in the refrigerator (2–8 °C).

4.4. Wash the beads by adding 2 mL (per 10^7cells) of MACS buffer, followed by centrifugation (300 × *g* for 10 min). Resuspend up to 10^7cells in 500 µL of MACS buffer.

4.5. Magnetic separation with an MS Column (**Table of Materials**)

4.5.1. Place an MS column in the magnetic MACS Separator, and rinse it with 500 µL of MACS buffer. Apply the cell suspension onto the column, and wash the column three times with 500 µL of MACS buffer. Collect the flow-through containing unlabeled cells.

4.5.2. For elution of the CD36-positive, labeled cells, remove the column from the separator and place it on a collection tube. Pipette 1 mL of MACS buffer onto the column, and immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

5. Cell culture

5.1. Centrifuge the cells (300 × *g*, 10 min, RT), discard the supernatant, and suspend the pellet in IMDM/MCDB complete medium. Distribute the cell suspension into wells for culture up to 5 days in CO_2 incubator (37 °C, 5% CO_2, and 95% humidity).

NOTE: The duration of the purification procedure for CD36-positive cells is ~8 h.

REPRESENTATIVE RESULTS:

After selection, all purified cells were found to co-express CD36 along with α-gustducin (**Figure 2A**). The expression of CD36 (**Figure 2B**) and *α-gustducin* (**Figure 2C**) was high compared to that of CD36-negative cells or cells before selection. Thus, these purified cells are fat taste receptor cells (type 2 cells). As CD36 represents the main sensor of dietary long-chain fatty acids (LCFAs) in taste buds, we investigated the effects of several fatty acids on changes in $[Ca^{2+}]_i$ by using FURA-2 as a fluorescent probe. For this, a saturated medium-chain fatty acid (SMFA)—capric acid (CA, C10:0), a saturated long-chain fatty acid (SLFA)—palmitic acid (PA, C16:0), and two long-chain polyunsaturated fatty acids (PUFAs)—linoleic acid (LA C18:2n–6) and arachidonic acid (AA, C20:4n–6) were used.

The results showed that in selected CD36-positive cells, both saturated and unsaturated long-chain fatty acids (LCFAs) elicited a rapid increase in $[Ca^{2+}]$i, but with different magnitude: PA ≥ LA > AA (**Figure 3A**). By contrast, the medium-chain fatty acid, capric acid, failed to induce a significant rise in $[Ca^{2+}]$i (**Figure 3A**). It should be noted that the increase in $[Ca^{2+}]$i, induced by the LCFAs, is mediated by CD36 as sulfo-succinimidyl oleate (SSO), a CD36 inhibitor, inhibited Ca^{2+}

signaling in these cells (**Figure 3B**). This result is in accordance with previous reports indicating that CD36 represents a high-affinity receptor for LCFAs in TBCs[10] and provides evidence of the physiological function of CD36 as a fat taste receptor[11].

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic representation of CD36-positive cell selection using a MicroBead kit. Taste bud cells were suspended in MACS buffer and incubated with a primary antibody, anti-CD36-PE, followed by a secondary antibody, anti-PE-coupled MicroBeads. After washing, labeled "CD36-positive cells" were transferred onto an MS Column placed in magnetic MACS Separator. CD36-negative cells were eluted through the column, whereas CD36-positive cells were retained. CD36-positive cells were flushed out after removing the column from the magnetic support. Abbreviations: CD36 = cluster differentiation 36; MACS = magnetic-activated cell sorting; PE = phycoerythrin.

Figure 2: Characterization of purified CD36-positive gustatory cells for their expression of CD36 and α-gustducin. CD36-positive cells were subjected to immunocytochemistry to detect CD36 and α-gustducin. Briefly, cytospin-prepared slides were fixed and blocked with fetal calf serum before incubation with (**A**) anti-rat CD36 antibody and (**B**) a polyclonal anti-mouse α-gustducin antibody. Cell nuclei were labeled using Hoechst 33342. Slides were analyzed under a fluorescence microscope, magnification 60x. Comparison by quantitative real-time PCR of CD36 mRNA (primers: forward GGCCAAGCTATTGCGACATG; reverse CCGAACACAGCGTAGATAGAC) and α-gustducin (primers: forward ATCACCATCTTCTAGTGTATTTGCC; reverse ACACATTGCAGTCCATCCTAGC) levels in gustatory cells before and after selection. Results are expressed with reference to β-actin (primers: forward AGAGGGAAATCGTGCCTGAC, reverse CAATAGTGATGACCTGGCCGT). Scale bars for A = 10 µm. Abbreviations: CD36 = cluster differentiation 36; PCR = polymerase chain reaction.

Figure 3: Measurement of Ca^{2+} signaling. The CD36-positive gustatory cells (2×10^6/assay) were loaded with the fluorescent probe, Fura-2/AM, as previously described[12]. Experiments were performed on CD36-positive cells in the presence or absence of CD36 blocker, SSO, at 50 µM (20 min pre-incubation). The arrowheads indicate when the fatty acids (20 µM) were added into the cuvette without interruptions in the recording. Upward deflection indicates increases in $[Ca^{2+}]_i$. (**A**) Single traces of observations representing the effects of various fatty acids on the increases in $[Ca^{2+}]_i$ in isolated CD36-positive gustatory cells. (**B**) Effect of SSO on fatty acid-mediated rise in $[Ca^{2+}]_i$ in CD36-positive cells, values are Means $\pm$ SD (n=6), *P < 0.001. Abbreviations: CA = capric acid (C10:0); PA = palmitic acid (C16:0); LA = linoleic acid (C18:2n–6); AA = arachidonic acid (C20:4n–6); SSO = sulfo-succinimidyl oleate.

Table 1: Recipes of media and solutions.

DISCUSSION:

CD36-positive taste bud cells were isolated from tongue fungiform and circumvallate papillae using a positive selection approach with anti-CD36 antibodies, which offers greater purity due to the specificity of the reaction in comparison to negative selection. All these cells express CD36

and are type II cells as they co-express α-gustducin. This was in accordance with previous work[6] demonstrating colocalization of CD36 and α-gustducin by immunohistochemistry on a whole tongue and the restriction of CD36 expression to the lingual papillae, which confirms that CD36-selected cells are taste receptor cells. It has been reported CD36 is coupled to an increase in free intracellular Ca^{2+} concentrations during its activation by dietary LCFAs[3].

A disadvantage of positive selection using anti-CD36/antibodies coupled to magnetic beads is that isolated cells will carry bead-bound antibodies, which could affect downstream signaling. However, by employing a CD36 blocker, sulfo-*N*-succinimidyl oleate ester (SSO), we observed that an LCFA, but not a medium-chain FA, activated CD36 and triggered an increase in $[Ca^{2+}]i$ in CD36-positive TBCs. This demonstrates that in CD36-selected cells, CD36 calcium signaling was not affected by the antibodies or the beads.

It has been reported that obesity, in humans and rodents, leads to decreased fat taste sensitivity with an increase in fat preference and intake. Furthermore, the downregulation of *CD36* or its genetic[13] or epigenetic[14] modification may be responsible for altered fat taste perception in obesity. In this context, the mouse model is largely used for laboratory experiments, and the isolation of CD36-positive TBCs can be of great help for *in vitro* investigations to study taste bud physiology.

ACKNOWLEDGEMENTS:

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COMPETING INTERESTS:

The authors declare no competing interests.

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Figure 1

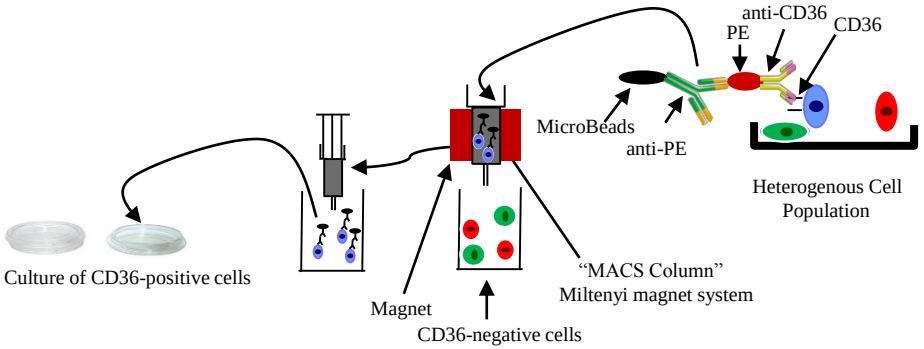

Figure 2

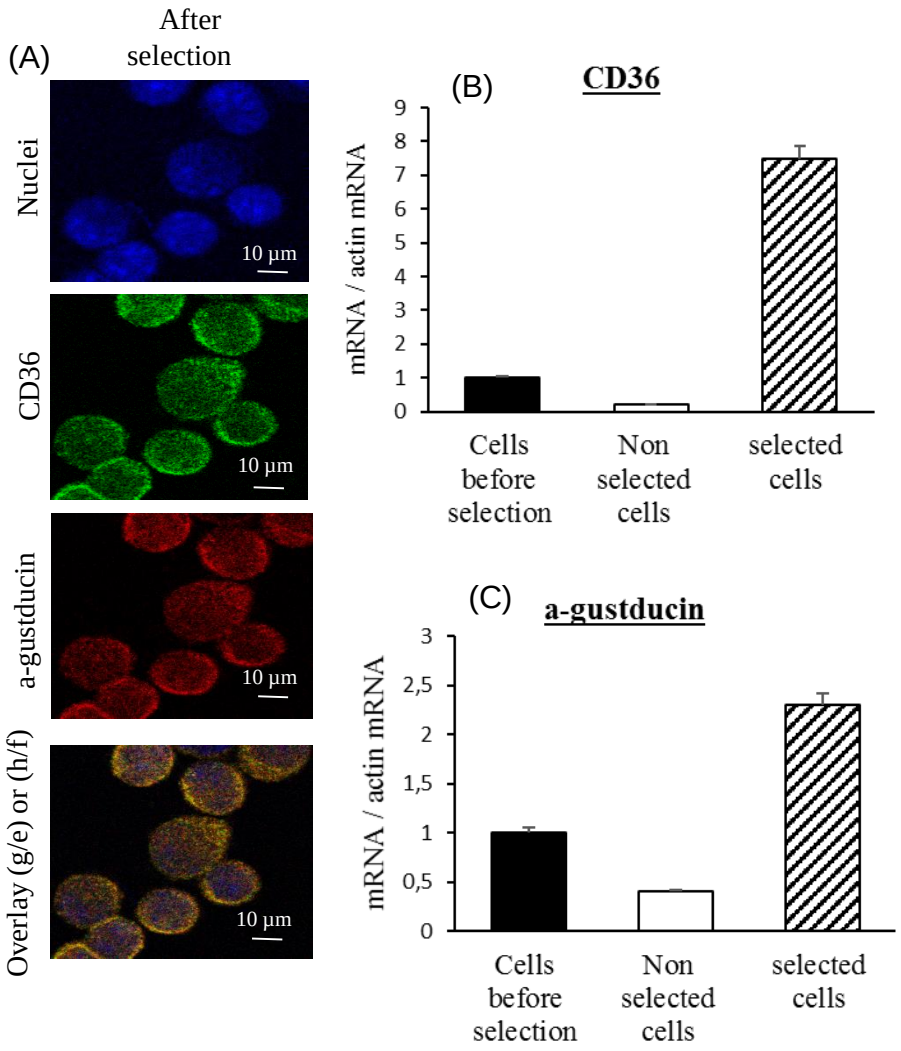

Figure 3

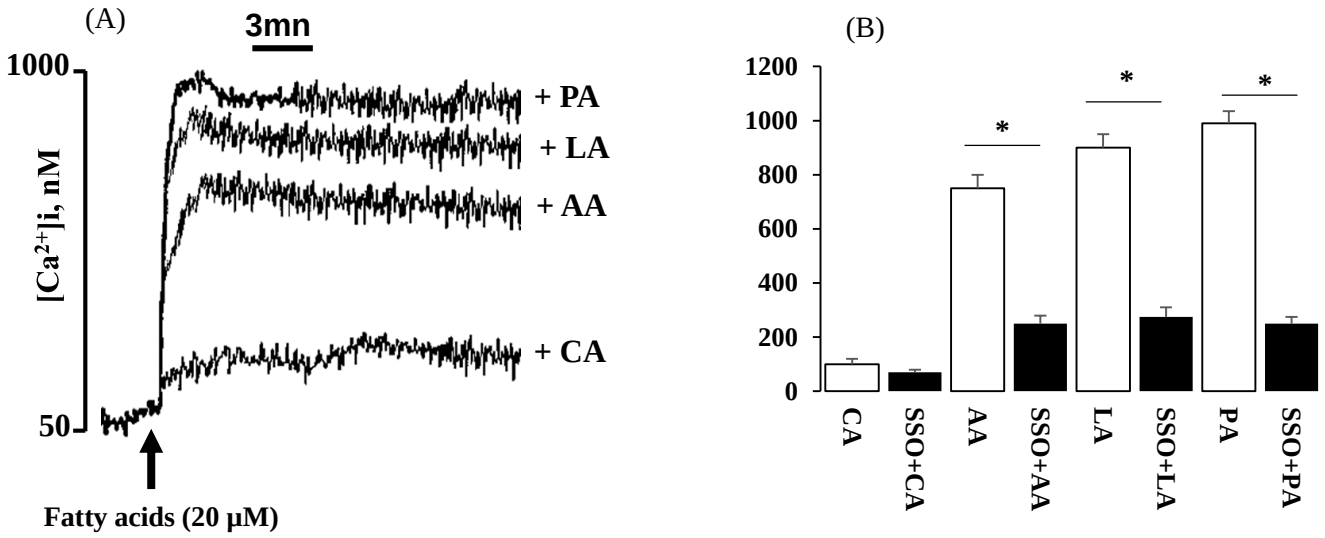

Table 1		
Medium/solutions	Reagent	concentration
IMDM/MCDB complete medium	Mix of IMDM and MCDB-153	5:1 (v/v)
	Fetal calf serum (FCS)	10%
	Penicillin	100 U/mL
	Streptomycin	100 µg/mL
	Amphotericin B	0.5 µg/mL
Tyrode solution with calcium (pH 7.4)	NaCl	120 mM
	KCl	5 mM
	Hepes	10 mM
	CaCl2	1 mM
	$MgCl_2$	1mM
	Glucose	10 mM
	Na-pyruvate	10 mM
Ca^{2+}-free tyrode solution (pH 7.4)	NaCl	120 mM
	KCl	5 mM
	Hepes	10 mM
	EGTA	2 mM
	Glucose	10 mM
	Na-pyruvate	10 mM
IMDM/MCDB complete medium with 0.2 mM EDTA	IMDM/MCDB complete medium	-
	EDTA	0.2 mM
Elastase (8 mg/mL)	Tyrode solution with calcium	-
	Elastase	8 mg/mL
Collagenase I (4 mg/mL)	IMDM/MCDB complete medium	-
	Collagenase I	4 mg/mL
Trypsin inhibitor (4 mg/mL)	IMDM/MCDB complete medium	-
	Trypsin inhibitor	4 mg/mL
Elastase/Dispase enzyme mix	IMDM/MCDB complete medium with 0.2 mM EDTA	600 µL
	Elastase (8 mg/mL)	150 µL
	Collagenase I (4 mg/mL)	150 µL
	Trypsin inhibitor (4 mg/mL)	50 µL

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
amphotericin B (250 µg/mL)	PAA	P11-001	
anti-CD36	Atlas Antibodies	HPA002018	
anti-α-gustducin	Santa Cruz	sc-395	
Anti-PE MicroBeads	Miltenyi Biotec	130-048-801	
collagenase	Worthington Biochemical	LS004196	
CD36 Antibody, anti-mouse, PE, REAfinity	Miltenyi Biotec	130-122-084	
Dispase	Worthington Biochemical	LS02109	
Elastase	Worthington Biochemical	LS02292	
fetal calf serum (FCS)	Dominique Dutscher 500105EE	500105EE	
gentamycin (10 mg/mL)	PAA Laboratories P06-03050	P06-03050	
Iscove's Modified Dulbecco's Medium (IMDM)	Pan biotech	P04-20150	
MACS buffer BSA Stock Solution	Miltenyi Biotec	130-091-376	
MCDB 153	Alphabioregen	PG053	
MS Columns	Miltenyi Biotec	130-042-201	
N-succinimidyl oleate ester (SSO)	Sigma	SML2148	
OctoMACS Starting Kit	Miltenyi Biotec	130-042-108	
penicillin/streptomycin (10,000 U/10,000 µg/mL)	Thermo Fisher 15140-122	15140-122	
Pre-Separation Filters (70 µm)	Miltenyi Biotec	130-095-823	
Trypsin inhibitor	Worthington Biochemical	LS0028292	

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1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
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2. Please revise the following lines to avoid previously published work: 25-31,33-34,132-133,151-154,183-186,198-199,201-204,209-210.
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3. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).
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4. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).
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a) A clear statement of the overall goal of this method
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c) The advantages over alternative techniques with applicable references to previous studies
d) A description of the context of the technique in the wider body of literature
e) Information to help readers to determine whether the method is appropriate for their application
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12. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique
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13. Please include an Acknowledgements section, containing any acknowledgments and all funding sources for this work.
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18. Figure 2: Please include scale bars in all the images of the panel (Figure 2A). Please include the details of the magnification in Figure Legends. Please ensure that the Figure Labels depicted in the figure are included in the Figure Legend (label "C" is missing in the Figure Legend).
Answer : we insert scale bars in all the images of the panel (Figure 2A) and magnification in legend.

19. Please sort the Table of Materials in alphabetical order.

Answer : We have sorted the Table of Materials in alphabetical order.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The protocol "Purification and characterization of fat taste receptor-positive cells from mouse tongue papillae" describes a protocol to isolate CD36+ taste receptor cells from mice and to culture them for subsequent studies. There are a few concerns.

Major Concerns:

1. There are inconsistencies between the abstract and the protocol. In the abstract, the fungiform and CV are isolated under local anesthesia, while in the protocol, mice are killed via cervical dislocation. It would be very difficult to harvest papillae from the tongues of mice that were only under local anesthesia. Additionally, in the abstract, it states that papillae were "picked off" by scissors, which is not how the papillae were harvested in the protocol.

Answer : We are agree reviewer and we have corrected the inconsistency and we changed it in the Abstract as follow: "After cervical dislocation, tongue was removed and injected with Elastase/Dispase enzyme mix under the epithelium and around circumvallate papillae, then epithelium containing taste buds were picked off……"

2. Section 4: Purification of CD36 positive cells. This section is for the separation of type I cells, but CD36 is on Type II cells, so how will this isolate CD36 positive cells?

Answer: We are agree reviewer that CD36 positive cells belong to type II cells, We have changed it in Section 4: type I cells by CD36 positive cells.

3. Figure 1 does not clearly explain the process.

Answer**:** Schematic representation of CD36 positive cell selection in fig 1 is improved and we have explained briefly the protocol in the legend of figure 1

4. Methods: Tongue isolation, protocol states that tongues are removed and left in medium until all tongues are removed. There should be some time limit or time estimate included. Additionally, there should be an estimated time for the entire protocol. Is this a 1 day or a 2 day, etc.

Answer: We are agree with the reviewer, we have included in the revised MS the estimated time (around 50 min) to remove tongues of 10 mice. Also, we have added at the end of methods section that 8 h are required for entire protocol.

5. Methods: Isolation of lingual epithelium. Add information on trouble-shooting removal of the epithelium here. The CV is harder to remove than the fungiform, and tips for removal would be helpful.

Answer: We agree with reviewer that the CV is harder to remove. Hence, we have included in the "methods " the need of scissors and forceps to remove circumvallate papillae under microscope.

Minor Concerns:
6. Under "solutions", be consistent with measurement abbreviations. Ex. ml vs mL
Answer: We have revised the expression of units and use in the revised MS the standard abbreviations for units example mL , µL.

7. Add "x" when referring to centrifuge speed, ex. 600xg
Answer: We have revised this point as suggested by reviewer.

8. Section 3.3, is not clear. Supernatant now contains released cells and "a pellet is an undigested tissue"? What does this refer to?
Answer :
We have clarified this point in revised MS. Indeed, in the current protocol, we performed a short time of enzymatic digestion (10 min) which is not sufficient to dissociate all the epithelium but gives an optimal viability for the dissociated cells. Hence, the remaining indigested tissues was subjected to three rounds of enzymatic digestion.

9. Section 3.4, the supernatant is used for dissolving the undigested tissue? Unclear.
Answer: We have clarified this point in revised MS, we remove this sentence

10. 3.6, for repeat step2-5, if would be helpful to label them 3.2-3.5
Answer: We have changed as suggested by reviewer. We changed the repeated step 2-5 with repeating three times steps 3.1-3.4.

11. 4.2 says Cells (the pellet) were suspended in buffer. It would be helpful to add to 4.1, if this step ends in a pellet
Answer: We have revised this point as suggested by reviewer.

Reviewer #2:
Manuscript Summary:
Study on the presence of the fatty taste receptor from the mouse tongue

Major Concerns:
The results are not novel, there are several papers showing the implications of CD36 in fat perception in mice. The cell isolation method shows that CD36+ cells opportunely selected are also expressing alpha gustducin and this is a condition sufficient to state that cells from papillae were isolated. The calcium uptake experiment was performed only with linoleic acid but different fats should be tested.

Answer: We are agree with reviewer that it's well admitted that CD36 plays a pivotal role in fat taste detection. The aim of this article is to describe the technical purification of taste bud cells that express CD36.

To my opinion a colocalization of CD36 and alpha gustducin by ICC or IHC on a whole (undigested tongue) is the experiment necessary to show that this receptor is in the tongue papillae and different fat tastant should be checked in calcium uptake experiment to state that this receptor is implicated in fat taste perception.

Answer: colocalization of CD36 and alpha gustducin by IHC on a whole tongue (undigested tongue) has been investigated by Laugerette et al (Laugerette at al 2005) and they observed that CD36 was

co-expressed with α-gustducin, demonstrating that CD36-selected cells are taste receptor cells. In this work authors used anti-mouse CD36 antibody UA009 obtained from Graham Mayrhofer (Zhang X et al. 2003) and commercialized by (Novus bio-techne) but this antibody is no more available. Unfortunately, we cannot repeat this kind of colocalization by IHC staining, as we did not find any antibody anti-CD36 that works in this type of staining.

As suggested by reviewer we have tested other fatty acid on calcium response. We have used two saturated fatty acids; capric acid (CA, C10:0) and palmitic acid (PA, C16:0), and long chain polyunsaturated fatty acid, and arachidonic acid (AA, C20:4 n-6). We observed that in selected CD36-positive cells, both saturated and unsaturated long chain fatty acid evoked a rapid increase in $[Ca^{2+}]i$, but with different magnitude PA ≥LA>AA; however, the medium-chain fatty acid, capric acid, failed to induce significant rise in $[Ca^{2+}]i$

Zhang, X., Fitzsimmons, R., Cleland, L. *et al.* CD36/Fatty Acid Translocase in Rats: Distribution, Isolation from Hepatocytes, and Comparison with the Scavenger Receptor SR-B1. *Lab Invest* **83,** 317–332 (2003).

Minor Concerns:
Expand a little the rationale; update the bibliography; improve the abstract with the main conclusion of the work.
Answer: We have improved the rationale of the work, the abstract and we have added conclusion in the abstract.

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Dr. Aziz Hichami, aziz.hichamiu-bourgogne.fr, Tel : 0380393851 /Fax : 0380396330

Dijon, 18/03/2021

Subject: ***Purification and characterization of fat sensor-positive taste bud cells from mouse tongue papillae***

Dear Editors Dr.Vineeta Bajaj and Dr. Lyndsay Troye

Please find the revision of our manuscript entitled as above and submitted today via the JoVE web site. Our manuscript is pertinent to the scope of the issue of JoVE video methods collection "Current tools and methods in taste science", created under the leadership of Dr. Dany Gaillard

Our article has not been sanctioned by any Editorial committee and all of the authors have no conflict of interest. Our article is not currently under consideration at any journal other than special issue of JoVE

.

Aziz Hichami

Associate Professor