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1 **Butyrate, valerate, and niacin ameliorate anaphylaxis by suppressing**
2 **IgE-dependent mast cell activation: Roles of GPR109A, PGE_2, and epigenetic**
3 **regulation**

4 Running title: Effects of SCFAs in IgE-dependent MC activation

5

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23 **Abstract**

24 Short chain fatty acids (SCFAs) were recently shown to modulate the development and
25 functions of immune-related cells. However, the molecular mechanisms by which
26 SCFAs regulate mast cells (MCs) are not fully understood. We found that the oral
27 administration of valerate or butyrate ameliorated passive systemic anaphylaxis in mice.
28 Butyrate and valerate suppressed the IgE-mediated degranulation of bone
29 marrow-derived MCs, which were eliminated by pertussis toxin and by the knockdown
30 of *Gpr109a*. A treatment with trichostatin A suppressed IgE-mediated MC activation
31 and reduced the surface expression level of FcεRI on MCs. Acetylsalicylic acid and
32 indomethacin attenuated the suppressive effects of SCFAs on degranulation. The
33 degranulation degree was significantly decreased by the treatment with PGE_2 whose
34 release from MCs was markedly enhanced by SCFAs. The SCFA-mediated amelioration
35 of anaphylaxis was exacerbated by COX inhibitors and an EP3 antagonist. The
36 administration of niacin, a ligand of GPR109A, alleviated the symptoms of passive
37 cutaneous anaphylaxis, which was inhibited by COX inhibitors and the EP3 antagonist.

38

39 **Key words:** IgE, mast cell, short-chain fatty acid, G protein-coupled receptor, PGE_2,
40 anaphylaxis

41

42 **Abbreviations used:** AERD, aspirin-exacerbated respiratory diseases; Ag, antigen;
43 ASA, acetylsalicylic acid; BM, bone marrow-derived; cysLT, cysteinyl leukotriene; DC,
44 dendritic cell; GPCR, G protein-coupled receptor; HDAC, histone deacetylase; i.p.,

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45 intraperitoneal; i.v., intravenous; MC, mast cell; NSAID, non-steroidal
46 anti-inflammatory drug; PCA, passive cutaneous anaphylaxis; PGs, prostaglandins; p.o.,
47 per os ; PSA, passive systemic anaphylaxis; PTX, pertussis toxin; RALDH,
48 retinaldehyde dehydrogenase; s.c., subcutaneous; SCFA, short chain fatty acid; siRNA,
49 small interfering RNA; TSA, trichostatin A.
50
51 **Key Messages:**
52 Short chain fatty acids (SCFAs), particularly butyrate and valerate, suppress the
53 IgE-mediated activation of mast cells (MCs) *in vivo* and *in vitro*.
54 SCFAs enhance the release of PGE_2 from MCs, which inhibits the IgE-mediated
55 activation of MCs.
56 Niacin, a ligand of GPR109A, ameliorates IgE-dependent anaphylaxis.
57 The administration of COX inhibitors or an antagonist of PGE_2 receptor 3 (EP3)
58 inhibited the suppressive effects of butyrate and niacin on IgE-dependent anaphylaxis.
59

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60 **Introduction**

61 Dietary fibers that are barely digested in the mammalian digestive system are fermented
62 by the bacteria that constitute the intestinal flora. Short chain fatty acids (SCFAs),
63 including acetate, butyrate, propionate, and valerate, are produced in the colon during
64 the fermentation process as secondary metabolites. The effects of intestinal SCFAs on
65 immune responses have recently become topics of interest. Previous studies indicated
66 that SCFAs contributed to the maintenance of homeostasis by modulating the
67 development and function of immune-related cells [1,2]. SCFAs have been shown to exert
68 beneficial effects on immune-related diseases. Mice kept under germ-free conditions or
69 fed a low-fiber diet showed an exacerbated pathology and symptoms of inflammatory
70 bowel disease, airway hypersensitivity, experimental autoimmune encephalomyelitis,
71 and rheumatoid arthritis due to a deficiency in SCFAs [3-8]. SCFAs attenuate these
72 inflammatory diseases by accelerating Treg development [4,5,7], increasing IL-10
73 production by lymphoid cells [4,8], suppressing the activation of neutrophils and
74 eosinophils [3], and regulating T cell development via the modulation of monocyte
75 functions [6]. Intestinal epithelial cells and immune cells take up SCFAs through
76 monocarboxylate transporters and/or sense SCFA signals via G protein-coupled
77 receptors (GPCRs) on the cell surface. SCFAs exhibit an additional function as HDAC
78 inhibitors, inducing the expression of genes involved in the anti-inflammatory function
79 of immune cells.

80 Mast cells (MCs), which develop in bone marrow and terminally differentiate in the
81 mucosa and connective tissues of the whole body, play a key role in IgE-mediated

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82 allergic diseases, such as pollinosis and food allergy. The cross-linking of the
83 high-affinity receptor for IgE, FcεRI, by the IgE and antigen (Ag) complex induces
84 rapid degranulation, eicosanoid release, and cytokine production in MCs, resulting in
85 allergic symptoms. Although accumulating evidence recently showed the anti-allergic
86 effects of SCFAs targeting MCs [9-11], the molecular mechanisms by which SCFAs
87 modulate MC functions remain unknown. In the present study, we demonstrated that the
88 oral administration of butyrate and valerate ameliorated IgE-mediated anaphylaxis in
89 mice, and that SCFAs significantly reduced the degree of IgE-induced degranulation
90 and cytokine production of bone marrow-derived MCs (BMMCs). We also investigated
91 the molecular mechanisms underlying the suppressive effects of SCFAs *in vitro* and *in*
92 *vivo*, by examining the roles of GPR109A, HDAC inhibition (HDACi) activity, the
93 NRF2 pathway, and prostaglandins (PGs) in the effects of SCFAs on MCs. Based on the
94 present results, we concluded that butyrate and valerate regulated MCs via GPR109A
95 and by the HDACi activity, with the accelerated synthesis of PGE_2, resulting in the
96 amelioration of anaphylaxis.
97

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98 **Results**

99 **Orally administered butyrate and valerate suppress IgE-mediated PSA**

100 To evaluate the efficacy of SCFAs to prevent MC-mediated allergic responses *in vivo*,
101 we utilized the PSA model, and found that the decrease in body temperature caused by
102 the IgE-mediated activation of MCs was significantly abated by the administration of
103 butyrate (Fig. 1A) and valerate (Fig. 1B). To confirm whether the intake of SCFAs
104 under this experimental condition affected the development of Treg, which is known to
105 be enhanced by SCFAs, we examined the frequency of Treg cells in the mesenteric
106 lymph node (MLN) and spleen. In the mice that received 100 μmol butyrate or valerate
107 once per day for 4 to 6 days, an increase in Treg cells was not observed in the MLN or
108 spleen (Figs. 1C, 1D). Footpad swelling following a subcutaneous (s.c.) injection of IgE
109 and i.v. injection of Ag was also reduced in mice that received orally administered
110 butyrate (Fig. 1E), suggesting that the uptake of SCFAs via the digestive tract
111 suppressed not only systemic anaphylaxis, but also peripheral cutaneous anaphylaxis.
112 These results indicate that butyrate and valerate exerted suppressive effects on the
113 MC-mediated allergic responses *in vivo* independent of Treg development.

114

115 **Effects of butyrate and valerate on the degranulation and cytokine production in**
116 **IgE-stimulated MCs**

117 BMMCs pretreated with various SCFAs were stimulated with IgE and Ag to evaluate
118 the effects of SCFAs on MC activation. The activity of β-hexosaminidase released from
119 MCs was assessed as an index of the immediate response level (Fig. 2A). As shown in

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120 Fig. 2B, the frequency of DAPI-stained cells remained unchanged in the presence of 10
121 mM SCFAs, suggesting that SCFAs did not induce apparent toxicity. Under this
122 experimental condition, five out of the six SCFAs examined, namely, propionate,
123 butyrate, valerate, isobutyrate, and isovalerate, suppressed the degranulation of
124 IgE-stimulated BMMCs in a dose-dependent manner (Fig. 2A). The suppressive effects
125 of these SCFAs at 1 mM were significant, whereas acetate did not affect the degree of
126 degranulation even at 10 mM (Fig.2A). These results suggest that these five SCFAs
127 suppressed the IgE-induced degranulation of MCs. We also examined sodium butyrate
128 to exclude the effects of pH changes caused by the acids, and confirmed that sodium
129 butyrate significantly reduced IgE-stimulated degranulation as well as butyrate (Fig.
130 2C).

131 The effects of SCFAs on cytokine production by MCs was examined. As shown in Fig.
132 2D, IL-13 release from IgE-stimulated MCs was significantly suppressed by the
133 pretreatment with butyrate, valerate, and isovalerate, and was slightly suppressed by the
134 isobutyrate treatment. The IgE-induced production of TNF-α was significantly inhibited
135 by the treatment with valerate, isobutyrate, and isovalerate, whereas the butyrate
136 treatment promoted the production of TNF-α.

137 The IgE-induced activation of MCs was initiated by the binding of IgE to FcεRI. The
138 expression level of FcεRI is associated with the degree of degranulation and is a risk
139 factor for allergic diseases [12-15]. To evaluate the effects of SCFAs on FcεRI expression,
140 we performed a flow cytometric analysis and found that FcεRI levels on the cell surface
141 were decreased by the pretreatment with butyrate and valerate (Fig. 3A). FcεRI

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142 comprises 3 subunits, namely, α, β, and γ, and the transcription factors PU.1, GATA1,
143 and GATA2 regulate the cell type-specific expression of α and β [16-20]. To clarify
144 whether the down-regulation of surface FcεRI occured in a transcription-dependent
145 manner, we measured the mRNA levels of FcεRI subunits (Fig. 3B) and related
146 transcription factors (Fig. 3C) by quantitative PCR. The obtained results revealed that
147 butyrate and valerate did not reduce but rather tend to increase the mRNA levels of
148 *Fcer1a*, *Ms4a2*, *FceR1g*, *Spi1*, *Gata1*, and *Gata2*. Therefore, valerate and butyrate ma
149 have reduced FcεRI levels on the cell surface without inhibiting the transcription of
150 FcεRI subunit genes.
151 These results indicate that butyrate and valerate suppressed the IgE-mediated
152 degranulation and cytokine release of MCs, and also that the decrease observed in the
153 cell surface level of FcεRI was partly involved in the suppressive effects of SCFAs on
154 the IgE-mediated activation of MCs.
155
156 **Butyrate and valerate suppress the activation of MCs via GPR109A**
157 To elucidate the molecular mechanisms by which butyrate and valerate modify the
158 function of MCs, we examined cell surface molecules in order to identify a candidate
159 transporter and/or receptor for SCFA. A quantitative PCR analysis showed that BMMCs
160 expressed detectable amounts of mRNAs for the solute carrier group of membrane
161 transport proteins (*Slc5a8*, *Slc5a12*, and *Slc16a1*) and GPCRs (*Gpr41*, *Gpr43*, and
162 *Gpr109a*). Moreover, the mRNA expression levels of *Slc16a1* and *Gpr109a* were higher
163 than those of other mRNAs (Figs. 4A, 4B). To confirm the involvement of the

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164 transporter and receptor in SCFA signaling, we pretreated BMMCs with a reagent that
165 inhibits the transporter or receptor for 1 h prior to the addition of SCFAs. As shown in
166 Fig. 4C, the presence of 2-cyano-4-hydroxyphenyl acrylic acid (monocarboxylate
167 transporter inhibitor) did not affect the SCFA-mediated suppression of degranulation. In
168 contrast, PTX, an inhibitor of Gi/o proteins, counteracted the suppressive effects of
169 butyrate and valerate (Fig. 4D), suggesting that Gi/o-type GPCR functions are required
170 for the butyrate- and valerate-mediated suppression of MC activation. We also found
171 that mRNAs for GPR43 and GPR109A were expressed in human MCs (Fig. 4E). To
172 clarify the involvement of GPR109A, which was a Gi-GPCR that was expressed at
173 higher levels than other Gi-GPCRs (GPR41 and GPR43) in mouse and human MCs
174 (Figs. 4B, 4E), we performed a knockdown experiment using siRNA. When butyrate
175 and valerate significantly suppressed the degranulation of control siRNA-introduced
176 BMMCs, *Gpr109a* siRNA transfectants in which *Gpr109a* mRNA was effectively
177 knocked down (Fig. S1A) exhibited markedly enhanced degranulation (Fig. 4F). This
178 result supports the hypothesis that GPR109A is a receptor for butyrate and valerate on
179 MCs. The increase in degranulation by the knockdown of *Gpr109* was more restrictive
180 for butyrate-treated MCs than for valerate-treated MCs, suggesting that the effects of
181 butyrate were partly dependent on GPR109A. The knockdown of *Gpr109a* up-regulated
182 the degranulation of non-treated control BMMCs. This result suggests that GPR109A
183 signaling constitutively suppressed MC activation. Therefore, to clarify the role of
184 GPR109A in the IgE-dependent activation of MCs, we conducted a GPR109A
185 overexpression experiment. When GPR109A was constitutively overexpressed in

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186 BMMCs using a retroviral vector (Fig. S1B), the suppressive effects of SCFAs were
187 enhanced and the degree of degranulation was markedly reduced even in the absence of
188 SCFAs (Fig. 4G).
189 Based on these results, we concluded that GPR109A was involved in the suppression of
190 the sIgE-dependent degranulation of MCs and that valerate and butyrate functioned as
191 ligands for GPR109A.
192
193 **Trichostatin A (TSA)-treatment suppresses the IgE-mediated activation of MCs**
194 SCFAs exhibit HDACi activity, which enhances the anti-inflammatory functions of
195 immune cells by inducing the expression of genes including Foxp3 and IL-10 [5,8]. The
196 order of anti-allergic effects in Fig. 2A (butyrate > valerate >> acetate) is consistent
197 with the order of HDACi activity among SCFAs [8]. To evaluate the effects of HDACi
198 activity on the IgE-mediated activation of MCs, we analyzed MCs that were treated
199 with TSA, an inhibitor of class I and II HDACs. The pretreatment of MCs with TSA (5
200 – 20 nM) for 24 h before the IgE-mediated stimulation significantly and
201 dose-dependently suppressed degranulation (Fig. 5A) without affecting the frequency of
202 DAPI-stained dead cells (Fig. 5B). The release of IL-13 and TNF-α from
203 IgE-stimulated MCs was significantly inhibited by the pretreatment with 20 nM TSA
204 (Fig. 5C).
205 The TSA treatment reduced the surface expression levels of FcεRI (Fig. 5D), even
206 though the mRNA levels of FcεRIα, β, and γ were not decreased in TSA-treated MCs
207 (Fig. 5E), which is consistent with the results obtained from butyrate-treated MCs (Fig.

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208 2). We also found that the suppressive effects of butyrate on FcεRI expression levels in
209 MCs were not inhibited by the PTX treatment (Fig. 5F).
210 These results suggest that SCFA-induced reductions in the cell surface expression level
211 of FcεRI were mediated by the HDAC inhibition rather than by the
212 Gi-GPCR-stimulation.
213

214 **NRF2 is activated by SCFA, but is not involved in suppressive effect of SCFAs**

215 Butyrate has been shown to activate the transcriptional regulator NRF2, which
216 alleviates oxidative stress, in various cells [21-23]. Furthermore, the activation of NRF2 is
217 frequently observed in MCs treated with anti-allergic compounds [24-26]. Hmox1, a target
218 gene of NRF2, exhibited the greatest increase in its mRNA level following the treatment
219 of human MCs with butyrate [9], however, the role of NRF2 in human MCs was not
220 analyzed. Based on these findings, we investigated whether NRF2 was activated in
221 SCFA-treated MCs and played a role in the suppressive effects of SCFAs on MCs. As
222 shown in Figs. 6A and 6B, the mRNA levels of Hmox1 and Nrf2 were elevated in
223 butyrate-treated BMMCs, and these increases were sustained for at least 48 h under our
224 experimental conditions. In addition, we found that Hmox1 mRNA levels, which were
225 increased by the IgE-mediated stimulation, were further up-regulated in the presence of
226 butyrate (Fig. 6C), and that the TSA treatment increased Hmox1 mRNA levels in MCs
227 (Fig. 6D). To clarify whether the SCFA-induced activation of NRF2 in MCs was
228 involved in the suppression of IgE-mediated activation, we conducted an experiment
229 using an NRF2 inhibitor and found that the inhibition of the NRF2 pathway did not

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230 reduce the suppressive effects of butyrate on the degranulation and cytokine release (Fig.
231 6E).
232 These results indicate that the activation of the NRF2-HO-1 pathway was not involved
233 in the suppression of IgE-mediated activation in SCFA-treated MCs; however, SCFAs
234 and TSA activated this pathway in MCs.
235

236 **Butyrate and valerate increase the release of PGE_2, which suppresses**
237 **degranulation**

238 GPR109A is classically known as a receptor for niacin/nicotinic acid/vitamin B3.
239 Moreover, the intake of excess amounts of niacin induces a niacin flash due to the
240 enhanced release of PGs. Based on these findings, we hypothesized that the treatment of
241 MCs with butyrate and valerate may induce the production of PGs, which affects the
242 activation degree of MCs. To evaluate the roles of PGs in MC activation, we analyzed
243 the degranulation degree of SCFA-treated MCs in the presence of the non-steroidal
244 anti-inflammatory drugs (NSAIDs), ASA and indomethacin. The suppression of
245 degranulation in valerate-treated MCs was counteracted by the addition of ASA,
246 whereas the effects of ASA and indomethacin on butyrate-induced suppression were
247 moderate (Figs. 7A, 7B).
248 PGD_2 and PGE_2 are preferentially produced by activated MCs. Therefore, we evaluated
249 the effects of PGD_2 and PGE_2 on the degranulation levels. Although pretreatment with
250 1-100 nM PGD_2 for 24 h did not affect the degranulation of MCs (Fig. 7C), that with
251 PGE_2 exerted inhibitory effects (Fig. 7D). Similar suppressive effects of PGE_2 were

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252 observed following its addition 2 h prior to the stimulation (Fig. 7F), whereas PGD_2 did
253 not inhibit degranulation at either time point (Fig. 7E). The measurement of PGE_2
254 concentrations in culture media revealed that the IgE stimulation strongly promoted the
255 release of PGE_2, which was already enhanced by butyrate, valerate, and propionate (Fig.
256 7G). Furthermore, SCFA-treated MCs released slightly more PGE_2 than non-treated
257 MCs, even in the steady state (Fig. 7G).
258 We recently reported that a treatment with ASA or the knockdown of cyclooxygenases
259 (COXs), particularly COX-1, promoted the IgE-induced activation of BMMCs [27]. To
260 reveal the role of COX-1 in the suppression of degranulation in SCFA-treated MCs, we
261 evaluated the effects of the COX-1-specific inhibitor SC-560 on MC degranulation.
262 When BMMCs were pretreated with SC-560 prior to the addition of SCFAs, SC-560
263 attenuated the suppressive effects of SCFAs in a dose-dependent manner (Fig. 7H).
264 Collectively, these results demonstrated that the SCFAs increased the *de novo* synthesis
265 and release of PGE_2, which suppressesd the activation of MCs.
266
267 **Involvement of PGs in the SCFA-mediated suppression of anaphylaxis**
268 To investigate whether prostanoid production is involved in the effects of SCFAs *in vivo*,
269 we performed anaphylaxis analyses of mice that were administered an NSAID. When
270 PSA was induced in mice administered butyrate and/or indomethacin, the effects of the
271 administration of butyrate were significantly inhibited by supplementation with
272 indomethacin (Fig. 8A). In mice administered ethylvalerate (to avoid the corrosivity of
273 valerate) and/or ASA, the decrease of body temperature following the Ag injection was

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274 reduced by the administration of ethylvalerate, and the suppression of PSA by
275 ethylvalerate was counteracted by additional supplementation with ASA (Fig. 8B).
276 The results showing that NSAIDs suppressed the SCFA-mediated amelioration of PSA
277 suggest the involvement of PGs in the effects of SCFAs on passive anaphylaxis. To
278 further clarify the role of PGE_2, we conducted a PGE_2 receptor antagonism experiment
279 *in vivo*, and found that the administration of the EP3 antagonist inhibited the attenuating
280 effects of butyrate on PSA (Fig. 8D) and PCA (Fig. 8E), whereas the EP4 antagonist did
281 not affect the butyrate-mediated suppression of PCA (Fig. 8F).
282
283 **Effects of the niacin treatment on anaphylaxis**
284 To elucidate the roles of the GPR109A stimulation on IgE-mediated anaphylaxis, we
285 evaluated the effects of niacin on PCA. Niacin is general term for two kinds of vitamin
286 B3, nicotinic acid and nicotinamide. Although both compounds similarly plays
287 important roles in nutrition as vitamin B3, nicotinic acid and nicotinamide are
288 distinguishable on the basis of GPR109A stimulation activity, namely, nicotinic acid
289 stimulates GPR109A, but nicotinamide does not. As shown in Fig. 8G, nicotinic acid
290 significantly reduced footpad swelling induced by PCA, whereas nicotinamide did not.
291 The suppression of PCA by the nicotinic acid treatment was inhibited by the additional
292 administration of NSAIDs (data not shown) or the EP3 antagonist (Fig. 8H).
293 These results demonstrated that the stimulation of GPR109A was involved in the
294 suppression of anaphylaxis by modulating the production of prostanoids, particularly
295 PGE_2.

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297 **Discussion**

298 MCs play important roles in IgE-dependent allergic responses. The cross-linking of
299 IgE-binding FcεRI on MCs via allergens induces rapid responses, including
300 degranulation and the release of eicosanoids, and late-phase inflammation accompanied
301 by the production of cytokines. Current therapeutic medicines for allergies, such as
302 histamine receptor antagonists, leukotriene receptor antagonists, and humanized
303 anti-human IgE, target steps in the IgE-MC axis, suggesting that the inhibition of MC
304 activation is useful for the prevention of allergic diseases.

305 In the present study, we identified GPR109A-mediated signaling as a key event for the
306 suppression of MC-mediated allergic responses. A recent study demonstrated that
307 GPR109A was involved in colonic homeostasis based on a *Gpr109a* gene deficiency
308 exacerbating dextran sodium sulfate (DSS)-induced colonic inflammation [28].
309 Furthermore, the aforementioned study indicated that the activation of GPR109A by
310 niacin or butyrate suppressed colitis and colon cancer, and colonic macrophages,
311 dendritic cells (DCs), and the epithelium were considered to be the main target cells
312 expressing GPR109A. GPR109A signaling amplified the production of retinoic acid and
313 anti-inflammatory cytokines from macrophages and DCs and subsequently accelerated
314 the development of Tregs. The involvement of butyrate in $CD103^+$ DC-mediated Treg
315 differentiation was supported by another study using a food allergy mouse model with
316 high-fiber feeding [29]. High-fiber feeding enhanced retinaldehyde dehydrogenase
317 (RALDH) activity in $CD103^+$ DCs in a GPR109A-dependent manner. As reported in
318 previous studies, DCs and macrophages were identified as GPR109A-expressing

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319 myeloid cells. In contrast, the role of GPR109A in MCs was largely unknown. In the
320 present study, we found that *GPR109A* mRNA levels were higher than those of *GPR43*
321 (and *GPR41*) in BMMCs and human MCs (Figs. 4B, 4E). The knockdown of *Gpr109a*
322 completely and partially canceled the suppressive effects of valerate and butyrate,
323 respectively, and exacerbated the degranulation of control MCs. The overexpression of
324 GPR109A reduced the degranulation degree and enhanced the sensitivity of MCs to
325 SCFAs. These results demonstrated the following: i) GPR109A signaling inhibits MC
326 activation, ii) valerate is one of the ligands for GPR109A, and iii) endogenous ligands
327 for GPR109A are present in the MC culture medium. Furthermore, the administration of
328 butyrate, valerate, or niacin prior to passive anaphylaxis significantly ameliorated
329 IgE-dependent anaphylaxis (Figs. 1, and 8). The results of *in vitro* and *in vivo*
330 experiments revealed the potential of the butyrate/valerate/niacin-GPR109A axis as a
331 therapeutic target for MC-mediated allergy. In a previous study, the provision of
332 butyrate in drinking water *ad libitum* for 3 weeks reduced the anaphylaxis score of food
333 allergy in mice, and this was accompanied by a decrease in serum IgE levels and
334 increase in $CD103^+$ DCs and the Treg population in MLNs [29]. Although the effects of
335 butyrate on MC functions were not evaluated [29], the oral intake of butyrate or valerate
336 for 1 week suppressed PSA and PCA in the present study (Figs. 1, and 8); therefore,
337 butyrate may have contributed to the decrease observed in the anaphylaxis score with
338 the suppression of MC functions in the previous study. In the present study, Treg levels
339 were not elevated in mice orally administered butyrate or valerate for one week (Fig. 1).

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340 Therefore, we concluded that butyrate and valerate ameliorated anaphylaxis by directly
341 modulating MC functions even when Treg development was not affected by SCFAs.
342 The protective effects of niacin against colitis were also reported in a study using a
343 DSS- or 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced mouse model [30]. The
344 administration of niacin up-regulated PGD_2 levels in the colon and subsequently
345 ameliorated DSS/TNBS-induced colitis in mice via the DP1 receptor [30]. MCs are a
346 major source of PGD_2. A previous study using mice with food allergy revealed an
347 anti-allergic role for PGD_2 derived from MCs [31]. However, under our experimental
348 conditions, PGE_2, but not PGD_2, suppressed MC activation in an autocrine manner (Fig.
349 7). Another study using ptges$^{-/-}$ mice demonstrated that a PGE_2 deficiency caused
350 aspirin-exacerbated respiratory diseases (AERD) in which overproduced cysteinyl
351 leukotrienes (cysLTs) and activated MCs were involved in airway inflammatory
352 disorders [32]. PGE_2 have been shown to attenuate the cysLT-mediated IL-33-dependent
353 activation of MCs [32,33], whereas MC-derived PGD_2 is one of the hallmarks of the
354 severity of AERD [34,35]. Furthermore, PGE_2 exhibited immunosuppressive effects in the
355 colonic mucosa [36]. SCFAs, which enhanced PGE_2 production by MCs (Fig. 7), may
356 contribute to the prevention and/or treatment of AERD and inflammatory bowel disease.
357 A recent study reported that PGE_2 levels inversely correlated with the severity of
358 anaphylaxis in humans and mice [37]. In that study, an EP2 agonist and EP4 agonist
359 suppressed the IgE-induced activation of BMMCs and IgE-mediated PSA. In contrast,
360 the EP3 antagonist, but not the EP4 antagonist inhibited the protective effects of
361 butyrate and niacin against anaphylaxis under our experimental conditions (Fig. 8).

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362 Although we cannot explain this discrepancy, EP3 plays protective roles in
363 MC-mediated allergic responses because *Ptger3*$^{-/-}$ mice had more severe airway
364 inflammation accompanied by the hyperactivation of MCs [38].
365 The characteristics of butyrate and valerate differed in the present study. For example,
366 TNF-α production by MCs was decreased by valerate and increased by butyrate (Fig. 2).
367 Valerate primarily suppressed MC activation via GPR109A, whereas butyrate affected
368 multiple pathways in MCs, including those associated with other GPCRs, in addition to
369 GPR109A (Fig. 4). Two other GPCRs, GPR41 and GPR43, which are receptors for
370 SCFAs [2-4,6], may be involved in MC functions. However, we were unable to clarify this
371 aspect because siRNAs for GPR41 and GPR43 were not functional in MCs, in which
372 mRNAs for GPR41 and GPR43 were rarely detected. Furthermore, a previous study
373 demonstrated that the deficiencies in GPR41 and GPR43 in mouse MCs did not affect
374 the butyrate-mediated suppression of degranulation [9]. An observation that acetate,
375 whose specificity is restricted to GPR43 (EC_{50} for acetate is ~250-500 μM) and barely
376 to GPR41 (propionate > butyrate >> acetate) but not to GPR109A [39], dose not suppress
377 IgE-mediated activation of MCs ([9] and Fig. 2), may support the irrelevance of GPR41
378 and GPR43 in the activation of MCs.
379 *In vivo* experiments using TSA revealed that HDACi, which is one of the activities of
380 butyrate and valerate, inhibited the IgE-induced degranulation and cytokine release of
381 MCs (Fig. 5). The suppressive effects of HDACi on the IgE-dependent activation of
382 MCs were reported in previous studies, in which the down-regulation of tyrosine
383 kinases [9] and the suppression of transcription factor recruitment [40] were observed. In the

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384 present study, the cell surface expression level of FcεRI was decreased by both SCFAs
385 and TSA (Figs. 3, and 5). Although the down-regulation of BTK, SYK, and LAT in
386 butyrate-treated MCs was transcriptional due to a decrease in the acetylation of these
387 promoters [9], the mRNA levels of the three subunits of FcεRI slightly increased in
388 SCFA-treated MCs (Fig. 3) and TSA-treated MCs (Fig. 5). Further studies are
389 warranted to clarify the mechanisms by which SCFAs reduce the surface expression of
390 FcεRI on MCs via HDACi activity.

391 The composition of the daily diet has been shown to affect the microbiota population
392 and SCFA concentrations, which are closely associated with human and animal health
393 [41,42]. In contrast to acetate, butyrate, and propionate, which are major SCFAs in the
394 colon, limited information is currently available on valerate, isobutyrate, and isovalerate,
395 the colonic concentrations of which are low. *Clostridia* and Bacteroides have the
396 potential to produce valerate [41,43,44]. Isobutyrate and isovalerate, which suppressed MC
397 activation (Fig. 2), are produced as secondary metabolites from amino acids in *Bacillus*
398 [45]. Both of these branched SCFAs are present in the Japanese traditional food "Natto",
399 which is made of soybeans through a fermentation process by *Bacillus subtilis natto*.
400 Although branched SCFAs in Natto have been recognized merely as the source of a
401 unique flavor, they may exert anti-allergic effects and/or contribute to colonic
402 homeostasis. Changes in dietary habits in Japan in the past few decades, from a typical
403 Japanese diet including Natto and high-fiber dishes to Western diets may be associated
404 with the incidence of allergic diseases. We intend to investigate the relationship between
405 the mucosal environment and immunoregulation by focusing on valerate, butyrate, and

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406 branched SCFAs, which may lead to the proposal of the health benefits of certain foods

407 in the near future.

408

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409 **Methods**

410 **Mice and cells**

411 BMMCs were generated from the BM cells of C57BL/6 mice (Japan SLC, Hamamatsu,
412 Japan) by cultivation in the presence of 5 ng/mL of mouse IL-3 (BioLegend) as
413 previously described [18,46,47]. All animal experiments were performed in accordance with
414 the approved guidelines of the Institutional Review Board of Tokyo University of
415 Science, Tokyo, Japan. The Animal Care and Use Committees of Tokyo University of
416 Science approved this study (K22005, K21004, K20005, K19006, K18006, K17009,
417 K17012, K16007, K16010).
418

419 **Reagents**

420 SCFAs (acetate, butyrate, sodium butyrate, isobutyrate, propionate, valerate,
421 ethylvalerate, and isovalerate), a monocarboxylate transporter inhibitor
422 (α-cyano-4-hydroxycinnamic acid, CHCA) (Cat. 476870), and acetylsalicylic acid
423 (ASA) (Cat. A5376) were purchased from Sigma-Aldrich (St. Louis, MO). Pertussis
424 toxin (PTX), which is used as a Gi/o-type G protein inactivator, was purchased from
425 Calbiochem (Cat. 516561, San Diego, CA), and indomethacin was obtained from
426 Fujifilm Wako Pure Chemical (Cat. 095-02472, Japan). PGD_2 (Cat. 12010), PGE_2 (Cat.
427 14010), and an inhibitor of COX-1, SC-560 (CAY-70340) were supplied by Cayman
428 Chemical (Ann Arbor, MI). Nicotinic acid/niacin (#72309, Sigma-Aldrich) and
429 nicotinamide/niacinamide (#N0078, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan)
430 were obtained. ONO-AE5-599, an antagonist of PGE_2 receptor 3 (EP3), and

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431 ONO-AE3-208, an antagonist of EP4 were kindly provided by Ono Pharmaceutical Co.
432 Ltd. (Osaka, Japan)
433
434 **Quantification of mRNA**
435 The purification of total RNA and reverse transcription to synthesize cDNA were
436 performed using a ReliaPrep RNA Cell Miniprep System (Promega) and ReverTra Ace
437 qPCR RT Master Mix (TOYOBO, Osaka, Japan), respectively. The mRNA levels were
438 measured by quantitative PCR using a Step-One Real-Time PCR system (Applied
439 Biosystems) with THUNDERBIRD probe qPCR Mix (TOYOBO) or THUNDERBIRD
440 SYBR qPCR Mix (TOYOBO). The TaqMan primers *Gata1* (#Mm00484678_m1),
441 *Gata2* (#Mm00492300_m1), *Spi1* (#Mm01270606_m1), *Fcer1a* (#Mm00438867_m1),
442 *Ms4a2* (#Mm00442780_m1), *Fcer1g* (#Mm00438869_m1), and *Gapdh* (#4352339E)
443 were purchased from Applied Biosystems. The following oligonucleotides were
444 synthesized for PCR primers: *Gpr41* forward, 5'-GTGACCATGGGGACAAGCTTC-3',
445 reverse, 5'-CCCTGGCTGTAGGTTGCATT-3', *Gpr43* forward,
446 5'-GGCTTCTACAGCAGCATCTA-3', reverse,
447 5'-AAGCACACCAGGAAATTAAG-3', *Gpr109a* forward,
448 5'-ATGGCGAGGCATATCTGTGTAGCA-3', reverse,
449 5'-TCCTGCCTGAGCAGAACAAGATGA-3', *Slc5a8* forward,
450 5'-CATTCGTCTCTGTGGCACAATC-3', reverse,
451 5'-GGGCATAAATCACAATTCCAGTGT-3', *Slc5a12* forward,
452 5'-ACAACAACAGTAGCCCCACAGA-3', reverse,

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453 5’-GGTAGGAGAGTGAGTACCATGTGTCA-3’, *Slc16a1* forward,
454 5’-ACAACAACAGTAGCCCCACAGA-3’, reverse,
455 5’-GGTAGGAGAGTGAGTACCATGTGTCA-3’.
456

457 **Degranulation assay**

458 Two hundred nanograms of anti-TNP mouse IgE (clone IgE-3, BD Bioscience, San Jose,
459 CA) was added to 1 mL of culture medium containing BMMCs (5×10^5). After a 2-h
460 incubation, cells were washed with Tyrode’s buffer and resuspended in Tyrode’s buffer
461 containing TNP-BSA (final concentration of 3 ng/mL) (LSL, Tokyo, Japan). Thirty
462 minutes after the TNP-BSA stimulation, the culture supernatant was harvested, and
463 β-hexosaminidase activity in the supernatant was assessed as previously reported [17].
464

465 **Flow cytometry**

466 Cell surface FcεRI and c-kit stained by PE-labeled anti-mouse FcεRIα Ab (MAR-1,
467 eBioscience) and FITC-labeled anti-mouse CD117 Ab (2B2, BioLegend) were detected
468 by a MACS Quant (Miltenyi Biotech). To detect Foxp3$^+$ cells in CD4$^+$ T cells, cells
469 treated with a Foxp3/Transcription factor staining buffer kit (Cat. TNB-0607, TOMBO
470 Bioscience) were stained with FITC-labeled anti-mouse CD4 Ab (GK1.5, BioLegend)
471 and allophycocyanin-labeled anti-mouse Foxp3 Ab (3G3, TOMBO Bioscience).
472

473 **Knockdown by small interfering RNA (siRNA)**

474 siRNA for mouse Gpr109a (MSS234551), and control siRNA (Stealth RNAi Negative

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475 Universal Control, #12935) were purchased from Invitrogen (Carlsbad, CA). Ten
476 microliters of 20 μM siRNA was introduced into BMMCs (5×10^6) by a Neon
477 Transfection System (Invitrogen) set at Program #5 using a Neon 100 μL Kit.
478
479 **Overexpression of mouse GPR109A on MCs by a retroviral vector**
480 Mouse GPR109A cDNA was amplified by PCR using mouse BM macrophage cDNA as
481 a template, and the following oligonucleotides as primers:
482 5'-ggc*ggatcc*atgagcaagtcagaccattttctag-3' (the inserted *Bam*HI sequence is shown in
483 italics, and the initiation codon is underlined) and 5'-ggg*ctcgag*ttaacgagatgtggaagccag-3'
484 (the inserted *Xho*I site and termination codon are shown in italics and underlined,
485 respectively). The *Bam*HI/*Xho*I-digested PCR fragment encoding GP109A cDNA was
486 inserted into the *Bam*HI/*Xho*I region in the multicloning site of pMXs-puro [48] to obtain
487 pMXs-puro-mGPR109A. The preparation of retroviral vectors using pMX plasmids and
488 packaging cells, the transfection of BMMCs by retroviral vectors, and the selection of
489 transfectants were performed as previously described [49,50].
490
491 **ELISA**
492 The concentrations of IL-13 and TNF-α in the culture media were measured by using
493 mouse IL-13 DuoSet ELISA (R&D Systems, Minneapolis, MN) and ELISA MAX
494 Deluxe Set mouse TNF-α (BioLegend), respectively. Prostaglandin E_2 ELISA
495 Kit-Monoclonal (Cayman Chemical, # 514010) was used to determine the concentration
496 of prostaglandin E_2 (PGE_2).

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497

498 **Passive systemic anaphylaxis (PSA) and the oral administration of SCFAs**

499 C57BL/6 mice were orally administered 435 mg/kg/day butyrate or 510 mg/kg/day
500 valerate for 4 to 6 days using saline and ethanol-PEG as a vehicle. On the last day of the
501 administration, mice received an intravenous (i.v.) injection of 3 μg of TNP-specific
502 mouse IgE. Twenty-four hours after the IgE injection, mice were i.v. transfused with 20
503 μg of TNP-BSA. The body temperature of each mouse was measured every 10 min for
504 1 h.

505

506 **Passive cutaneous anaphylaxis (PCA)**

507 After 4 days of the administration of butyrate, 0.02 μg of TNP-specific mouse IgE or
508 saline as a control was injected into the footpad to establish PCA. Twenty-four hours
509 after the IgE injection, mice were i.v. transfused with 20 μg of TNP-BSA. Footpad
510 thickness was measured 0.5 h after the TNP-BSA injection.

511

512 **Administration of ASA, indomethacin, an EP3 antagonist, an EP4 antagonist,**
513 **niacin/nicotinic acid, and niacinamide**

514 Mice were orally administered ASA (25 mg/kg), indomethacin (1 mg/kg),
515 ONO-AE5-599 (10 mg/kg), ONO-AE3-208 (10 mg/kg), or vehicle (0.5 % methyl
516 cellulose, 0.2 mL) twice per a day using a disposable feeding needle with a 200-μL
517 scale (#7202, Fuchigami Co., Ltd., Kyoto, Japan). Niacin/nicotinic acid (100 mg/kg),
518 niacinamide (100 mg/kg), or vehicle (saline, 0.2 mL) was administered via an

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519 intraperitoneal (i.p.) injection once per a day.

520

521 **Statistical analysis**

522 A two-tailed Student's *t*-test was used to compare two samples, and a one-way
523 ANOVA-followed by Tukey's multiple comparison test, Dunnett's multiple comparison
524 test, and Sidak's multiple comparison test were used to compare more than three
525 samples. p values < 0.05 were considered to be significant.

526

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539

540 **Authorship contribution**

541 K.N. performed the experiments and wrote the manuscript; D.A. performed the
542 experiments and analyzed data; T.A., K.I., R.M., I.F., M.A., N.I., H.K., Y.I., M.I., and
543 T.Y. performed the experiments; M.H. analyzed data and wrote the manuscript; K.K.
544 designed the research and performed the experiments; C.N. designed the research and
545 wrote the manuscript.

546

547 **Disclosures:** The authors have no financial conflict of interest.

548

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549 **Additional information**

550 Supplemental information includes two figures.

551

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705 **Legends**

706 **Fig. 1 Butyrate and valerate alleviate the IgE-induced anaphylaxis reaction.**

707 (**A**) Changes in the body temperature of control and butyrate-administered mice
708 following PSA. Mice were orally administered 100 μmol (200 μl of 500 mM) butyrate
709 or saline once a day for 4 days prior to PSA. Open circle, control (n = 10); closed circle,
710 butyrate administration (n = 9). Data were pooled from 2 independent experiments.
711 (**B**) Changes in the body temperature of control and valerate-administered mice
712 following PSA. Mice were orally administered 100 μ mol (200 μl of 500 mM) valerate
713 or ethanol-PEG solution once a day for 6 days prior to PSA. Open circle, control (n =
714 6); closed circle, valerate administration (n = 5). Data were pooled from 2 independent
715 experiments.
716 (**C**, **D**) Frequency of $Foxp3^{+}$ cells in $CD4^{+}$ T cells isolated from the MLN (left) and
717 spleen (right) of mice administered butyrate (B) or saline as the control of butyrate (C)
718 on the same schedule as in Figure 2A (**C**), or mice administered valerate (V) or
719 ethanol-PEG as the control (C) on the same schedule as in Figure 2B (**D**).
720 (**E**) Anaphylaxis induced on the footpad by the s.c. injection of IgE (right footpad) or
721 vehicle (left footpad) and a subsequent i.v. injection of Ag. Footpad thickness was
722 measured before and after the Ag injection. Footpad swelling (mm) was calculated as
723 follows.
724 (Footpad swelling) = (Footpad thickness after the Ag injection) – (Footpad thickness
725 before the Ag injection)
726 Sidak's multiple comparison tests (**A**, **B**, **E**), and a two-tailed Student's *t*-test (**C**, **D**)

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727 were used for statistical analyses. *, $p<0.05$

728

729 **Fig. 2 SCFAs suppress the degranulation of IgE-stimulated MCs.**

730 (**A**) Effects of SCFAs on the degranulation degree of IgE-stimulated BMMCs. BMMCs
731 were treated with the indicated concentrations of SCFAs for 48 h. Data represent the
732 mean ± SD of triplicate samples. A typical result is shown, and representative results
733 were obtained from 3 independent experiments.

734 (**B**) Viability of BMMCs incubated in the presence of 10 mM SCFAs for 48 h. A;
735 acetate, P; propionate, B; butyrate, V; valerate, IB; isobutyrate, IV; isovalerate. Survival
736 rates were assessed by DAPI staining using flow cytometry. Data represent the mean ±
737 SD of 3 independent experiments.

738 (**C**) Degranulation degree of IgE-stimulated BMMCs pretreated with 1 mM butyrate (B),
739 1mM sodium butyrate (NaB), or without SCFA (C; control). The data represent the
740 mean ± SEM of 3 independent experiments.

741 (**D**) Effects of SCFAs on the amount of cytokines produced by IgE-stimulated BMMCs.
742 Data represent the mean ± SEM of 3 independent experiments. C; control, B; butyrate,
743 V; valerate, IB; isobutyrate, IV; isovalerate.

744 A two-tailed Student's t-test (left and middle in **D**) and Dunnett's multiple comparison
745 tests (**A**, **B**, **C**, right in **D**) were used for statistical analyses. *, $p<0.05$.

746

747 **Fig. 3 Effects of butyrate and valerate on the cell surface expression level of FcεRI**
748 **and on the mRNA levels of FcεRI subunits.**

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749 (**A**) Cell surface expression levels of FcεRI and c-kit on SCFA-treated BMMCs. A
750 typical flow cytometry profile (top) and relative FcεRI expression level based on data
751 obtained from 3 independent experiments (bottom). C; control, B; butyrate, V; valerate.
752 Dunnett's multiple comparison test was used for statistical analysis. *, $p<0.05$.
753 (**B**, **C**) mRNA levels of FcεRI subunits (**B**) and FcεRI-related transcription factors (**C**)
754 in BMMCs treated with butyrate, or valerate. *Fcer1a* (encoding FcεRI α subunit),
755 *Ms4a2* (FcεRI β), *Fcer1g* (FcεRI γ), *Spi1* (PU.1), *Gata1* (GATA1), and *Gata2* (GATA2).
756 BMMCs incubated in the presence of 1 mM butyrate (B), or valerate (V) for 48 h were
757 harvested to measure mRNA levels. Tukey's multiple comparison test was used. *,
758 $p<0.05$ versus the control without the SCFA treatment (C).
759
760 **Fig. 4 Involvement of GPR109A in the butyrate- and valerate-mediated**
761 **suppression of degranulation.**
762 (**A, B**) Expression levels of mRNAs for transporters (**A**) and GPCRs (**B**) in MCs. Data
763 represent the mean ± SEM of 3 (*Slcs*) and 4 (*Gprs*) independent experiments.
764 (**C**) Degranulation degree of BMMCs treated with the transporter inhibitor, CHCA, in
765 the presence and absence of 1 mM SCFAs. The indicated concentrations of CHCA were
766 added to the culture media 1 h before the SCFA treatment. Data represent the mean ±
767 SEM of 3 independent experiments.
768 (**D**) Effects of 0.1 μg/mL of PTX, a Gi protein inhibitor on SCFA-dependent
769 suppression. Data represent the mean ± SD of triplicate samples, and representative
770 results were obtained from 3 independent experiments.

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771 (**E**) Expression levels of mRNAs for GPCRs in human MCs. Data were obtained from
772 "processed expression data of all samples for CAGE human PRJDB1099 (FANTOM5)"
773 (https://figshare.com/articles/dataset/RefEx_expression_CAGE_all_human_FANTOM5
774 _tsv_zip/4028613).
775 (**F**) Degree of degranulation in MCs with the knockdown of GPR109A. Data represent
776 the mean ± SD of triplicate samples, and the representative results were obtained from 3
777 independent experiments.
778 (**G**) Degranulation degree of GPR109A-overexpressing and control (mock) BMMCs.
779 GPR109A-overexpressing and control BMMCs were stimulated with IgE plus Ag after
780 a pre-incubated in the presence or absence of the indicated concentrations of SCFAs.
781 Data represent the mean ± SD of triplicate samples, and representative results were
782 obtained from 3 independent experiments.
783 Dunnett's multiple comparison test (**C**) and Tukey's multiple comparison test (**D**, **F**, and
784 **G**) were used. *, p<0.05.
785
786 **Fig. 5 Effects of TSA on the IgE-mediated activation of and FcεRI levels in MCs.**
787 (**A**) IgE-mediated degranulation of TSA-treated BMMCs. BMMCs were preincubated
788 with the indicated concentrations (nM) of TSA for 24 h. Data represent the mean ±
789 SEM of 3 independent experiments.
790 (**B**) Percentage of DAPI-stained cells in BMMCs pretreated with the indicated
791 concentrations of TSA for 24 h.
792 (**C**) IgE-induced cytokine release from TSA-treated BMMCs. BMMCs incubated in the

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793 presence or absence of 20 nM TSA for 24 h were stimulated with IgE plus Ag, and
794 culture supernatants at 3 h after the stimulation were harvested to measure the
795 concentrations of TNF-α and IL-13. Data represent the mean ± SEM of 3 independent
796 experiments.
797 (**D**, **E**) Surface expression levels of FcεRI (**D**) and mRNA levels of *Fcer1a*, *Ms4a2*, and
798 *Fcer1g* (**E**) in BMMCs treated with 20 nM TSA for 24 h. Data represent the mean ±
799 SEM of 3 independent experiments.
800 (**F**) FcεRI expression levels on BMMCs, which were pretreated in the presence or
801 absence of 0.1 μg/mL PTX for 1 h and further incubated with or without 1 mM butyrate
802 for 48 h.
803 Dunnett's multiple test (**A**), a *t*-test (**C**, **E**), and Tukey's multiple comparison test (**F**)
804 were used. *, $p<0.05$.
805
806 **Fig. 6 SCFA-induced activation of the NRF2 pathway in MCs.**
807 (**A**, **B**) Effects of butyrate on the transcription levels of NRF2 pathway-related genes.
808 The time-course of the relative mRNA levels of *Hmox1* (encoding HO-1) (**A**) and
809 *Nfe2l2* (NRF2) (**B**) in butyrate-treated BMMCs.
810 (**C**) mRNA levels of *Hmox1* (left) and *Nfe2l2* (right) in butyrate-treated and/or
811 IgE-stimulated BMMCs. BMMCs pretreated with 1 mM butyrate for 48 h were
812 stimulated with IgE plus Ag, and then harvested after an additional incubation for 1 h.
813 (**D**) mRNA levels of *Hmox1* in BMMCs treated with or without 20 nM TSA for 24 h.
814 (**E**) The butyrate-mediated suppression of degranulation was not affected by an NRF2

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815 inhibitor. BMMCs were pretreated with 1 mM butyrate and 5 μM ML385 (an NRF2
816 inhibitor) for 48 h before the IgE-induced degranulation assay.
817 Data represent the mean ± SEM of 2 (**A**, **B**) and 3 (**C**, **D**, **E**) independent experiments.
818 Tukey's multiple comparison test (**C**, and **E**), and a *t*-test (**D**) were used for statistical
819 analyses. *, $p<0.05$.
820
821 **Fig. 7 Roles of COX and PGs in suppressive effects of butyrate and valerate.**
822 (**A**, **B**) Effects of the ASA and indomethacin pretreatment on the butyrate (**A**)- or
823 valerate (**B**)-mediated suppression of degranulation. Data represent the mean ± SD of
824 triplicate samples and the representative results were obtained from 3 independent
825 experiments.
826 (**C**, **D**) Effects of PGD_2 and PGE_2 on the degranulation of MCs. BMMCs were
827 pretreated with the indicated concentrations of PGD_2 (**C**) or PGE_2 (**D**) for 24 h prior to
828 the degranulation assay. Data represent the mean ± SEM of 3 independent experiments.
829 (**E**, **F**) Degranulation degree of MCs pretreated with PGs. A total of 100 nM PGD_2 (**E**)
830 or PGE_2 (**F**) was added to the culture media 2 h or 24 h before IgE stimulation. Data
831 represent the mean ± SEM of 3 (PGD_2) and 4 (PGE_2) independent experiments.
832 (**G**) SCFAs up-regulate the amount of PGE_2 released from MCs. Data represent the
833 mean ± SD of triplicate samples, and representative results were obtained in another
834 independent experiment.
835 (**H**) Effects of the inhibitor of COX-1, SC-560, on the SCFA-mediated suppression of
836 MC degranulation. BMMCs pretreated with the indicated concentrations of SC-560 for

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837 2 h were incubated for an additional 48 h in the presence or absence of 1 mM of SCFAs.
838 Data represent the mean ± SD of triplicate samples, and representative results were
839 obtained from 3 independent experiments.
840 Tukey's multiple comparison test (**A**, **B**, **G**, **H**) and Dunnett's multiple comparison test
841 (**C**, **D**, **E**, **F**) were used for statistical analyses. *, p<0.05.
842

843 **Fig. 8 Involvement of PGs and GPR109A-signaling in the SCFA-induced**
844 **suppression of anaphylaxis.**
845 (**A**) Schematics of the administration schedule of butyrate and indomethacin (I.D.) (top).
846 Changes in body temperature after the Ag challenge (bottom). Control, the
847 administration of saline as vehicle (n = 8); Butyrate, butyrate administration without
848 indomethacin (n = 8); I.D., indomethacin intake without butyrate administration (n = 6);
849 Butyrate + I.D., butyrate administration with indomethacin intake (n = 5).
850 (**B**) Schematics of the administration schedule of ethyl valerate and ASA (top). Changes
851 in body temperature after the Ag challenge (bottom). Control, the administration of
852 ethanol-PEG as vehicle (n = 12); Ethyl valerate, ethyl valerate administration without
853 ASA (n = 12); ASA, ASA intake without ethyl valerate administration (n = 12); Ethyl
854 valerate + ASA, ethyl valerate administration with ASA intake (n = 11).
855 (**C**) Administration schedule of EP antagonists, butyrate, and/or niacin on passive
856 anaphylaxis in **D**-**H** of Fig. 8.
857 (**D**) The administration of the EP3 antagonist suppressed the butyrate-mediated
858 amelioration of PSA. Changes in body temperature after the Ag challenge for PSA.

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859 Control, vehicle (n = 12); Butyrate (B), butyrate administration without the EP3
860 antagonist (n = 9); EP3 ANT, the EP3 antagonist treatment without butyrate (n = 12); B
861 + EP3 ANT, butyrate administration and the EP3 antagonist treatment (n = 10).
862 (**E**, **F**) Effects of the EP3 antagonist (**E**) and EP4 antagonist (**F**) on the
863 butyrate-mediated suppression of PCA. PCA was induced in mice treated with IgE (IgE;
864 right footpad) or vehicle (-; left footpad) by an i.v. injection of Ag. Swelling was
865 assessed by measurements of footpad thickness before and after the Ag injection.
866 (**G**) Effects of niacin on IgE-dependent PCA.
867 (**H**) The EP3 antagonist attenuated the suppressive effects of niacin on PCA.
868 Tukey's multiple comparison test was used in all statistical analyses in Fig. 8. Data were
869 pooled from 2 (**A**, **G**, **H**), 3 (**D**), or 4 (**B**) independent experiments. *, $p<0.05$.

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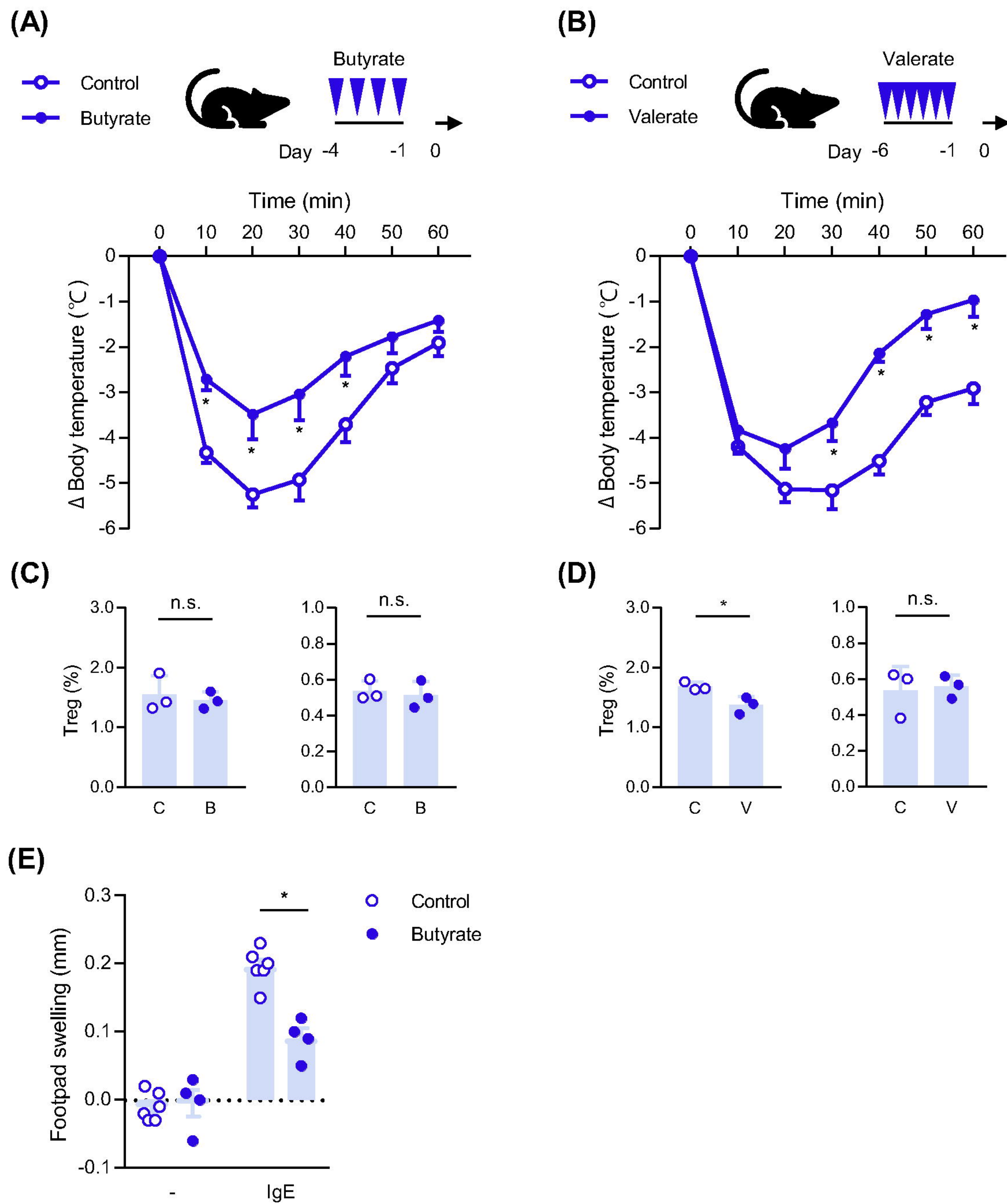

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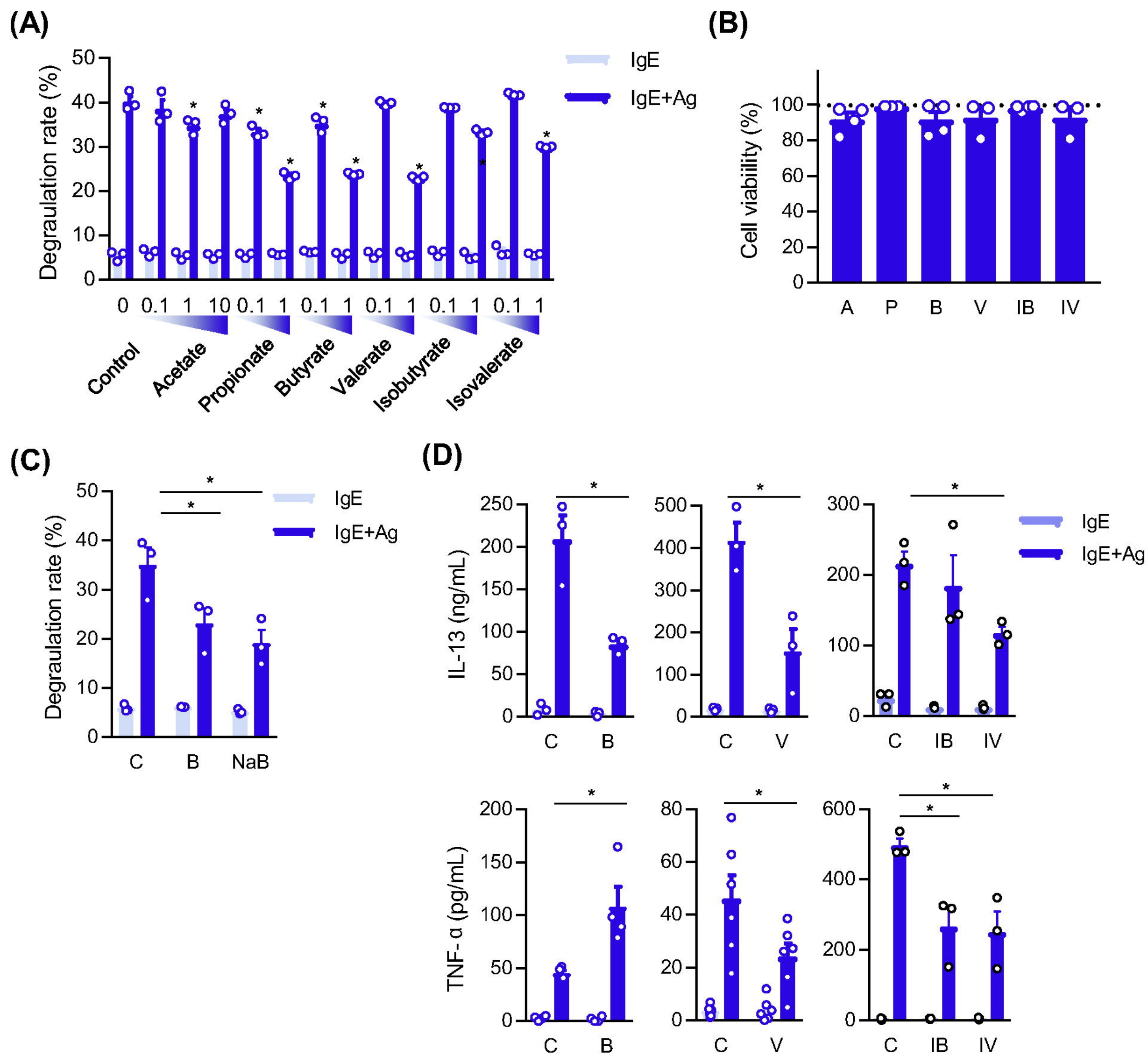

Figure 2_Nagata et al.

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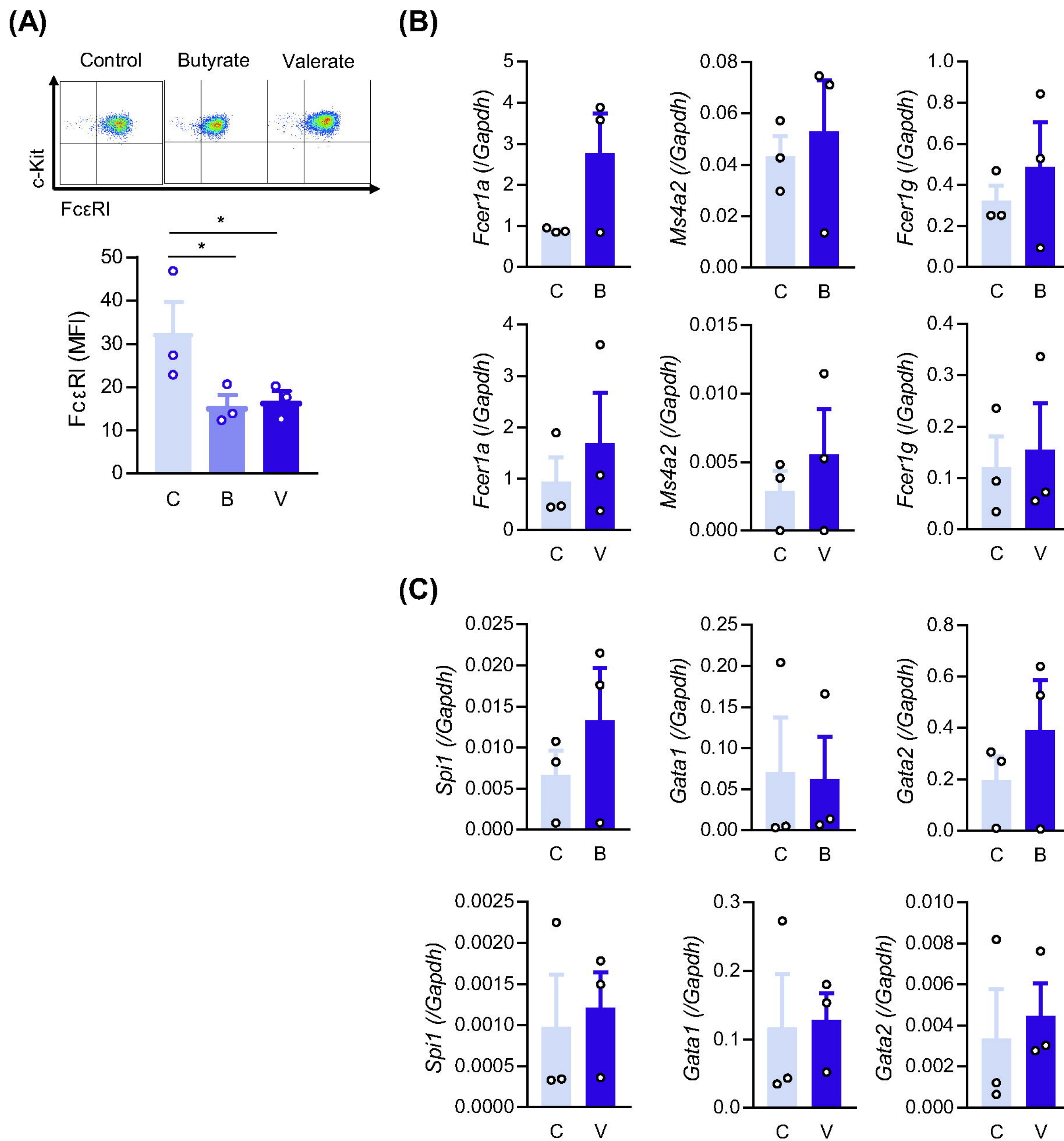

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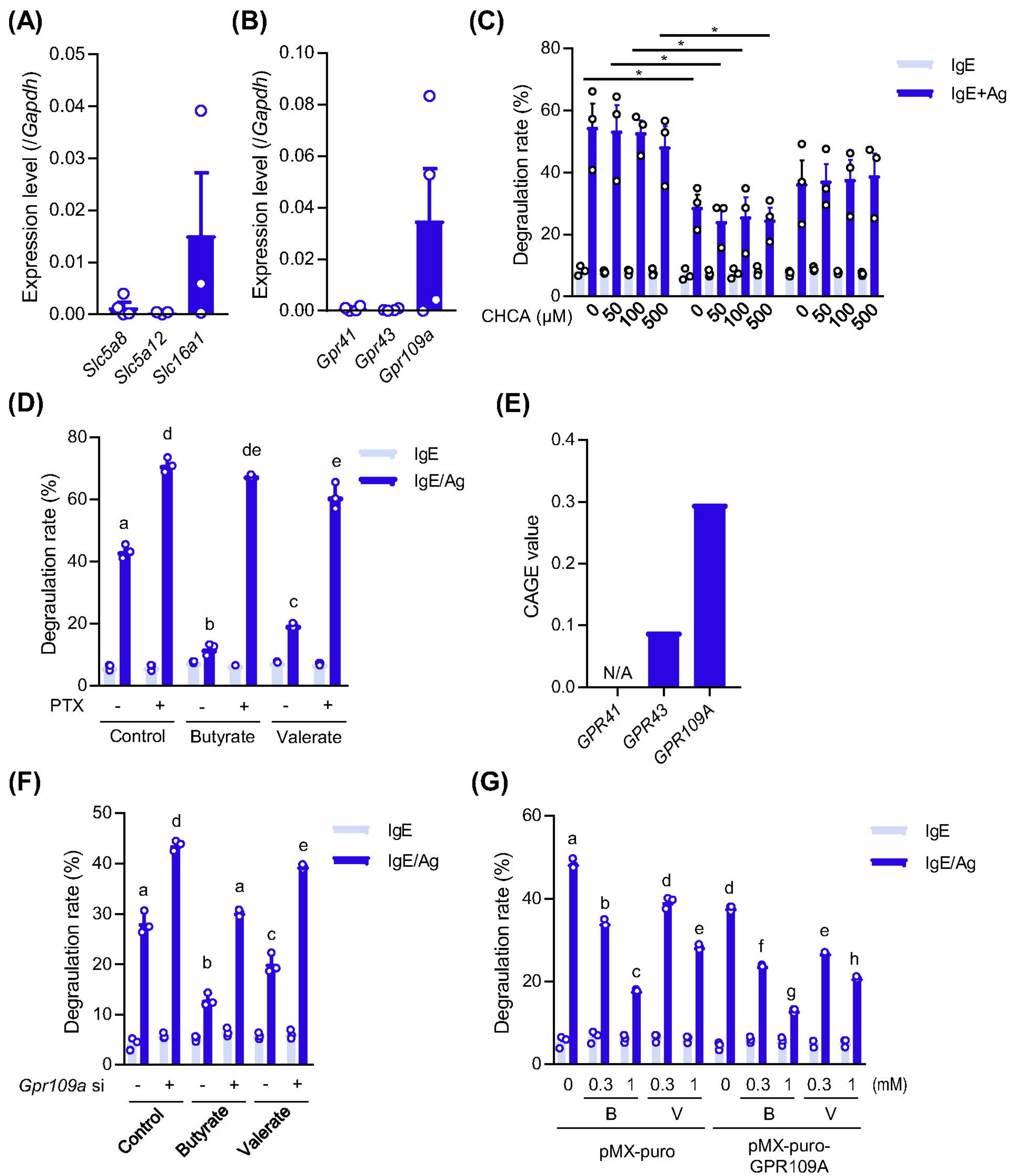

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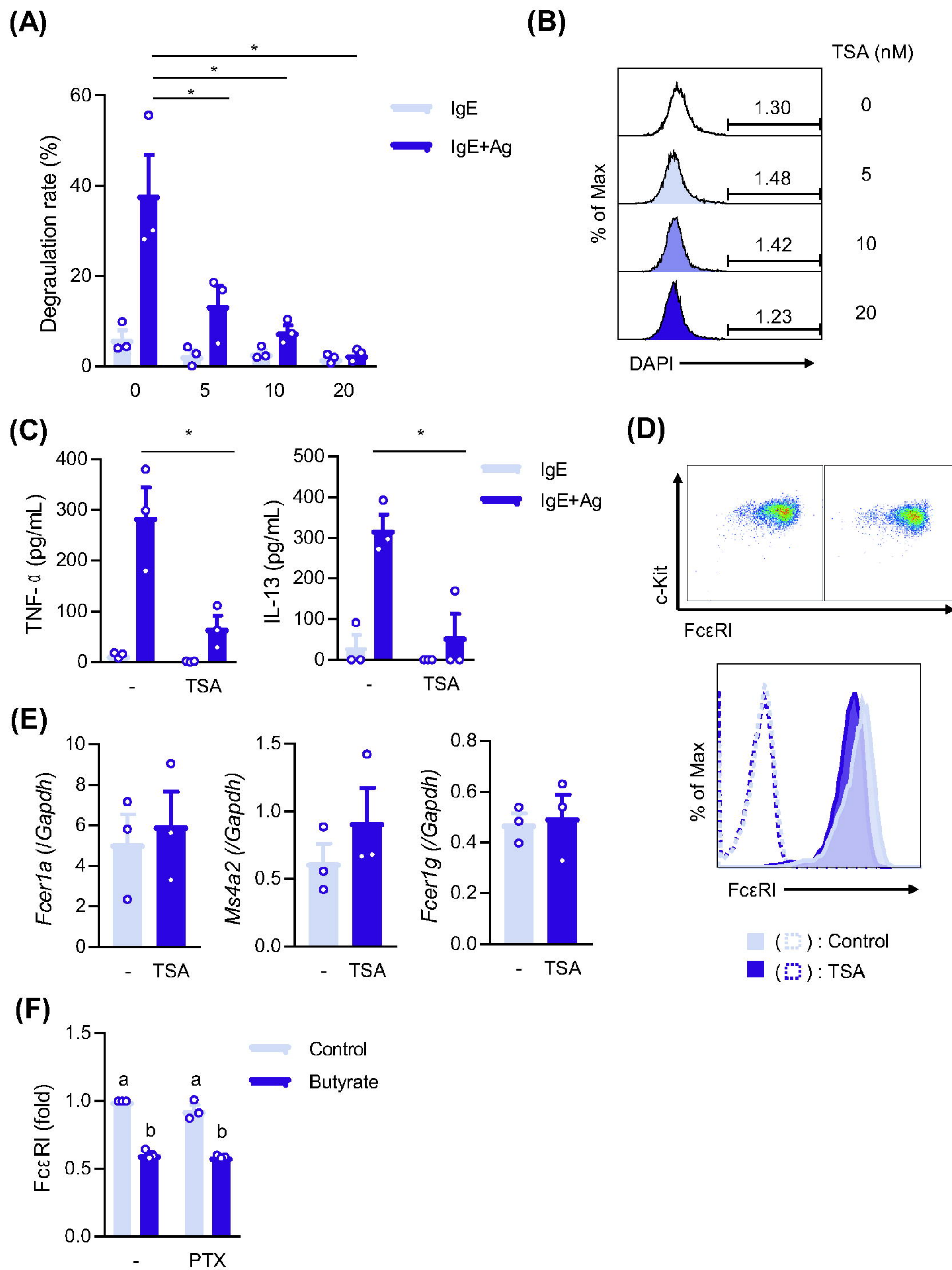

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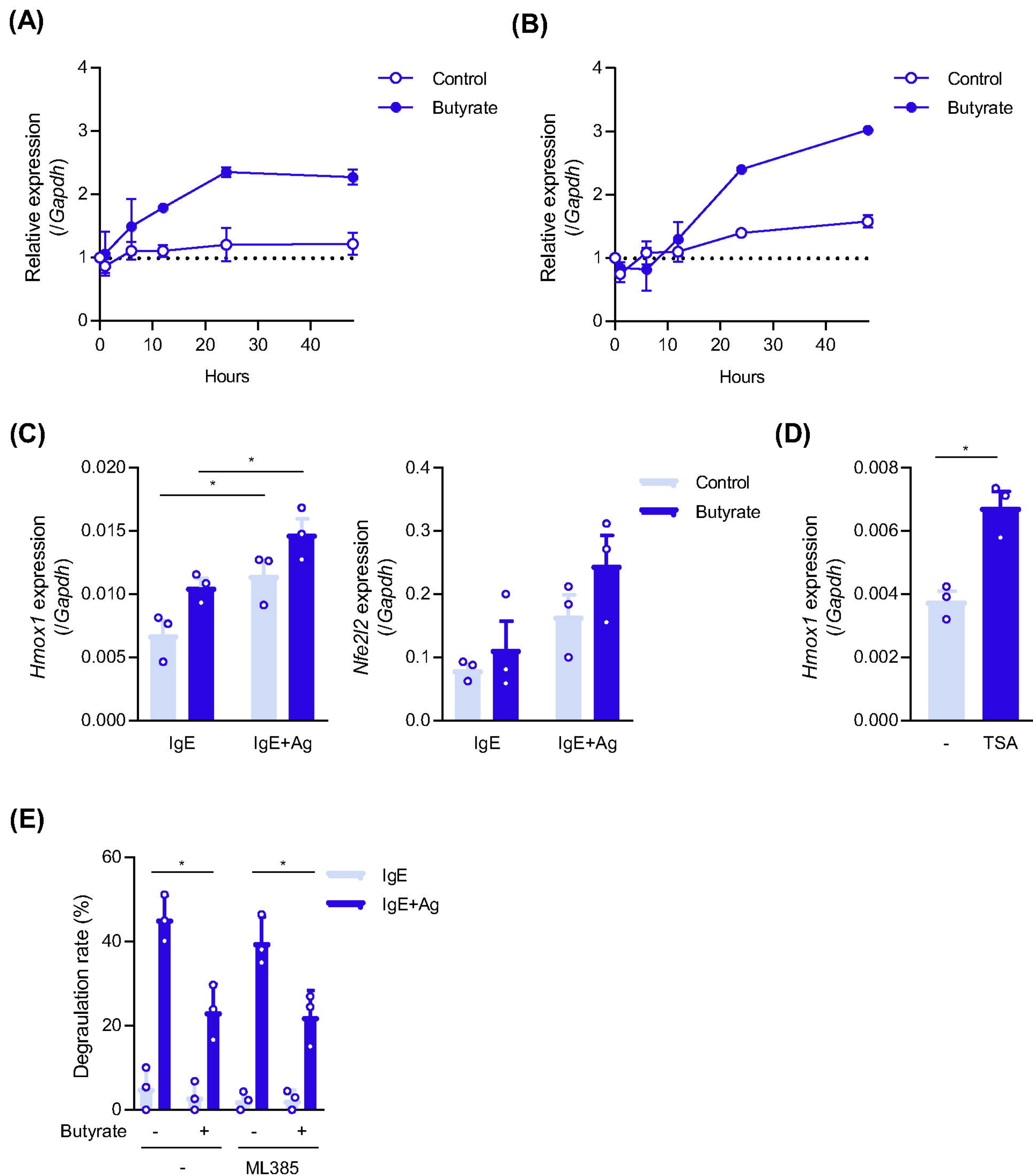

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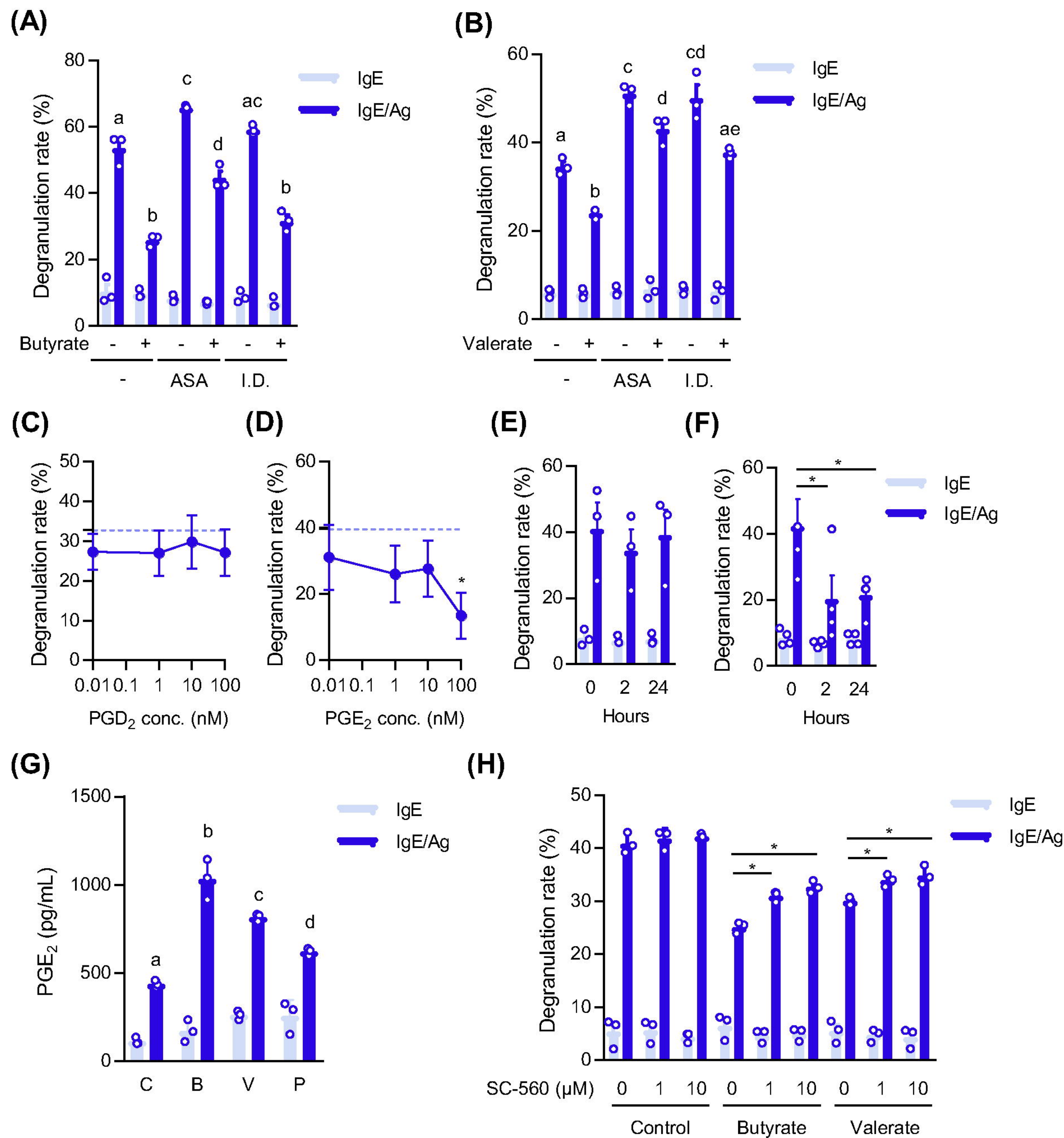

Figure 7_Nagata et al.

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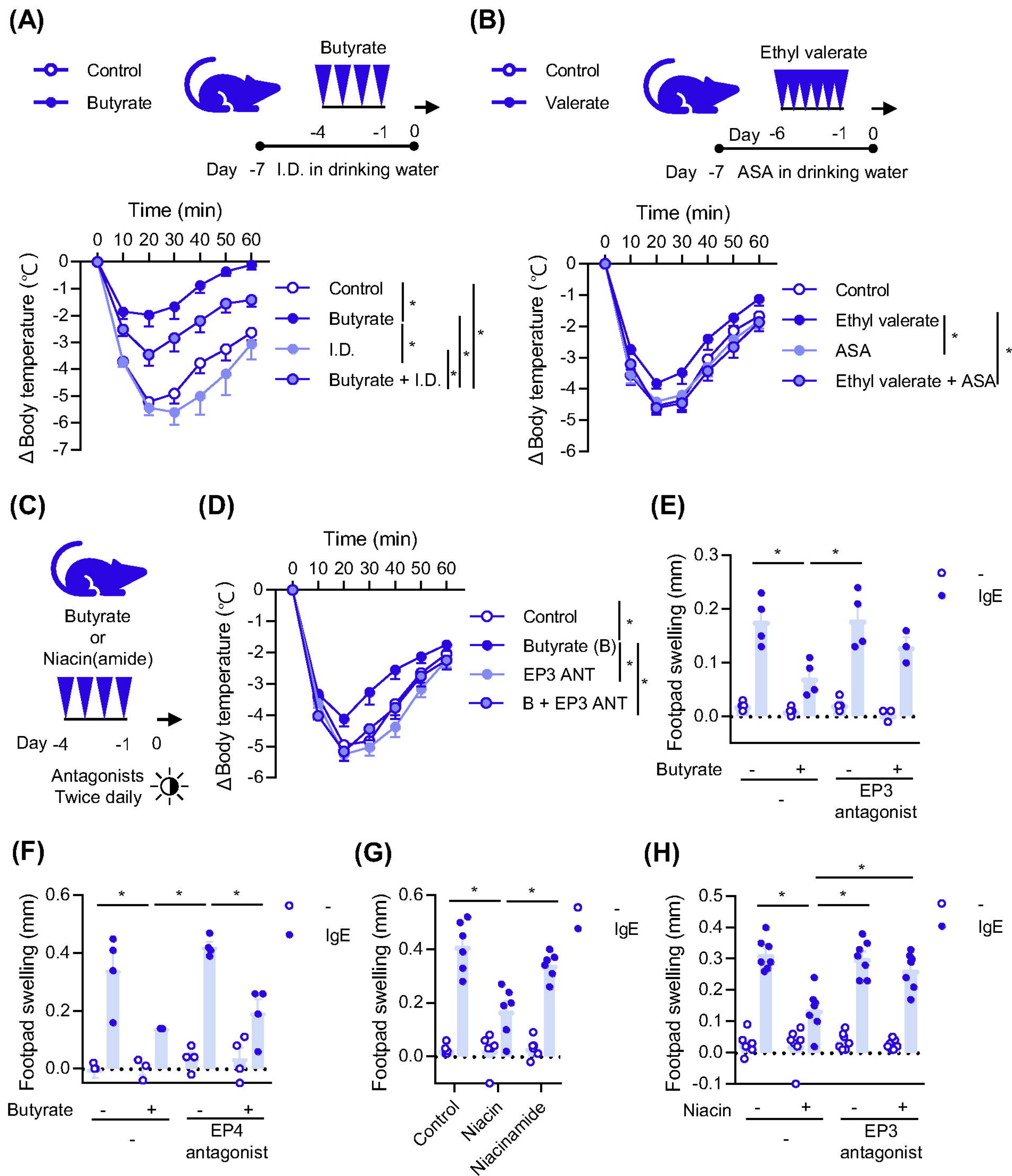

Figure 8_Nagata et al.