

**Lipid-mediated release of GLP-1 by mouse taste buds from
circumvallate papillae: putative involvement of GPR120
and impact on taste sensitivity**

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Abbreviation title: **GPR120, GLP-1 and taste sensitivity.**

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ABSTRACT

Glucagon-like peptide-1 (GLP-1) signaling modulates sweet taste sensitivity in the mouse. Since circumvallate papillae (CVP) express both GLP-1 and its receptor, a local regulation has been suggested. However, whether dietary lipids are involved in this regulation, as shown in the gut, is unknown. By using a combination of biochemical, immunohistochemical and behavioral approaches, the present data *i)* confirm the role of GLP-1 signaling in the attraction for sucrose, *ii)* demonstrate that minute quantities of long-chain fatty acids (LCFA) reinforce the attraction for sucrose in a GLP-1R-dependent manner, *iii)* suggest an involvement of the LCFA receptor GPR120 expressed in taste buds in this system and *iv)* support the existence of a regulation by GLP-1 of the lipid sensing mediated by lingual CD36. Therefore, oro-sensory detection of LCFA may affect sweet and “fatty” tastes responsiveness by controlling the secretion of lingual GLP-1. This regulatory loop, likely triggered by the LCFA-GPR120 interaction, might contribute to the high palatability of foods rich both in fat and sugar.

Supplementary key-words: Long-chain fatty acids; CD36; eating behaviour; obesity risk; health.

ABBREVIATIONS: ALA, α -linolenic acid; CVP, circumvallate papillae; DPP4, dipeptidyl peptidase 4; FFAR1, free fatty acid receptor 1; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; LCFA, long-chain fatty acids; OLA, oleic acid; TBC, taste bud cells.

INTRODUCTION

Substantial evidence supports the existence of a specific detection system devoted to the oro-sensory perception of dietary lipids in both rodents and human. Long-chain fatty acids (LCFA) are the main molecules detected by this system and are thought to play a significant role in the spontaneous preference for fatty foods (1-2). The plasma membrane glycoprotein CD36 has been the first plausible candidate identified to exert the function of a lipid-sensor in the oral cavity (3). Indeed, it displays a very high affinity for LCFA (4), is specifically found in the gustatory papillae in rat (5), mouse (3) or human (6) and ablation of *CD36* gene expression renders mice unable to recognize and prefer LCFA in a textured solution during two bottle preference tests (3, 7-8). In human subjects, the common single nucleotide polymorphism rs1761667, known to reduce *CD36* gene expression (9), is also associated with a deep attenuation of oro-sensory sensitivity for fat (10).

Two unrelated members of the G protein-coupled receptor family, the free fatty acid receptor 1 (FFAR1, also termed GPR40) and GPR120, have also been recently identified as playing a role in the spontaneous preference for fat in the mouse (11). Such a function is likely indirect for FFAR1 since it is not found in taste buds in rat (12) and human (13) and is not systematically detected in circumvallate papillae (CVP) in the mouse (8, 11), in contrast to GPR120. This last observation raises the question of the respective role(s) played by CD36 and GPR120 in the coding mechanisms for fat taste at the periphery. The fact that CD36 expression is subjected to a short-term lipid-mediated down-regulation in mouse taste buds during food intake, while *GPR120* gene expression remains unchanged (8), is consistent with distinct functions.

A biological action for GPR120 was first identified in the entero-endocrine L cells in which its activation by LCFA triggers the secretion of the glucagon-like peptide-1 (GLP-1) (14). Besides its insulinotropic effect, GLP-1 exerts multiple physiological functions including a role in the regulation of eating behavior (15). Interestingly, GLP-1 and its receptor (GLP-1R) have also been identified in mouse taste buds suggesting an involvement of this incretin in the sense of taste (16). Consistent with

this assumption, it has been shown that GLP-1 signaling modulates taste sensitivity in the mouse, decreasing sour taste but enhancing the responsiveness to sucrose (16). However, mechanisms by which this regulation takes place are not yet determined.

Compelling evidence supports the existence of a functional *continuum* along the oro-intestinal tract responsible for the permanent analysis and control of ingestion, digestion, absorption and metabolic fate of energy nutrients. For fat, cells from taste buds and entero-endocrine cells share common lipid-sensors (e.g. GPR120), express similar hormones and their respective receptors (e.g. GLP-1, GLP-1R), are connected to afferent nerve fibers involved in feeding behavior (i.e. gustatory nerves and vagus). A continuum being “*a set of elements such that one can pass from one to another continuously*”, we propose that fundamental knowledge from the gut can be used to better understand the functional characteristics of the oro-sensory tract, and reciprocally. Consistent with this hypothesis, the goal of the present work was to determine whether LCFA, GPR120 and GLP-1 are functionally linked in the tongue as found in the gut and to explore the impact of such a regulatory system on sweet and fatty tastes responsiveness.

MATERIALS AND METHODS

Ethics Statement

French guidelines for the use and the care of laboratory animals were followed and experimental protocols were approved by the animal ethics committee of Burgundy University (approval codes B1010, B0210 and C1011).

Animals

Animals were housed in a controlled environment (constant temperature and humidity, darkness from 7 pm to 7 am) and were fed a standard laboratory chow (4RF21, Mucedola, Italy). C57Bl/6J wild-type mice were purchased from Charles River Laboratories (France). *CD36*^{-/-} (17) and *Glp1r*^{-/-} (18) mice with a C57Bl/6J background were bred locally.

Behavioral experiments

CD36^{-/-} and *Glp1r*^{-/-} mice were used in the behavioural experiments. Two different tests, which consisted to offer successively in a randomized manner (licking test) or simultaneously (two bottle preference test) a control or an experimental solution, have been used.

- Licking test. This test consists of subjecting a mouse to the control or experimental solution successively to determine the number of licks given on each bottle using contact lickometer (Med Associates, USA). Mice were food and water deprived 6h before the test which took place 6h after the beginning of the dark period. After a training period required to learn the procedure, different groups of mice were subjected to different solutions. In a first experiment, mice were randomly subjected to a bottle containing a control solution (62mM of sucrose; Sigma-Aldrich, USA) or a bottle containing an experimental one (62mM of sucrose + 200µM of oleic acid (OLA) or α-linolenic acid (ALA); Sigma-Aldrich) for 15min. Then mice were offered the other bottle for an additional 15min session. OLA and ALA were previously dissolved in ethanol (0.1% final). The same quantity of ethanol was added in the control solution. In a second experiment, mice were randomly subjected to a bottle containing water (control solution) or a bottle containing 62mM sucrose, 200µM of OLA or 200µM of ALA in water. In a third experiment, mice were randomly subjected to a bottle containing mineral oil (control solution; Cooper, France) or

different concentrations of OLA in mineral oil. In each experiment, data were analyzed for 1min from the first lick to exclude post-ingestive signals.

- Two bottle preference test. Mice were submitted for 12h to a double choice test. Mice were offered a pair of bottles of water in experimental cages for 1 day. Since rapeseed oil was added in xanthan gum to facilitate solubilization and minimize textural cues, mice were subjected on day 2 to 0.3% xanthan gum (Sigma-Aldrich) alone to avoid neophobia. A double choice test between control solution (xanthan gum) and experimental solution (xanthan gum + rapeseed oil) was performed on day 3. Position of bottles (on the right or the left) was changed daily to avoid the development of side preference. Consumption of each solution (in grams) was analyzed for 12h after the beginning of the test and preference for the experimental solutions (ratio between the consumption on experimental bottle and the total consumption) was calculated.

Papillae isolation

CVP from wild-type or *Glp1r*^{-/-} mice were isolated according to previously published procedures (3). In brief, lingual epithelium was separated from connective tissue by enzymatic dissociation (elastase and dispase mixture, 2 mg/ml each in Tyrode buffer: 140 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM CaCl₂, 10 mM glucose, 1 mM MgCl₂, 10 mM Na pyruvate, pH 7.4) and papillae dissected under a microscope. Epithelium surrounding the papillae was also collected to serve as non-sensory control tissue. Samples were snap-frozen in liquid nitrogen and stored at -80°C until RNA or protein extraction or put in culture.

Tissue culture of circumvallate papillae

CVP of wild-type mice were isolated and incubated at 37°C in an oxygenized medium containing 33µM fatty acid-free BSA alone (Sigma-Aldrich; control solution), 200µM α-linolenic acid (ALA), 200µM oleic acid (OLA) or 50µM of a GPR120 agonist (GSK137647A). After 2h of incubation, the medium was collected and the active GLP-1 release was measured by enzyme-linked immunosorbent assay (Millipore, USA). We have postulated that secretion of GLP-1 by CVP might be very low. To be sure to detect active GLP-1 in the incubation medium, 10pM of pure GLP-1 were systematically added in each experimental well, but not in standard curve according to the manufacturers' recommendations. In these conditions, values under 2pM

become resolved. A dipeptidyl peptidase 4 (DPP4) inhibitor (0,1%; Millipore) was added to the medium to prevent GLP-1 degradation.

Compound profiling in recombinant GPR120 receptor assay using intracellular calcium mobilization

U2OS (human osteo-sarcoma ATCC HTB-96; ATCC, USA) cells were grown in Dulbecco's modified Eagle's medium (DMEM)/F-12 supplemented with 10% fetal bovine serum and 2mM L-glutamine. Recombinant GPR120 expressing cells were generated by transducing U2OS cells with BacMam viruses encoding the respective receptor and the chimeric G-protein Ga16 according to established protocols (19). In brief, cells were plate to a density of 2×10^5 cells/mL in cell culture medium containing human GPR120 (0.25%, v/v), mouse GPR120 (0.5%, v/v) or rat GPR120 (0.8%, v/v) BacMam virus. Ga16 BacMam virus (0.12%, v/v) was also transduced in preparation of recombinant human GPR120 cells to allow efficient coupling of human GPR120 receptor to phospholipase C pathway. This solution of cells/virus mixture was then plated at a density of 10^4 cells/well and cultured at 37°C, 5% CO₂, 95% humidity for 24h. Functional EC50 Studies cells were performed in cells incubated with Hank's buffered salt solution (HBSS) containing the cytoplasmic calcium indicator, Fluo-4 dye in the acetylmethyl form (4mM), 2.5mM probenecid and 250µM Brilliant Black at 37°C for 60min. Compound plates were generated containing 3% dimethyl sulfoxide in dye loading buffer. Compounds (*i.e.* GPR120 agonist GSK137647A or Histamine for host untransduced U2OS cells) were added to the cells at a 1:3 dilution and calcium mobilization was measured using a Fluorescence image plate reader (FLIPR, Molecular Devices). Data were converted into normalized responses with respect to assay standards GSK137647A (for GPR120) or Histamine (for Host U2OS cells). Data were further analyzed using a 4-parameter fit to calculate EC50 values.

Real-time RT-PCR

Total RNA from CVP and surrounding non-gustatory epithelium (negative control) were extracted using RNeasy mini-columns (Qiagen, USA). Genomic DNA digestion was performed using the RNase-free DNase Set (Qiagen). First-strand cDNA was generated by reverse transcription from total RNA (Omniscript Reverse Transcription, Qiagen). Levels of mRNA transcripts were determined by real-time RT-PCR (StepOnePlus apparatus, Applied biosystems, USA). RNA levels were normalized

against levels of 36B4 RNA transcripts. Primer probe sets were designed with Primer3 software tool using gene sequences from the GenBank database or purchased directly from Applied Biosystems. PCR amplification was done using Sybrgreen (Power SYBR Green PCR Master Mix, Applied biosystems) or Taqman (Universal Taqman PCR Master Mix, Applied biosystems) technology. The oligonucleotide sequences of primers and probes are shown in Table 1. The comparative $2^{-\Delta\Delta CT}$ method was used for relative quantification (20).

Western blotting

Samples were homogenized using a micro-potter in a TSE buffer (50mM Tris HCl, 150mM NaCl, 1mM EDTA, 1% nonidet P.40). Protein concentration in homogenates was assayed using a BCA kit (Perkin Elmer, USA). After being separated by SDS-PAGE, proteins were transferred to a PVDF membrane by electroblotting. After being blocked using a TBS buffer containing 5% BSA and 0.05% Tween 20, membranes were incubated overnight at 4°C with an anti-CD36 primary antibody raised in goat (1:1000 dilution; R&D systems, USA) or an anti-GPR120 primary antibody raised in rabbit (1:1000 dilution; MBL, USA). After a set of washes, an appropriate peroxidase-conjugated secondary antibody was added. Antibody labeling was detected by chemiluminescence (ECL-plus reagent, Perkin Elmer). GAPDH was used as an internal reference protein.

Immunohistochemistry

CVP from wild-type mice were fixed for 2-3 hours in 4% paraformaldehyde, cryoprotected overnight with 30% sucrose in 0.1M phosphate buffer (pH 7.4) and then embedded in OCT medium (Tissue-Tek, Sakura Finetek). Cryostat sections (10µm) were air dried for 2h at room temperature and then rehydrated in 0.1 M PBS (pH 7.4) for 10min. Rehydrated sections were incubated during 1h with PBS containing 0,3% Triton X-100, 30min with PBS 50mM glycine and then blocked with 10% fatty acid-free BSA in PBS for 40min. Next, the slices were incubated overnight at 4°C with an anti-GPR120 primary antibody raised in rabbit (1:500 dilution; MBL). Specificity of the GPR120 antibody was documented elsewhere (11). After washing, sections were incubated for 1h at room temperature with a fluorescent anti-rabbit secondary antibody (Alexa 568, 1:1000 dilution; Invitrogen, USA). After washing, slices were blocked again before adding an anti-CD36 primary antibody raised in

goat (1:250 dilution; R&D Systems) or an anti-GLP-1 primary antibody raised in goat (1:100 dilution; Santa Cruz Biotechnology). This GLP-1 antibody was used elsewhere (21). Sections were next incubated with a fluorescent anti-goat secondary antibody (Alexa 488, 1:1000 dilution; Invitrogen) and then counterstained with Hoechst reactive (0.05mg/ml; Sigma-Aldrich) to stain the nuclei. Slices were analyzed under a confocal microscope (Leica). In no cases was fluorescent staining observed when the primary antibody was omitted.

Statistics

Results are expressed as Means \pm SEM. The significance of differences between groups was evaluated with SigmaStat (Systat Software, Germany). We first checked that the data for each group were normally distributed and that variances were equal and then carried out ANOVA, two-tailed Student's *t* test or Mann-Whitney tests. A *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

LCFA enhance the GLP-1-mediated induction of sweet taste sensitivity

To explore the mechanisms by which GLP-1 can affect sweet taste sensitivity, drinking behavior of wild-type and *Glp1r*-null mice was compared using computer-controlled lickometers and a brief access procedure (1min). Consistent with published data (16), functional disruption of the *Glp1r* gene led to a decrease in the attraction for sucrose in conditions in which post-ingestive cues were known to be deeply minimized (Fig. 1-A). This effect took place independently of changes in both CVP structure (data not shown) and expression of key genes responsible for sweet taste perception (Fig. 1-B). Interestingly, addition of a small quantity of α -linolenic acid (ALA) or oleic acid (OLA) reinforced the avidity for the sucrose solution in wild-type mice, but was without effect in *Glp1r*-null mice (Fig. 1-C), suggesting that LCFA may modulate sweet taste sensitivity *via* the GLP-1 signaling pathway. It is unlikely that the greater preference for the fat-sweet mix was due to an additive effect of these two tastants since the concentration of LCFA used (*i.e.* 200 μ M \approx 0.005%) was not detected by mice when it was presented alone in a control solution (Fig. 2-A). This behavior is independent of any change in relative expression of genes encoding for the gustatory lipid-sensors GPR120 and CD36 in *Glp1r*-null mice (Fig. 2-B&C).

GLP-1 signaling in CVP is independent of CD36 gene expression

GLP-1 has been found in few taste bud cells (TBC) in various species (16, 22), but the mechanisms leading to its secretion by gustatory papillae are not yet fully understood. Since mouse CVP express both GPR120 and GLP-1, we hypothesized that the activation of GPR120 by LCFA leads to GLP-1 secretion by TBC as reported for intestinal entero-endocrine L cells (14). In support to this hypothesis, GPR120 and GLP-1 were found to be co-expressed in a large number of mouse taste cells from mouse CVP (Fig. 3A). No staining was detected when the GPR120 or the GLP-1 antibody were omitted (data not shown). The fact that CD36 was also found to be co-expressed with GPR120 in subsets of TBC (Fig. 3-B) raises the possibility of a direct or indirect implication of lingual CD36 in the GLP-1-dependent modulation of avidity for sucrose. To assess this assumption, sucrose licking tests in presence or absence

of 200 μ M ALA were performed in CD36-null mice. Interestingly, enhanced attraction for sucrose appeared to be independent of expression of the CD36 gene (Fig.3-C).

GPR120 is involved in the lipid-mediated release of GLP-1 by mouse circumvallate papillae

To assess the role of GPR120 in the lipid-mediated activation of GLP-1 signaling in TBC, freshly isolated mouse CVP were incubated for 2h in an oxygenized medium containing anti-DPP4, to prevent GLP-1 degradation, and 200 μ M LCFA or 50 μ M GSK137647A. This new drug (Fig. 4-A) was identified by screening a recombinant GPR120 receptor assay coupled with the calcium imaging as a potent and selective GPR120 agonist in various species (Fig. 4-B&C). ALA, which is a potent activator of GPR120 *in vitro* (14) led to a small but significant rise in active GLP-1 levels in culture medium (2.08pM \pm 0.09 vs.1.51pM \pm 0.16 in controls without ALA (data not shown). Because GPR120 is thought to be preferentially a ω 3 receptor (23), effect of ALA on GLP-1 secretion was compared to OLA. As shown in Fig. 5-A, addition of ALA and, in a lesser extent of OLA, increased the GLP-1 content of medium. Interestingly, addition of the specific GPR120 agonist GSK137647A fully reproduced the ALA effect suggesting that GPR120 might be responsible for the LCFA-mediated release of GLP-1 by the mouse CVP (Fig. 5-B).

Disruption of the *Glp1r* gene affects the detection threshold for lipids in the oral cavity.

GLP-1 signaling in mouse taste buds modulates sweet taste sensitivity (16). To determine whether such a regulatory system was also involved in the oro-sensory detection of dietary lipids, wild-type and *Glp1r*^{-/-} mice were subjected to a set of long-term (12h) two bottle preference tests using increasing amounts of rapeseed oil, known to contain both OLA and ALA. *Glp1r*^{-/-} mice were unable to detect low concentrations (from 0.02 to 0.5% w/v) of oil contrary to control animals. However, *Glp1r*^{-/-} mice responded to high lipid solutions (\geq 1% w/v) similarly to wild-type mice (Fig. 6-A) suggesting that GLP-1 signaling also plays a role in the fatty taste sensitivity.

It has been previously demonstrated that GLP-1 in TBC may act on local targets in a paracrine manner (16). To confirm that GLP-1-mediated modulation of the detection threshold for lipids took place in the oral cavity, mice were subjected to a computer-

controlled lickometer using a brief access procedure (1min) to minimize post-ingestive effects. As expected, *Glp1r*-deficient mice failed to detect small quantities of OLA (0.125% = 4.4mM), but shared similar high licking responses for the 0.5% OLA solution (= 17.7mM) compared to responses obtained with wild-type mice, suggesting a higher detection threshold for fat in *Glp1r*^{-/-} mice (Fig. 6-B).

Regulation of lingual CD36 is modulated by GLP-1 signaling

According to previous published data (3, 7-8), lingual CD36 plays a significant role in the spontaneous preference for fat (Fig. 7-A). Indeed, *CD36*^{-/-} mice failed to detect large quantities of OLA in a textured solution in contrast to wild-type mice. It has been recently reported in mouse CVP that CD36 is a lipid-sensitive receptor whom the down-regulation during a meal might lead to progressive sensory-specific satiety for lipid rich foods (8). Origin of this physiological regulation remains elusive. Since GLP-1 affects the detection threshold for lipids in oral cavity, it was tempting to hypothesize that CD36 expression levels in taste buds might be modulated by the GLP-1 signaling pathway during the post-prandial period. To explore this assumption, mice fasted overnight were refed a standard laboratory chow for 2h and CD36 expression levels were assayed by western blotting in wild-type and *Glp1r*^{-/-} mouse CVP. As expected, a 2-fold decrease in lingual CD36 protein levels were found in refed wild-type mice. By contrast, no change was detected in *Glp1r*-null mice (Fig. 7-B).

DISCUSSION

The sense of taste informs the organism about the quality of the food before it is ingested leading to stereotyped eating behavior (e.g. preference or aversion). Taste buds not only specifically detect tastants responsible for the basic taste modalities, but are also able to modulate gustatory perception in autocrine or paracrine manners. This last function, likely related to the body energy balance, is not yet fully understood. A better knowledge of physiological mechanisms modulating gustation is required to explain and, perhaps, predict the ingestive decision circuitry. It is a major health challenge, since it can be thought that a dysfunction of this regulatory system might lead to disturbances in eating behavior.

Subsets of taste bud cells synthesize and secrete gastro-intestinal hormones known to be controlled by energy nutrients, including lipids, and involved in the regulation of food intake, as GLP-1. Concomitant presence of receptor for GLP-1 (GLP-1R) in gustatory mucosa (16) suggests that this hormone is locally active and, thus, might directly affect the basic functions in mouse taste buds. Consistent with this assumption, it has been reported that GLP-1 signaling enhances sweet taste sensitivity (16). Because GLP-1 was found co-localized with the sweet taste receptor sub-unit T1R3 and α -gustducin in a subsets of type II TBC in mouse CVP, it was concluded that GLP-1-positive cells are likely sweet-sensitive (16). Data reported herein confirm that the knock-out of the *Glp1r* gene decreases the attraction for sucrose in the mouse. We show that it is not elicited by changes in the expression of key genes encoding for sweet taste transduction molecules, including T1R2 and T1R3 taste receptors, glucose/galactose transporter SGLT-1, α -gustducin, phospholipase C- β 2 (PLC β 2), the receptor for inositol 1,4,5-trisphosphate (IP3R3) or the transient receptor potential M5 (TRPM5) channel. Therefore, further investigations will be required to elucidate the involved mechanism.

We also show that attraction for the sucrose solution was reinforced by the presence of ALA or OLA suggesting the existence of an additive lipid-dependent regulatory system. Such an effect has also been found in the rat (24). In our experiments, this phenomenon occurred while the LCFA concentration used (i.e. 200 μ M \approx 0.005%) was undetectable by the mouse when it was presented alone during licking tests. It is

consistent with the results of Yoneda et al. showing that mice are unable to properly detect 0.01% LCFA (ALA, OLA or linoleic acid) during short-term behavioral tests (25). Therefore, the change in perceived intensity of sweet taste may not be attributed to addition of sucrose and LCFA effects. Interestingly, we have found that salient impact of ALA or OLA was abolished in *Glp1r*-null mice bringing the first demonstration that dietary lipids affect the perception threshold of sucrose *via* the GLP-1 signaling pathway. Because CD36 and GPR120 are lipid-sensors expressed in the gustatory epithelium, their implication in this regulation was possible. A role of CD36 seems unlikely since *CD36*-null mice display similar attraction for fat-sweet mixture than control mice during short-term licking tests. By contrast, several observations are in favor of an implication of GPR120. First, GPR120 and GLP-1 are found to be co-localized in subsets of TBC in mouse CVP. This observation correlated quite well with the fact that GPR120 (11, 26) and GLP-1 (16) are mainly expressed in type II TBC in the mouse. Second, using an original *ex-vivo* approach maintaining the morphological and functional integrity of taste buds, we found that LCFA lead to GLP-1 release by mouse CVP. ALA, which is known to be a potent activator of the GPR120 receptor (14), appears to be a stronger GLP-1 secretagogue than OLA. Third, use of the specific GPR120 agonist GSK137647A reproduces the secretion of active GLP-1 mediated by LCFA, especially ALA. Since LCFA, GPR120 and GLP-1 are functionally linked in the entero-endocrine L cells in the gut (14), these results suggest that the activation of lingual GPR120 by LCFA might induce the release of GLP-1 by TBC increasing the attraction for sucrose. This original function for the sense of taste does not exclude a direct implication of GPR120 in the oro-sensory perception of dietary lipids, as proposed by Damak and collaborators (11). Indeed, it is thought that the glucose sensor T1R3 plays a role both in sweet taste perception and hormone secretion (27-28).

GLP-1 signaling appears to be also involved in the oro-sensory perception of dietary fat. Invalidation of *Glp1r* gene leads to a significant reduction of sensitivity to rapeseed oil in long-term (12h) two bottle preference tests. While preference threshold for oil was 0.02% in age-matched wild-type controls, it was up to 0.5% in *Glp1r*^{-/-} mice. Mechanisms responsible for this eating behaviour mainly take place in the oral cavity. Indeed, similar data were reproduced when wild-type controls and *Glp1r*-null mice were tested with a computer-controlled lickometer using a brief-

access procedure (1min) known to minimize post-ingestive cues. We have recently reported that CD36 protein level in mouse CVP is subjected to a short-term down-regulation during food intake, contrary to GPR120 (8). It is a very sensitive regulation strictly dependent on the presence of lipid in the diet. Interestingly, direct oil deposition onto the tongue is sufficient to trigger the decrease of CD36 protein in CVP confirming a local regulation (8). However, the underlying mechanism(s) remained poorly understood. Data reported here demonstrate that GLP-1 signaling plays a significant role in this regulation. Indeed, no decrease in CD36 protein level was observed in CVP from refed *Glp1r*-null mice, contrary to wild-type animals. As reported for numerous surface receptors, this negative feedback might constitute a desensitization system during persistent exposure to dietary lipids. Consistent with this assumption, the post-prandial down-regulation of CD36 in CVP seems to be sufficient to affect the motivation for fat during a meal, initially high and then gradually decreasing secondary to the food intake (8). Therefore, it is tempting to speculate that the lower attraction for fat found in *Glp1r*^{-/-} mice is related with a dysfunction in the GLP-1 regulatory loop controlling CD36 protein level in CVP.

Existence of physiological links between oro-sensory perception of lipids, selection of energy-dense foods and obesity risk is gradually emerging. An inverse correlation between peripheral gustatory sensitivity to poly-unsaturated fatty acids and preference for lipid-rich foods has been reported in rats (29). In healthy humans, hypersensitivity to lipids seems to be associated with lower energy consumption, fat intake and body mass index (30). This phenomenon might be related to lipid-sensors found in taste buds. The fact that a common genetic polymorphism leading to the reduction of CD36 gene expression produces an attenuation of oro-sensory sensitivity for fat in Human (10) is consistent with this assumption. Studies have also shown synergy between oral fat sensitivity and attraction for sucrose in rodents. Ability of unsaturated LCFA to inhibit the delayed rectifying K⁺ (DRK) channels in rat TBC has been the first mechanism identified (31). Indeed, lipid-mediated cellular depolarization added to that triggered in own by sucrose should increase the sweet taste perception (24). Present data highlight an alternative mechanism suggesting the involvement of GLP-1 signaling. The relative physiological importance of these two mechanisms remains to be established.

In conclusion, our data support the existence of a functional link between unsaturated LCFA including ω 3, GPR120 and the secretion of GLP-1 by mouse CVP. This system, reminiscent of what happens in the entero-endocrine L cells, modulates the sensitivity thresholds for energy-dense nutrients (sucrose and LCFA). For lipids, it appears to be implicated in a regulatory loop targeting CD36. Since change of CD36 protein level in CVP modulates the motivation for fat during a meal (8), this LCFA/GPR120/GLP-1 axis might play a significant role in the sensory-specific satiety for lipids. Therefore, it is tempting to speculate that a dysfunction of this regulatory loop might lead to an increased motivation to obtain high fat foods. A better understanding of molecular mechanisms responsible for lipid sensing in the gustatory papillae and of their physiological impact on eating behavior should allow the development of new therapeutic and nutritional strategies for mitigating excess food intake and limit the obesity risk.

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FIGURES

Fig. 1: Minute quantities of LCFA increase sweet taste sensitivity *via* the GLP-1 signaling.

A: Short-term licking tests (1 min) in wild-type (Wt) and *Glp1r*^{-/-} mice subjected to a control solution or 62mM sucrose solution. Means ± SEM (n=11-15). * p<0.05; ** p<0.01.

B: mRNA levels of key genes involved in sweet taste perception assayed by real-time PCR in CVP from wild-type (Wt) and *Glp1r*^{-/-} mice. Each value corresponds to a pool of total RNA from 2 mice. Means ± SEM (n=6).

C: Short-term licking tests (1 min) in wild-type (Wt) and *Glp1r*^{-/-} mice subjected to a 62mM sucrose solution alone or in presence of 200µM α-linolenic acid (ALA) or oleic acid (OLA). Means ± SEM (n=11-13). * p<0.05; ** p<0.01.

Fig. 2: GLP-1R gene disruption does not affect gene expression of CD36 and GPR120 in mouse CVP.

A: Short-term licking tests (1 min) in wild-type (Wt) and *Glp1r*^{-/-} mice subjected to a control solution or a 200µM ALA or OLA solutions. Means ± SEM (n=11-13). **B:** CD36 and GPR120 mRNA levels assayed by real-time PCR in CVP from wild-type (Wt) and *Glp1r*^{-/-} mice. Each value corresponds to a pool of total RNA from 2 mice. Means ± SEM (n=6).

C: CD36 and GPR120 protein levels assayed by Western blotting in CVP from wild-type (Wt) and *Glp1r*^{-/-} mice. A representative blot corresponding to a pool of total proteins from 3 mice is shown. Means ± SEM (n=2-4).

Fig. 3: LCFA-induced sweet taste sensitivity is independent of the CD36 gene expression.

A: Immunolocalization of GPR120 and GLP-1 in mouse CVP. Nuclei were stained by Hoechst's dye. Scale bar is 40µm.

B: Immunolocalization of GPR120 and CD36 in mouse CVP. Scale bar is 40µm.

C: Short-term licking tests (1 min) in wild-type (Wt) and CD36-null mice subjected to a 62mM sucrose solution alone or in presence of 200µM ALA. Means ± SEM (n=10). ** p<0.01.

Fig. 4: Identification of a selective GPR120 agonist.

A: Formula of the specific GPR120 agonist GSK137647A.

B: *In vitro* potency (pEC₅₀) and efficacy (Max Response) of GPR120 agonists linoleic acid (LA) and GSK137647A for human, mouse and rat GPR120.

C: Calcium-response curves of the GPR120 agonist GSK137647A for human, mouse and rat GPR120.

For B&C, values are means of at least three experiments.

Fig. 5: LCFA and the selective GPR120 agonist GSK137647A induce active GLP-1 release by mouse CVP.

A: GLP-1 release by freshly isolated CVP incubated in presence of 33μM BSA alone (C, control) or with 200μM α-linolenic acid (ALA) or 200μM oleic acid (OLA). Each value corresponds to the GLP-1 released by a pool of CVP from 3 mice. Means ± SEM (n=3-4). * p<0.05.

B: GLP-1 release by freshly isolated CVP incubated in presence of 33μM BSA alone (C, control) or with 200μM α-linolenic acid (ALA) or 50μM of the specific GPR120 agonist (GSK137647A). Each value corresponds to the GLP-1 released by a pool of CVP from 3 mice. Means ± SEM (n=3). * p<0.05.

Fig. 6: Disruption of the *Glp1r* gene affects the lipid detection threshold in the mouse.

A: Long-term two bottle preference tests (12h) in wild-type (Wt) and *Glp1r*^{-/-} mice subjected to control solution (0.3% Xanthan gum in water) and growing levels of rapeseed oil (0.01-2%) in 0.3% xanthan gum. Xanthan gum was used to minimize textural cues and to emulsify rapeseed oil. Means ± SEM (n=10-12). Dotted line represents a lack of preference.

B: Short-term licking tests (1 min) in wild-type (Wt) and *Glp1r*^{-/-} mice subjected to a control solution (mineral oil) and 0.125 or 0.5% oleic acid (OLA) in mineral oil. Means ± SEM (n=11-20). * p<0.05; ** p<0.01.

Fig. 7: Regulation of lingual CD36 is modulated by the GLP-1 signaling.

A: Short-term licking tests (1 min) in wild-type (Wt) and *CD36*^{-/-} mice subjected to a control solution (mineral oil) and 0.5% oleic acid (OLA) in mineral oil. Means ± SEM (n=7). *** p<0.001.

B: CD36 protein levels determined by Western blotting in CVP from wild-type (Wt) or *Glp1r*^{-/-} mice fasted overnight or refed *ad libitum* with a standard laboratory chow for 2h. Each point corresponds to a pool of total proteins from 3-4 mice. Means ± SEM (n=4). * p<0.05.

Table 1: Sequences and GenBank numbers of primers employed for RT-PCR amplifications.

Gene name	Nucleotides sequences (5'→ 3') or Applied Biosystems Taqman Assay ID details	Pubmed accession number
CD36	Forward: GGCCAAGCTATTGCGACATG Probe: CACAGACGCAGCCTCCTTCCACCT Reverse: CCGAACACAGCGTAGATAGAC	NM_007643
GPR120	Applied Biosystem Mm01198944_m1	NM_181748.2
α -gustducin	Forward: ACACATTGCAGTCCATCCTAGC Probe: TGAAGTTGTTCTTGGTCCTCTCGGCTCC Reverse: ATCACCATCTTCTAGTGTATTTGCC	XM_144196
PLC β 2	Forward: GGCTTGAGTTCATCGTCATT Reverse: ACTCCCCCTGTCTTTTCCTA	NM_177568
T1R2	Forward: CCGCCAGGCTTTCTTCACC Probe: TTGCTTCTCCGTCTGCCTCTCCTGC Reverse: AGACGCACACAATCTGGAAGG	NM_031873.1
IP3R3	Forward: ACGGAGCTGCCACATTTAT Reverse: CTCGTCCTCTTCTACGATCTC	NM_080553
36B4	Forward: GCCACCTGGAGAACAACCC Probe: AGGTCCTCCTTGGTGAACACGAAGCC Reverse: GCCAACAGCATATCCCGAATC	NM_007475
TAS1R3	Applied Biosystem Mm00473459_g1	NM_031872.2
SGLT-1	Applied Biosystem Mm0041203_m1	NM_019810.4
TRPM5	Applied Biosystem Mm00498453_m1	NM_020277.2

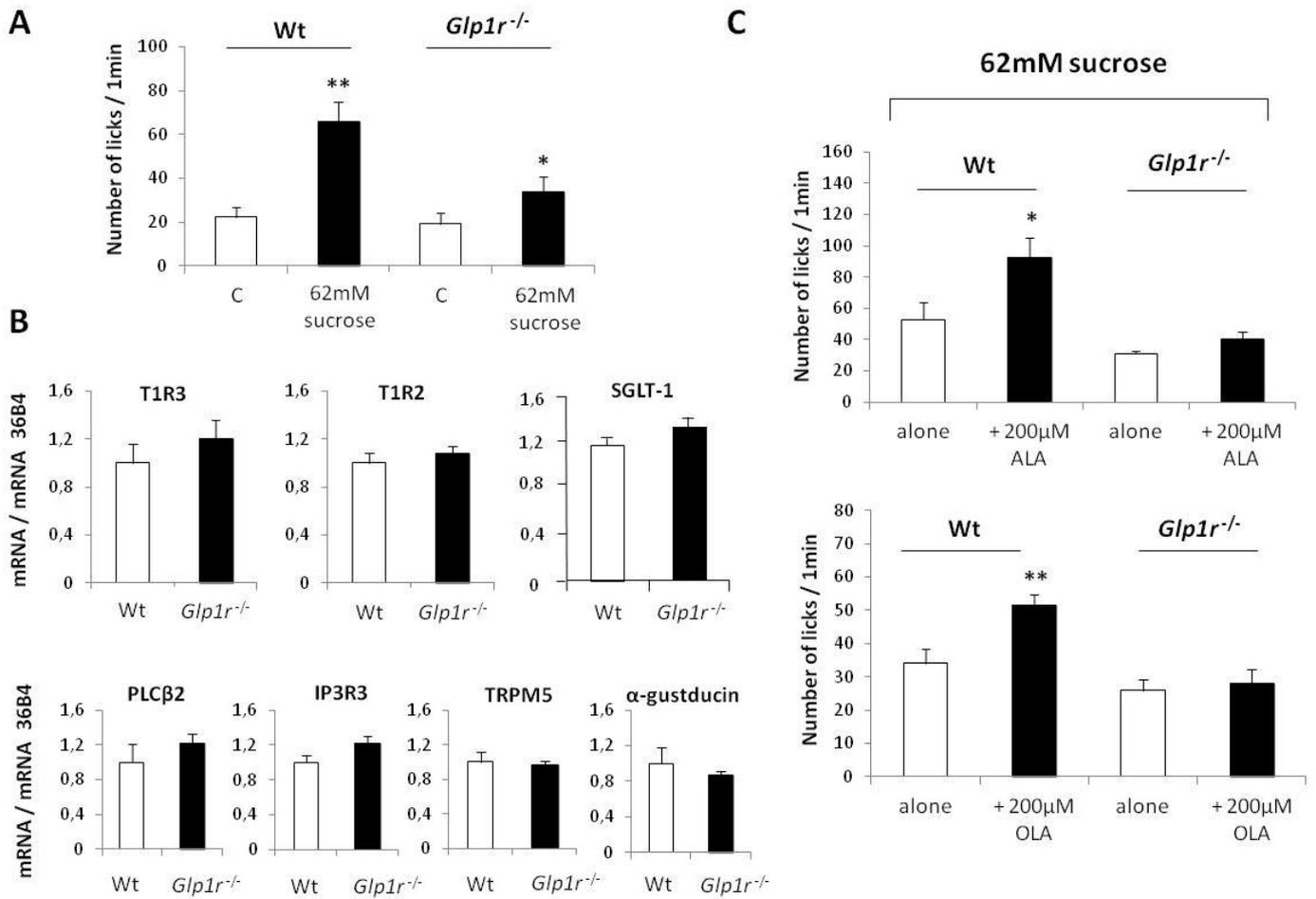


Fig. 1, Martin et al.

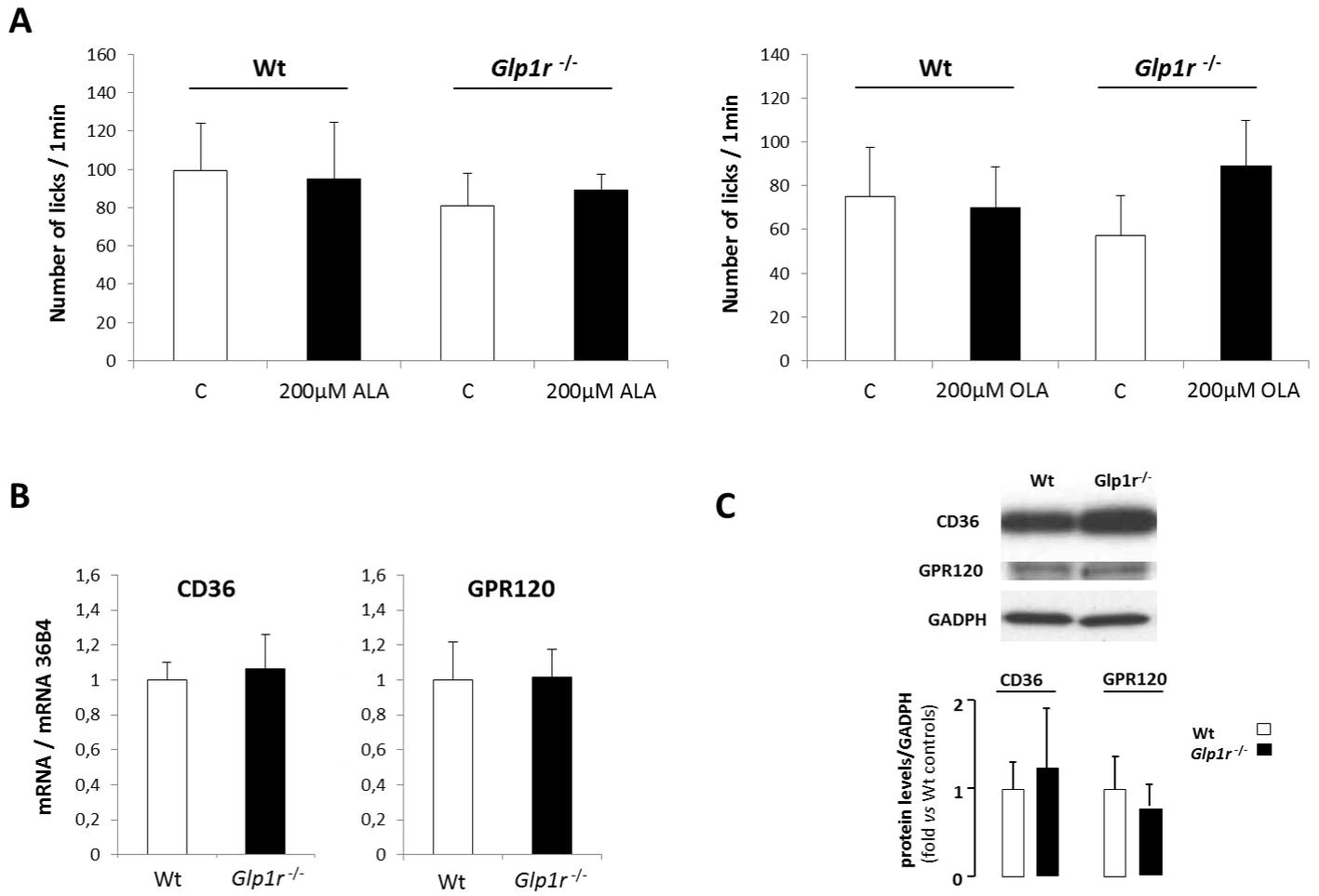
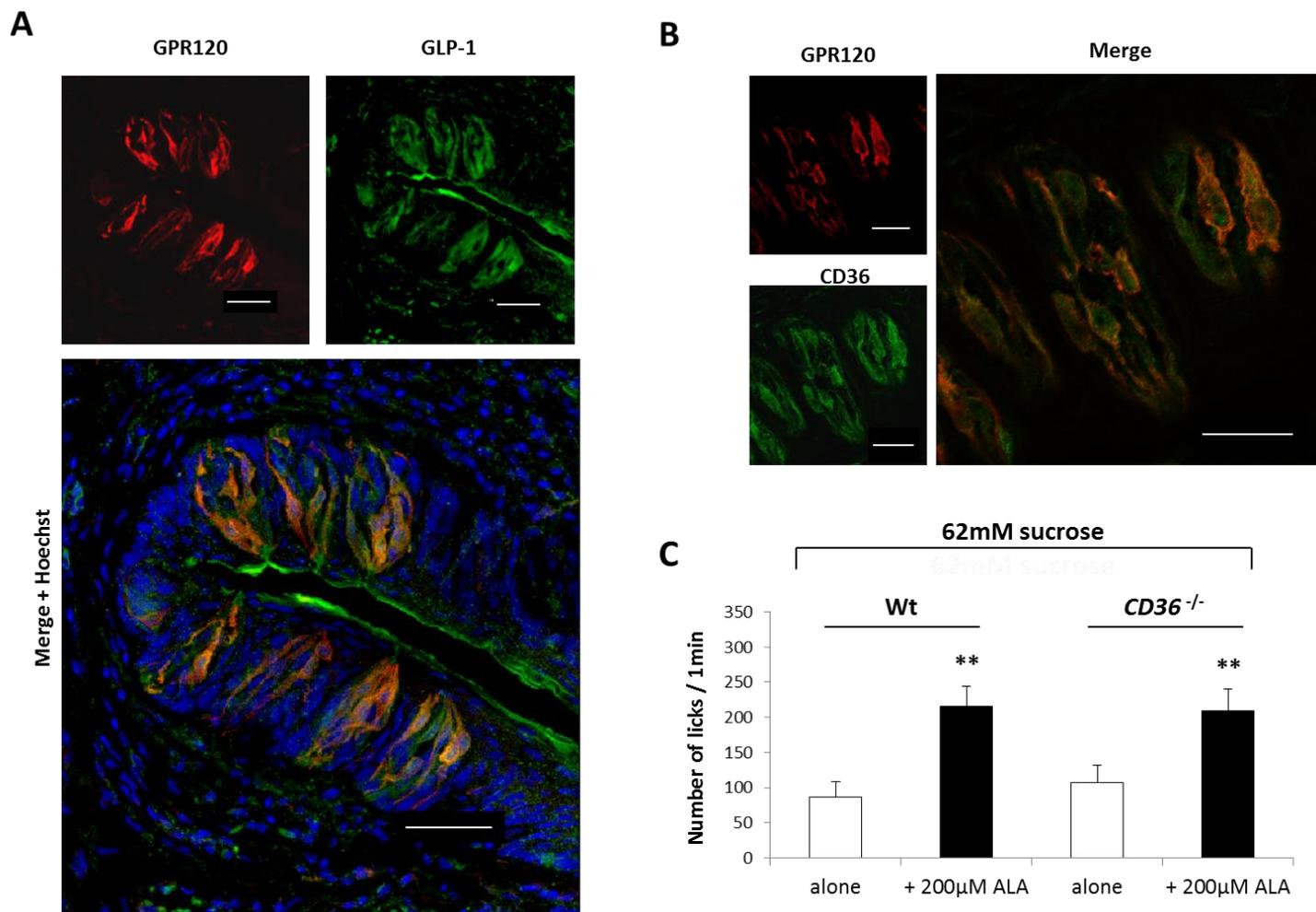
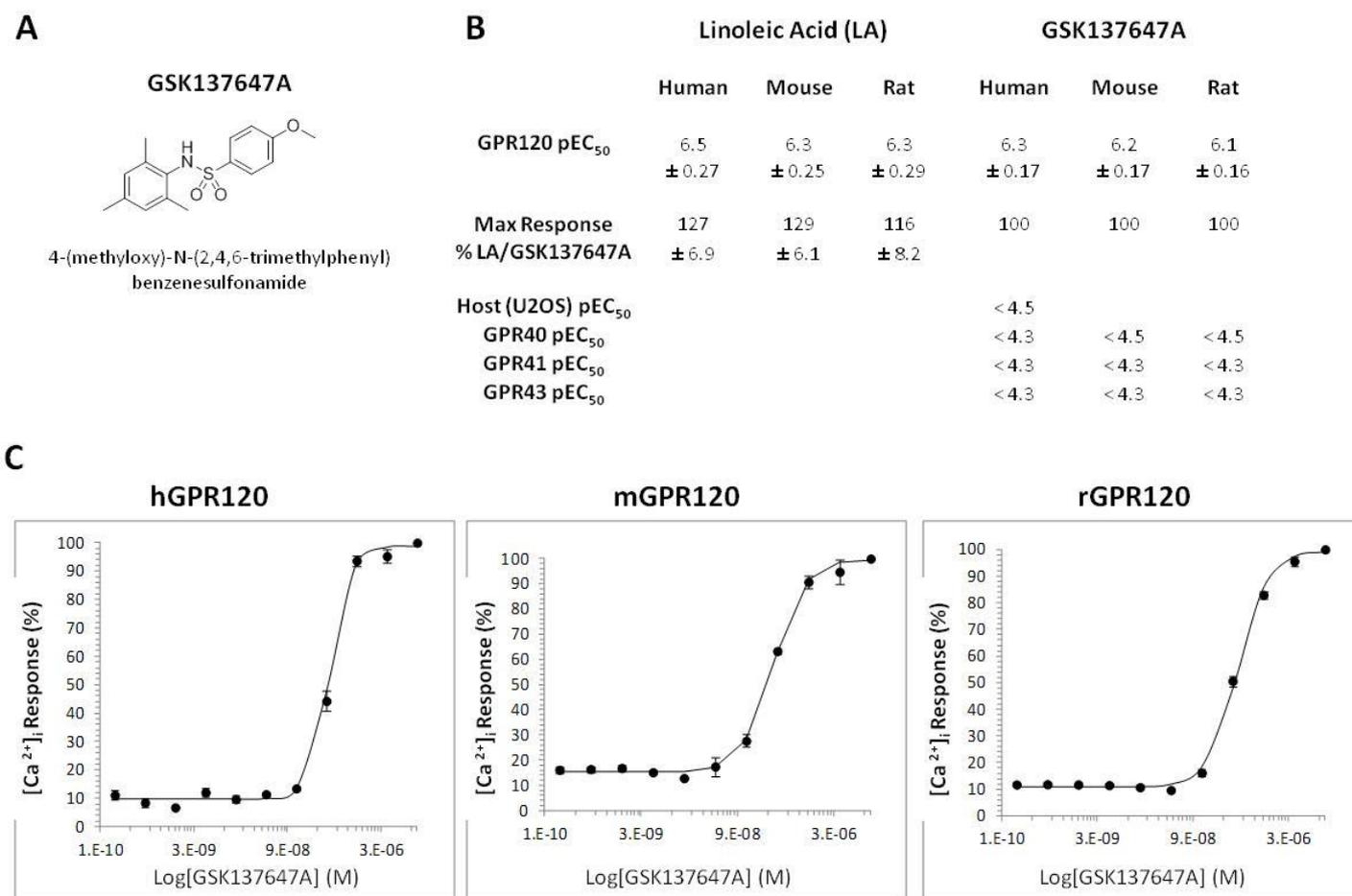


Fig. 2, Martin et al.

Fig. 3, *Martin et al.*

Fig. 4, *Martin et al.*

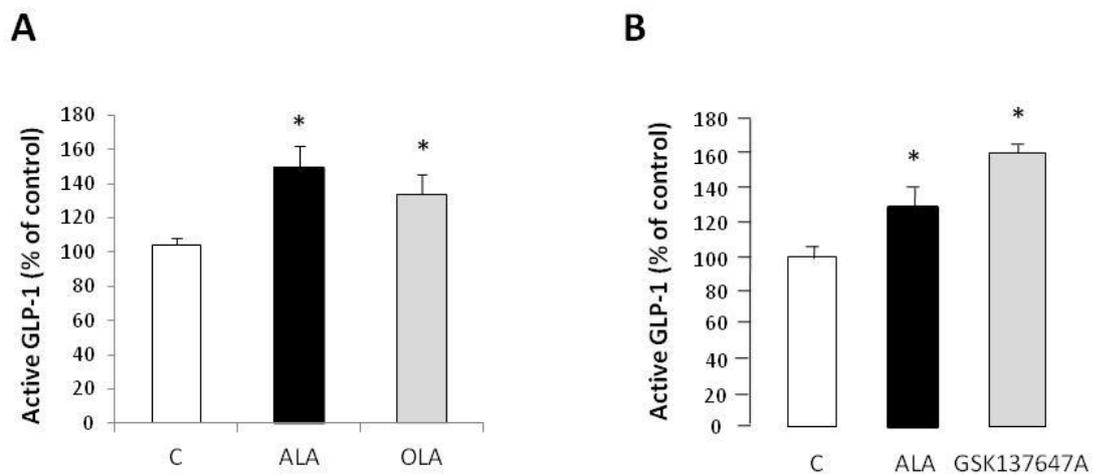


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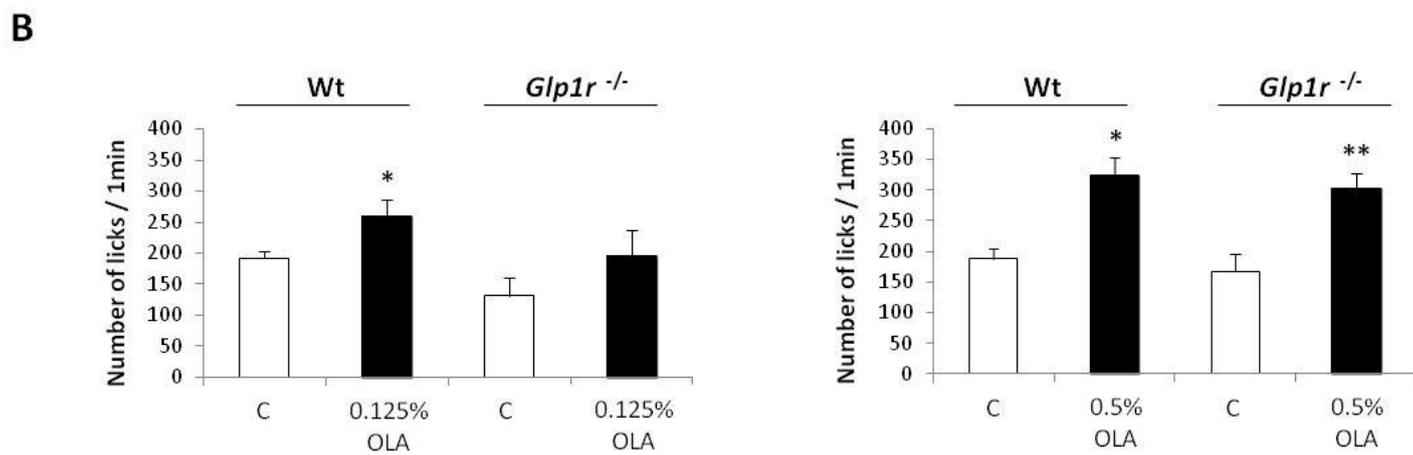
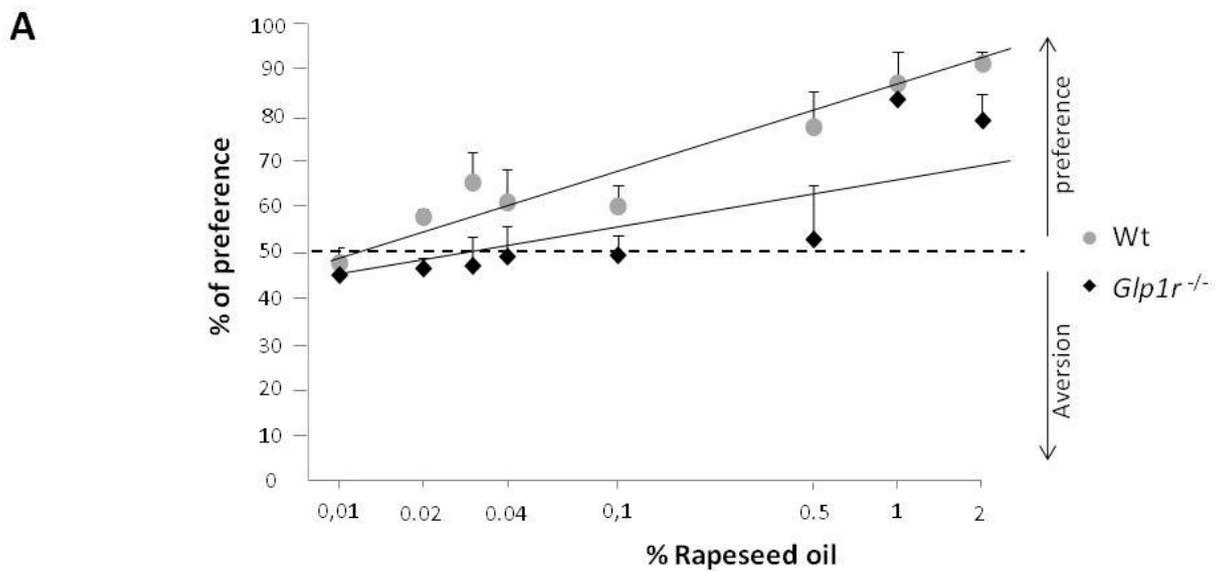
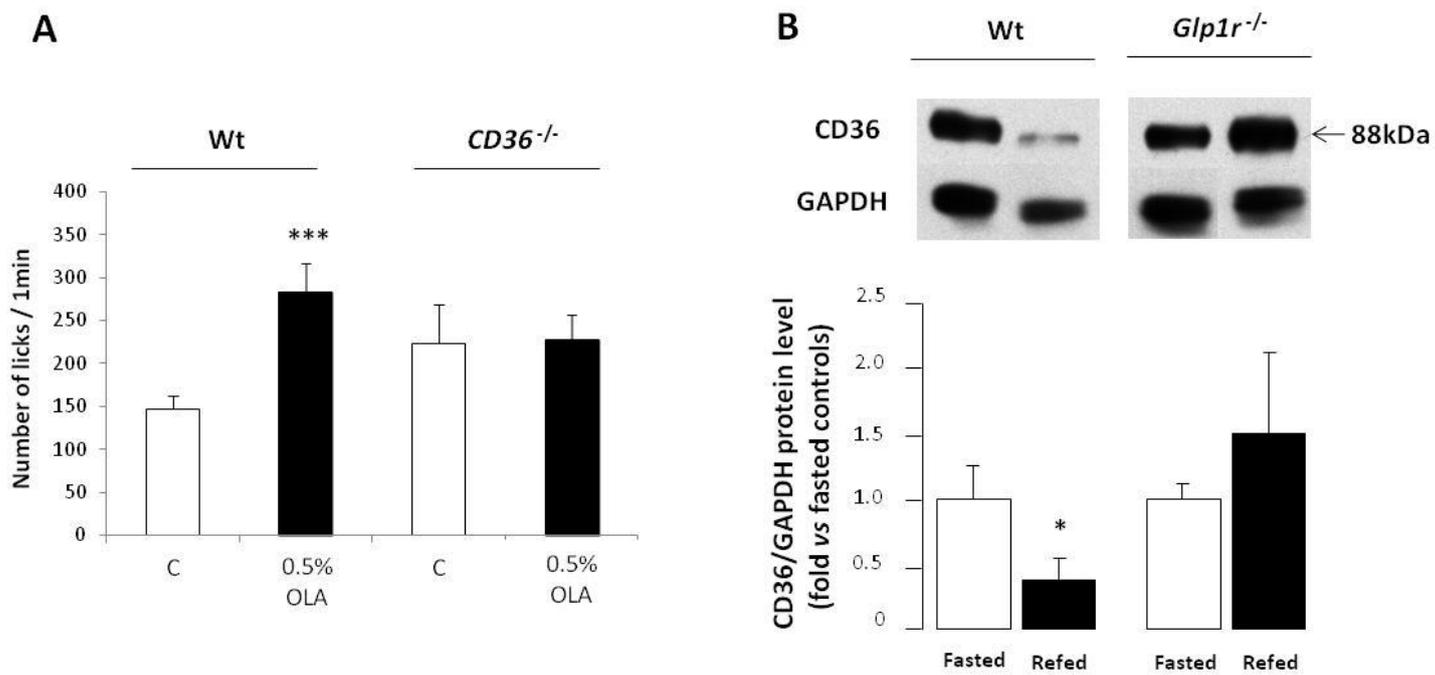


Fig. 6, *Martin et al.*

Fig. 7, *Martin et al.*