1. Title: Human RAMP1 overexpressing mice are resistant to migraine therapies for motion sensitivity: a mouse model of vestibular migraine

Abbreviated Title: Nausea and sway in RAMP1 overexpression

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4. Structured Abstract:

Background:

Both enhanced motion-induced nausea and increased static imbalance are \observed symptoms in migraine and especially vestibular migraine (VM). Motion-induced nausea and static imbalance were investigated in a mouse model, nestin/hRAMP1, expressing

elevated levels of human RAMP1 in the CNS, which enhances CGRP signaling in the nervous system.

Methods: Behavioral surrogates such as the motion-induced thermoregulation and postural sway center of pressure (CoP) assays were used to assess motion sensitivity.

Results: Tail vasodilation analysis revealed that this model exhibits an increased sensitivity to CGRP's effects at lower doses compared to control mice. In addition, the nestin/hRAMP1 mice exhibit a higher dynamic range in postural sway than their wildtype counterparts, along with increased sway observed in nestin/hRAMP1 male mice that was not present in male littermate controls. Results from migraine blocker experiments were challenging to interpret, but the data suggests that olcegepant is incapable of reversing CGRP-induced alterations in the nestin/hRAMP1 mice, while rizatriptan was ineffective in both the nestin/hRAMP1 and control mice. The results indicate that overexpression of hRAMP1 leads to heightened endogenous CGRP signaling. Results also suggest that both olcegepant and rizatriptan are ineffective in reducing CGRP-triggered nausea and sway in this hypersensitive CGRP mouse model.

Conclusions: This study suggests that hypersensitivity to CGRP may be a mouse model for difficult to treat cases of vestibular migraine.

5. Trial Registration: NA

6. Keywords: CGRP, RAMP1, vestibular, gastric distress, motion-sickness, nausea, postural sway, motion-sensitivity, migraine, mouse, olcegepant, rizatriptan.

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46 **Introduction**

47 Migraine is a chronic neurological disorder that is marked by pain in the head,
48 nausea, and heightened sensitivity to environmental stimuli such as light and sound.
49 Certain people may also experience an aura before the onset of migraine symptoms [1].
50 Approximately 40% of patients with a history of migraine may experience vestibular
51 migraine (VM), a unique type of migraine that makes patients vulnerable to recurrent
52 vertigo and imbalances [2-6]. VM affects about 1-4% of adults in the US, with three times as
53 many females affected as males. Furthermore, individuals with VM exhibit greater postural
54 sway when encountering challenging surroundings and are more prone to motion
55 sickness [7-12].

56 Calcitonin gene-related peptide (CGRP) is commonly considered a crucial factor
57 that causes migraines. It is a vasoactive peptide present in the nervous system that
58 contributes to vasodilation, inflammation, and pain modulation. CGRP signals through the
59 CGRP receptor consisting of three subunits: calcitonin-like receptor (CLR), receptor
60 activity-modifying protein 1 (RAMP1), and receptor component protein (RCP). RAMP1's
61 significance in CGRP signaling has been widely studied as the rate-limiting subunit. It is
62 essential for CLR translocation to the surface and for ligand binding [13-15].

63 A transgenic mouse model created by Russo et al. manifests sensitivity to CGRP
64 by upregulating the CGRP receptor [14]. The nestin/hRAMP1 mouse model shows

65 increased hRAMP1 expression in the nervous system and heightened sensitivity to the
66 neuropeptide CGRP, as compared to wild-type mice. The mice were subjects of preclinical
67 studies that analyzed surrogate behaviors for photophobia and allodynia. Their aversive
68 reaction to light is amplified by intracerebroventricular (ICV) CGRP injections [14, 16, 17].
69 Moreover, hind paw withdrawals are detected at markedly lower doses of centrally-
70 administered CGRP compared to their respective controls [18].

71 The CGRP receptor antagonist olcegepant (BIBN4096) effectively alleviates
72 CGRP-induced behaviors in both human and mouse receptors, as demonstrated by
73 mouse models. Moreover, preclinical models show that light aversion caused by CGRP is
74 reduced through selective serotonin receptor agonists like rizatriptan and sumatriptan.
75 Early studies targeting CGRP with olcegepant have led to the development of improved
76 "gepants" and monoclonal antibodies that can block either the CGRP receptor or the
77 peptide. Numerous treatment options for migraines based on antagonizing CGRP
78 signaling have been investigated in clinical trials, many of which have been approved by
79 the FDA since 2018, and a few studies have indicated that CGRP antagonists have been
80 successful in treating vestibular migraines, particularly in a subset of patients [19, 20].
81 Alternative treatments, such as vestibular suppressants, have not been shown to alleviate
82 vertigo and may cause drowsiness [21-23]. However, further research is needed to
83 investigate the potential role of CGRP sensitization in causing vestibular symptoms and to
84 elucidate the associated mechanisms. .

85 CGRP is widely distributed throughout the vestibular central nervous system
86 (CNS) and is present on vestibular efferent fibers that innervate the inner ear [24-26]. In this
87 study, we aim to characterize the effects of CGRP in nestin/hRAMP1 mice by employing
88 motion-induced thermoregulation and postural sway assays. Furthermore, we seek to
89 determine whether olcegepant or rizatriptan can provide protection against any CGRP-
90 induced changes and will compare to C57BL/6J and unaffected littermates.

91

92 **Materials & Methods**

93 **Animals**

94 A total of 175 mice (95F/80M) were tested across all experiments, and all studies
95 were sufficiently powered to detect sex differences. Mice were housed under a 12 to 12
96 day/night cycle at the University of Rochester's Vivarium under the care of the University
97 of Rochester's Veterinary Services personnel. All animal procedures were approved by
98 the University of Rochester's IACUC committee and performed in accordance with NIH
99 standards.

100 The nestin/hRAMP1 mice were bred by crossing heterozygous or homozygous
101 hRAMP1 loxP mice with heterozygous nestin-Cre mice to produce offspring that express
102 elevated hRAMP1 in the nervous system. A detailed synopsis of their breeding design

103 can be found in [15]. Control mice used in this study were hRAMP1 loxP mice not
104 expressing nestin-Cre. Mice were housed under a 12-hour day/night cycle under the care
105 of the University Committee on Animal Resources (UCAR) at the University of Rochester.
106 Mice were housed with ad libitum access to necessities (food, water, bedding, and
107 enrichment) with all studies sufficiently powered to detect sex differences. Mice were
108 equilibrated in a testing room with an ambient temperature between 22-23°C for at least
109 30 minutes prior to testing. Mice were tested between 3-6 months of age. Different
110 cohorts of mice were used to test motion-induced nausea and postural sway. Testing
111 occurred from 9:00 am to 5:30 pm.

112 **Drug administration**

113 All intraperitoneal (IP) injections were performed with a fine 33-gauge insulin
114 syringe. Dulbecco PBS (saline) served as vehicle control diluent. The CGRP-receptor
115 antagonist olcegepant and the selective serotonin receptor agonist rizatriptan were used
116 as migraine blockers in this study. The concentrations are listed: 0.1x, 0.5x, and 1.0x
117 CGRP were prepared at 0.01, 0.05, and 0.1 mg/kg (rat α-CGRP, Sigma), 1.0x olcegepant
118 was prepared at 1.0 mg/kg (BIBN4096, Tocris), and 1.0x rizatriptan was prepared at 0.6
119 mg/kg (Sigma-Aldrich). Mice were tested approximately twenty to thirty minutes after
120 injections. Animals were gently handled without the need for anesthesia. All animal

procedures were approved by the University of Rochester's (IACUC) and performed in accordance with the standards set by the NIH.

Motion-induced thermoregulation and center of pressure (CoP) testing for assessing motion-induced nausea and postural sway in mouse models

Fig. 1 provides details regarding experimental methodologies pursued in this study. Postural sway was evaluated using the center of pressure (CoP) assay. Around 10-12 CoP areas were recorded per mouse, and a 10% robust outlier removal (ROUT) was applied to eliminate outlier CoP areas from individual mouse trials. After this exclusion, each mouse had a minimum of six CoP areas available for averaging in each testing condition. No individual mice were excluded from the study.

We used a FLIR E60 IR camera (model: E64501) to head and tail temperatures of mice before, during, and after a vestibular perturbation. The measurements were conducted over a duration of 45 minutes. To summarize, we recorded baseline measurements for five minutes prior to initiating the perturbation (-5 mins ≤ t ≤ 0 mins). Subsequently, mice were recorded for 20 minutes (0 mins ≤ t ≤ 20 mins) during an orbital rotation at 75 rpm (2 cm orbital displacement). After the perturbation, mice were additionally recorded for 20 minutes to observe the recovery from hypothermia back to baseline (20 min ≤ t ≤ 40 min). FLIR Tools+ was used for analyzing data.

We defined Δ tail vasodilations (^{o}C) as the transient increases in mouse tail temperature in response to perturbation. These were computed by subtracting the baseline tail temperature at time t = 0 minutes from the maximum tail temperature measured during the first 10 minutes of the rotation ($0 \leq t \leq 10$). Additionally, we calculated the hypothermia magnitude (Δ heads) by subtracting the baseline head temperature at time t = 0 minutes from the minimum head temperature recorded throughout the entire experiment (0 mins $\leq t \leq$ 40 mins).

Data analysis and statistics

All statistical analyses were conducted in GraphPad Prism 9.5. Analyses were conducted separately in females and males unless otherwise noted. Multivariate ANOVAs and mixed-effect models were used to analyze Δ tail vasodilations and center of pressure (CoP) ellipse areas, and are further elaborated in the results. Group averages of Δ tail vasodilations and CoP ellipse areas are reported as mean ± SEM, and significance was set at $p < 0.05$ for all analyses.

Analysis of Δ tail vasodilations involved defining a threshold of 1.5^{o}C to examine the data as a binary outcome test. Tail temperature changes equal to or greater than +1.5^{o}C were designated a Δ tail vasodilation and those less than +1.5^{o}C did not meet the criteria, and were labeled as diminished tail vasodilations. Mice were excluded from

159 further testing if their Δ tail vasodilation after vehicle (saline) testing did not meet the
160 criteria.

161 Second-order curve fitting was used for fitting head temperatures ($B2*X^2 + B1*X +$
162 $B0$) and R^2 fit were calculated per curve. Head recovery (mins) was approximated by
163 normalizing head temperature at x = 0 mins to y = 0 ^{o}C. An x-intercept quadratic model
164 approximated the recovery by detecting x-intercepts passed 20 minutes (when we
165 stopped the perturbation).

166 **Results**

167 **No difference in Δ Heads/Magnitude of Hypothermia or Hypothermia Recovery**

168 Head temperatures are depicted after intraperitoneal (IP) delivery of vehicle
169 (saline), 1.0x CGRP, 1.0x CGRP + 1.0x Olcegepant, and 1.0x CGRP + 1.0x rizatriptan in
170 males and females (**Fig. 2A-H**). The magnitude of hypothermia was computed during an
171 animal's test – designated as the Δ head – and a 2-way ANOVA was computed across the
172 factors i) treatment and ii) strain in males and in females. No differences in hypothermia
173 magnitude were observed in mice regardless of treatment **(Fig. 2I-J).** In addition, no
174 differences in recovery were observed in the nestin/hRAMP1 mice compared to their
175 controls for either sex (**Table 1**).

176

177 **Dose response on tail vasodilations**

178 Motion-induced thermoregulation was examined at three different IP doses of
179 CGRP – 0.01 mg/kg, 0.05 mg/kg, and 0.1 mg/kg. These doses correspond to 0.1x CGRP,
180 0.5x CGRP, and 1.0x CGRP respectively. When mice are injected with vehicle (saline),
181 mice exhibit a transient delta tail vasodilation in response to the vestibular perturbation.
182 However, this response is diminished after 1.0x CGRP (**Fig. 3B, E**). Dunnett multiple
183 comparison's test was used to compare Δ tail vasodilations changes after CGRP dose to
184 vehicle, and a significant effect on was observed after intraperitoneal injections of 0.5x
185 CGRP and 1.0x CGRP (**Fig. 3C, F**). Of the male controls, 50% and 53% exhibited
186 diminished tail vasodilations after treatment of 0.5x and 1.0x CGRP (*adj. p = 0.1 and adj.*
187 *p < 0.001 respectively*), whereas 100% and 83% of male nestin/hRAMP1 mice were
188 affected by 0.5x CGRP and 1.0x CGRP (*adj. p = 0.004* and *adj. p = 0.02 respectively)*,
189 highlighting a potential sensitivity to CGRP in the male nestin/hRAMP1. A majority of
190 female controls (78% and 88%) exhibited diminished Δ tail vasodilations with doses of
191 0.5x and 1.0x CGRP (*adj. p < 0.0001 at each dose*). In comparison, male controls also
192 exhibited diminished Δ tail vasodilations at 0.5x and 1.0x CGRP, whereas 88% and 80%
193 of female nestin/hRAMP1 tested at 0.5x CGRP and at 1.0x CGRP exhibited diminished Δ
194 tail vasodilations (*adj. p = 0.0007 and adj. p = 0.001 respectively*). Based on the dose
195 response experiments, it is evident that titrating the dose of CGRP to 1.0x leads to
196 disruption in motion-induced thermoregulation in a strong majority of mice. It is also

observed that nestin/hRAMP1 males are more readily affected by incremental doses of CGRP than their complement controls (**Table 2**).

Olcegepant's efficacy on Δ tail vasodilations is similar between nestin/hRAMP1 and controls.

The CGRP-receptor antagonist olcegepant was assessed for its efficacy in motion-induced nausea. Raw temperatures after intraperitoneally delivered (IP) 1.0x CGRP + 1.0x olcegepant treatment are depicted (**Fig. 4A, D**). Post-hoc analysis was conducted using Tukey's multiple comparisons test. In female controls, 1.0x CGRP caused reduced Δ tail vasodilations, but co-administration of olcegepant helped to block this response in a portion of females. This observation was also seen in nestin/hRAMP1 mice, where some females showed restored Δ tail vasodilations with olcegepant (**Fig. 4B**). In males, olcegepant was effective in blocking 1.0x CGRP-induced changes in male controls and male nestin/hRAMP1 mice (**Fig. 4E**). A separate group of mice were tested at 0.5x CGRP, and mice with diminished Δ tail vasodilations were further tested with 0.5x CGRP + 1.0x olcegepant. We observed a majority of CGRP-affected female controls and nestin/hRAMP1 to regained their delta tail vasodilations with olcegepant blocking this sub-maximal dose. However, unlike male controls where a majority regained their tail vasodilation with olcegepant, male nestin/hRAMP1 still presented with diminished Δ tail vasodilations compared to their vehicle response (*0.5x CGRP vs 0.5x CGRP + 1.0x*

216 *Olceg, p = 0.04*), suggesting an enhanced sensitivity to CGRP at this submaximal dose
217 that is not being blocked with olcegepant (**Fig. 4F**).

218 **Rizatriptan does not block CGRP-induced changes on Δ tail vasodilations**

219 Rizatriptan was also examined across Δ tail vasodilations in a similar testing
220 scheme and analysis as olcegepant. Observed in both sexes of nestin/hRAMP1 and
221 controls, Δ tail vasodilations during CGRP+rizatriptan testing resembled CGRP-induced
222 changes rather than vehicle. Unlike olcegepant, rizatriptan had no protective effect on Δ
223 tail vasodilations (**Fig. 5A-D**).

224 A subset of nestin/hRAMP1 mice were also assessed after IP delivery of 0.5x
225 CGRP + rizatriptan (**Fig. 5E**). Even at the half dose, rizatriptan did not recover Δ tail
226 vasodilations in these mice.

227 **Nestin/hRAMP1 mice exhibit greater postural sway at baseline than controls**

228 Prior to testing CGRP and the effects of blockers, baseline center of pressure
229 (CoP) responses were compared between nestin/hRAMP1 and controls. Two-way ANOVA
230 with Dunnett's multiple comparisons test computed the following factors: i) male vs female
231 and ii) controls versus nestin/hRAMP1 on sway obtained after vehicle injections (saline).
232 While biological sex had no effect on sway ($F(1, 81) = 2.609$, $p = 0.11$), a significant
233 difference was observed between controls and nestin/hRAMP1 ($F(1, 81) = 35.45$, $p <$

234 0.001). Female controls exhibited a CoP of 0.91 ± 0.09 cm^2, whereas female
235 nestin/hRAMP1 mice had CoPs of 1.56 ± 0.12 (*adj. p < 0.001*). Similarly, male controls
236 had CoP 0.69 ± 0.06 cm^2, but male nestin/hRAMP1 had larger CoP at 1.41 ± 0.17 (*adj. p*
237 *< 0.001*). The higher sway in the nestin/hRAMP1 suggest the potential implications of
238 endogenous CGRP having a greater impact on static imbalance due to upregulated
239 hRAMP1 in the nervous system (**Fig. 6A**).

240 **Higher CGRP doses up to 0.1 mg/kg induce significant sway in female controls**

241 Changes in center of pressure (CoP) after intraperitoneally administered 0.1x
242 CGRP, 0.5x CGRP, and 1.0x CGRP were examined using the same statistical tools used
243 for the motion-induced thermoregulation experiments. 2-way mixed-effected models were
244 used to assess CGRP dose and controls versus nestin/hRAMP1, and Dunnett's multiple
245 comparisons was used to compare differences in CoP after each CGRP dose to vehicle.
246 In male nestin/hRAMP1 and controls, no differences were detected due to CGRP (**Fig.**
247 **6C**). Female controls exhibited significantly higher CoP after 1.0x CGRP compared to
248 their vehicle response (*adj. p < 0.001*) (**Fig. 6B**). In contrast, female nestin/hRAMP1 mice
249 did not significantly change their CoP in response to increasing doses of CGRP.

250

251 **Olcegepant and not rizatriptan blocks CGRP-induced changes in female control**
252 **sway, but nestin/hRAMP1 mice are not sensitive to CGRP or either migraine**
253 **blocker.**

254 Olcegepant and rizatriptan were examined for their ability to protect against 1.0x
255 CGRP-induced changes in CoP. 1.0x CGRP induced significantly higher CoP in female
256 controls than compared to their vehicle response, but co-administration of 1.0x CGRP +
257 1.0x Olcegepant led to lower CoP compared to 1.0x CGRP alone (*female controls: CGRP*
258 *vs CGRP+olcegepant, adj. p = 0.0004*) (**Fig. 7A**). In contrast, rizatriptan did not have an
259 effect on female control CoP, as 1.0x CGRP + 1.0x rizatriptan led to sway that resembled
260 1.0x CGRP induced changes (**Fig. 8A**). Interestingly, the CoP results on female controls
261 dramatically differ from their nestin/hRAMP1 counterparts. Female nestin/hRAMP1 mice
262 did not further increase their sway after 1.0x CGRP, nor did co-delivery of 1.0x olcegepant
263 or 1.0x rizatriptan modulate their CoP any further.

264 When examining male controls and nestinhRAMP1 (**Fig. 7B and 8B**), CGRP and
265 CGRP co-delivered with olcegepant/rizatriptan did not modulate their sway when
266 compared to their response to vehicle. It is clear that male nestin/hRAMP1 mice have
267 higher CoP values after vehicle and 1.0x CGRP than male controls, but olcegepant and
268 rizatriptan do not affect male CoP sway. We did not further test male controls with
269 olcegepant or rizatriptan, since no changes were observed after 1.0x CGRP, and male

270 controls had a much lower dynamic range in their CoP than did the nestin/hRAMP1. Due
271 to no real differences being detected in the nestin/hRAMP1 at 0.5x CGRP versus 1.0x
272 CGRP, we did not further examine the effects of olcegepant or rizatriptan at 0.5x CGRP
273 like we did during motion-induced thermoregulation studies.

274 **Table 2** is provided and gives specific details regarding the multivariate statistics
275 (F-statistics, p-values, and post-hoc analyses) used in this study for motion-induced
276 nausea and postural sway comparisons.

277

278 **Discussion**

279 Migraine treatment aims not only to address pain and ensure freedom from
280 headache, but also to alleviate the most bothersome symptoms (MBS), such as nausea,
281 vomiting, photophobia, or phonophobia. Photophobia is the most prevalent MBS and
282 affects 50% of migraine patients, while nausea -the second most frequent MBS- affects
283 30% of migraine patients [27]. Furthermore, individuals suffering from migraines also
284 encounter difficulty with postural control and balance, resulting in increased sway area
285 and velocity [3, 4, 28, 29]. A study examining various lighting conditions during bipedal and
286 unipedal stances of migraine patients concluded that migraine-associated photophobia is
287 linked to postural sway [30]. Additionally, nausea is linked to calcitonin gene-related peptide
288 (CGRP; and sensory pathways linked to nausea involve the vestibular system and the

289 area postrema, which is a circumventricular organ associated with nausea and vomiting
290 [31]. CGRP receptors have been detected in the area postrema through the localization of
291 RAMP1 and RAMP3 mRNA in this region [32]. Previous research has demonstrated that
292 the neuropeptide amylin, which has a similar structure to CGRP, can stimulates certain
293 subsets of area postrema neurons directly [33]. As the area postrema is not enclosed by
294 the blood-brain barrier, systemic delivery of CGRP can affect area postrema neurons and
295 potentially alter this region during motion-induced nausea and sickness. Moreover,
296 overexpression of RAMP1 may exacerbate this condition.

297 The nestin/hRAMP1 mouse model has been extensively studied to manipulate
298 CGRP's effects, simulate migraine-like surrogate behaviors, and assess the efficacy of
299 migraine drugs. The model first elucidated that the CGRP receptor is a critical aspect of
300 neurogenic inflammation [15]. Later studies examined the mouse model to assess
301 mechanical allodynia and photophobia. . This study examined the impact of CGRP on
302 motion-induced nausea and postural sway using the motion-induced thermoregulation and
303 center of pressure assays. Further, we investigated whether the CGRP-receptor
304 antagonist olcegepant or the selective serotonin receptor agonist rizatriptan could mitigate
305 CGRP-induced changes in the nestin/hRAMP1 and its littermate controls.

306 The study revealed several fascinating findings. Initially, the investigation did not
307 identify significant differences in hypothermia magnitude or time to recover from

308 hypothermia in mice irrespective of treatment. As a result, it is reasonable to conclude
309 that CGRP and its blockers do not partially shape this element of the nausea response in
310 mouse models. Notably, male and female nestin/hRAMP1 mice demonstrated lower
311 baseline head temperatures when compared to wildtype littermate control. Many prior
312 studies investigating cAMP signaling in the regulation of central body temperature have
313 demonstrated that an increase in cAMP levels leads to hypothermia, and conversely, a
314 decrease in cAMP levels leads to hyperthermia. The administration of the adenylyl
315 cyclase activator forskolin or treatment with dibutyryl-cAMP has induced hypothermia in
316 mice and rats [34-36]. The lower baseline temperature in the nestin/hRAMP1 at steady state
317 may be due to increased central cAMP levels resulting from elevated expression of
318 central/neural hRAMP1. The dose of IP CGRP had a significant impact on the
319 presentation of Δ tail vasodilations in both male and female mice. At higher doses (0.5x
320 and 1.0x CGRP), the majority of mice, including both nestin/hRAMP1 and control mice,
321 showed reduced Δ tail vasodilations. At the highest dose of 1.0x CGRP, which is 0.1
322 mg/kg, most mice do not show a significant increase in Δ tail vasodilation after being
323 stimulated with provocative rotation. The strong effect observed at the maximum dose of
324 CGRP prompted us to investigate the impact of the CGRP-receptor antagonist olcegepant
325 and the serotonin receptor agonist (5HT1B/1D) rizatriptan.

326 Olcegepant effectively restored tail vasodilations in male mice and, to a lesser
327 extent, in female mice, in both nestin/hRAMP1 mice and controls. However, rizatriptan did

328 not offer protection and resulted in changes comparable to those resulting from CGRP-
329 induced alterations. Rizatriptan and other triptans have been extensively researched as
330 drugs that indirectly inhibit the release of CGRP in migraine treatment. Further
331 investigation into the effects of half the maximum dose was conducted through the co-
332 administration of olcegepant or rizatriptan. It was discovered that at half the maximum
333 dose of CGRP, olcegepant improved the response of both nestin/hRAMP1 and control
334 mice; however, it was more effective in control male mice than nestin/hRAMP1 male mice.
335 Rizatriptan did not restore Δ tail vasodilations in nestin/hRAMP1 mice, even at 0.5 CGRP.

336 During the investigation of postural sway, we observed that nestin/hRAMP1 mice
337 showed higher baseline sway levels than control mice. Our previous research has
338 demonstrated that males do not encounter variations in their center of pressure while
339 being exposed to provocation rotation or CGRP. In contrast, female controls treated with
340 0.1 mg/kg CGRP encounter postural sway. In this study, we found that male mice have a
341 higher range of sway than their littermate controls. This change is associated with
342 increased levels of CGRP, which have an effect on sway due to elevated signaling
343 mediated by hRAMP1 overexpression. This finding is internally consistent with other
344 behavioral studies in nestin/hRAMP1 mice, who also exhibit light sensitivity to normal
345 room lighting that is not observed in littermate controls.

346 In conclusion, this study demonstrates a heightened sensitivity to motion in the
347 nestin/hRAMP1 model through the induction of nausea and static imbalance changes,
348 compared to unaffected control mice. It is important to understand the mechanisms
349 associated with CGRP signaling, not only for pain but also for other migraine symptoms
350 such as nausea and motion sensitivity, and to develop new therapies aimed at alleviating
351 these debilitating symptoms in migraine patients.

352

353 **Study Highlight Bullet points:**

354 • Enhanced motion-induced nausea is an observed symptom in migraine and
355 especially vestibular migraine (VM).
356 • Increased static postural sway is an observed symptom in migraine and especially
357 vestibular migraine (VM).
358 • We used two different motion-sensitivity assays to examine motion-sensitivity
359 (motion-sickness/nausea assay and postural sway assay) in mice
360 overexpressing human RAMP1 in the CNS and unaffected control mice.
361 • Differences in motion-induced thermoregulation after systemic CGRP were
362 blocked by olcegepant but not by triptans in both unaffected control mice and
363 mice over-expressing human RAMP1 in their CNS.

364 • Mice overexpressing RAMP1 in their CNS exhibit greater postural sway at
365 baseline than unaffected controls
366 • Olcegepant and not rizatriptan blocks CGRP-induced changes in female control
367 sway, but nestin/hRAMP1 mice are not sensitive to CGRP or either migraine
368 blocker.
369

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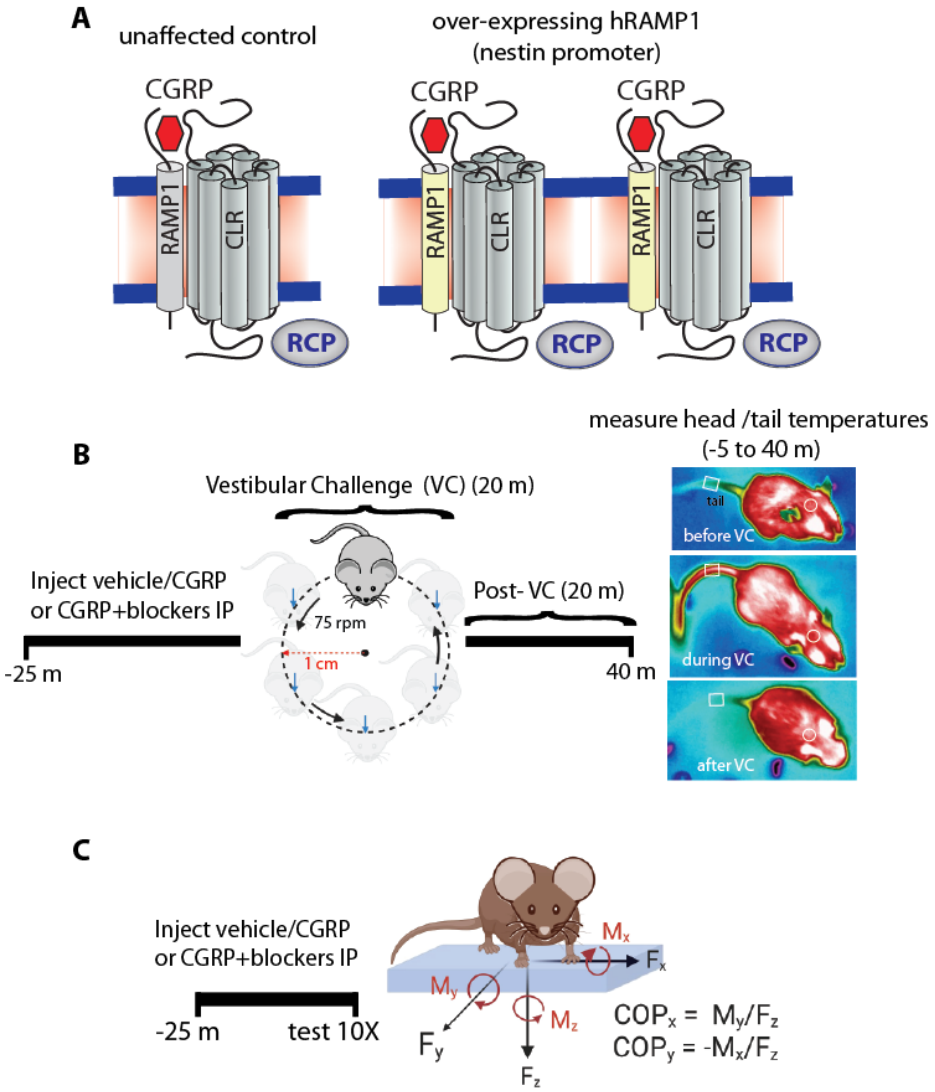

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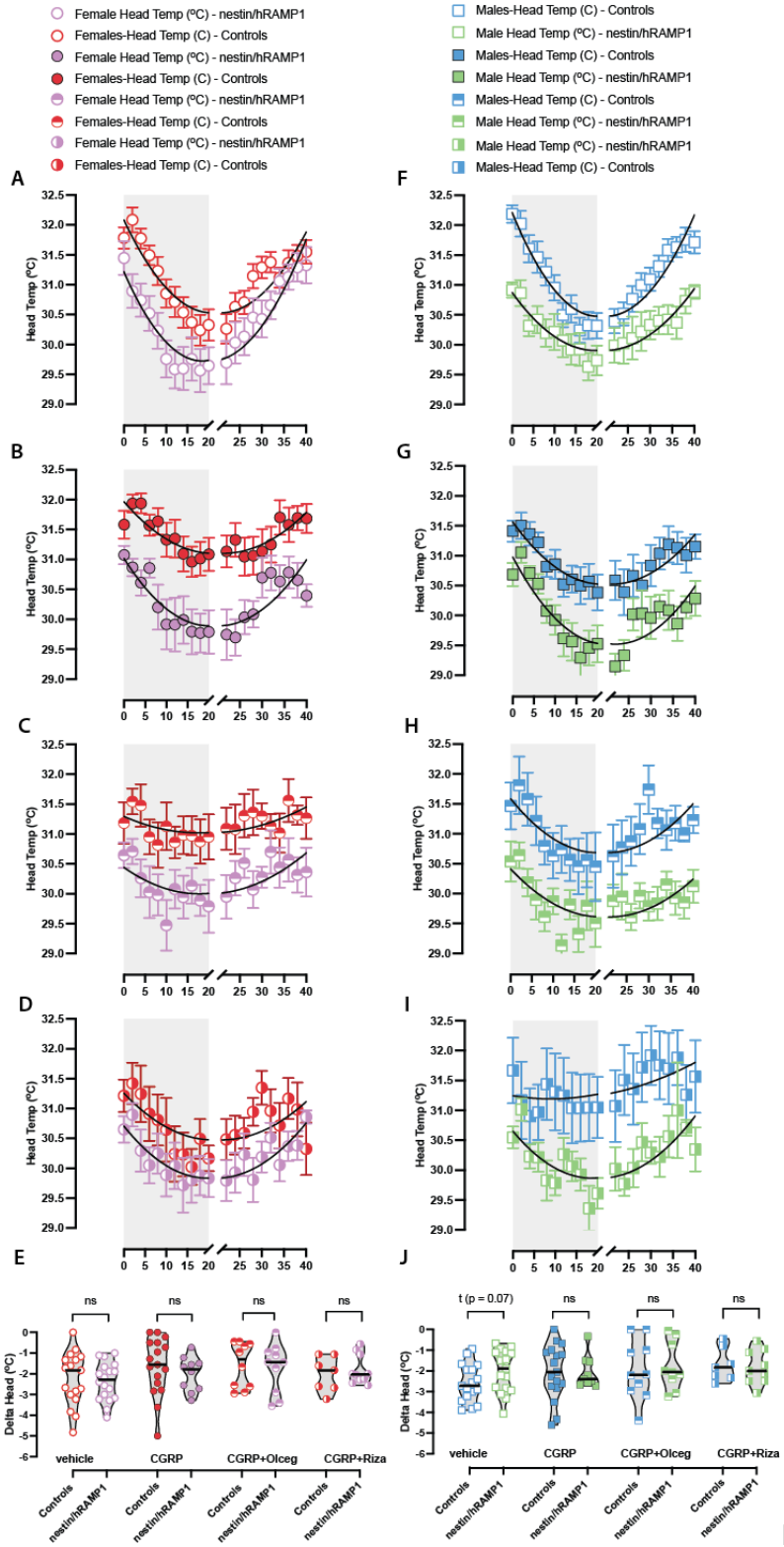
491 Fig 1.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Females-Head Temp (C) - Controls
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Females-Head Temp (C) - Controls
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Females-Head Temp (C) - Controls
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (C) - Controls
Males-Head Temp (C) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (C) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (C) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (C) - Controls
A
B
C
D
E
F
G
H
I
J
Head Temp (ºC)
Delta Head (ºC)
ns
t (p = 0.07)
vehicle
CGRP
CGRP+Olceg
CGRP+Riza
Controls
nestin/hRAMP1

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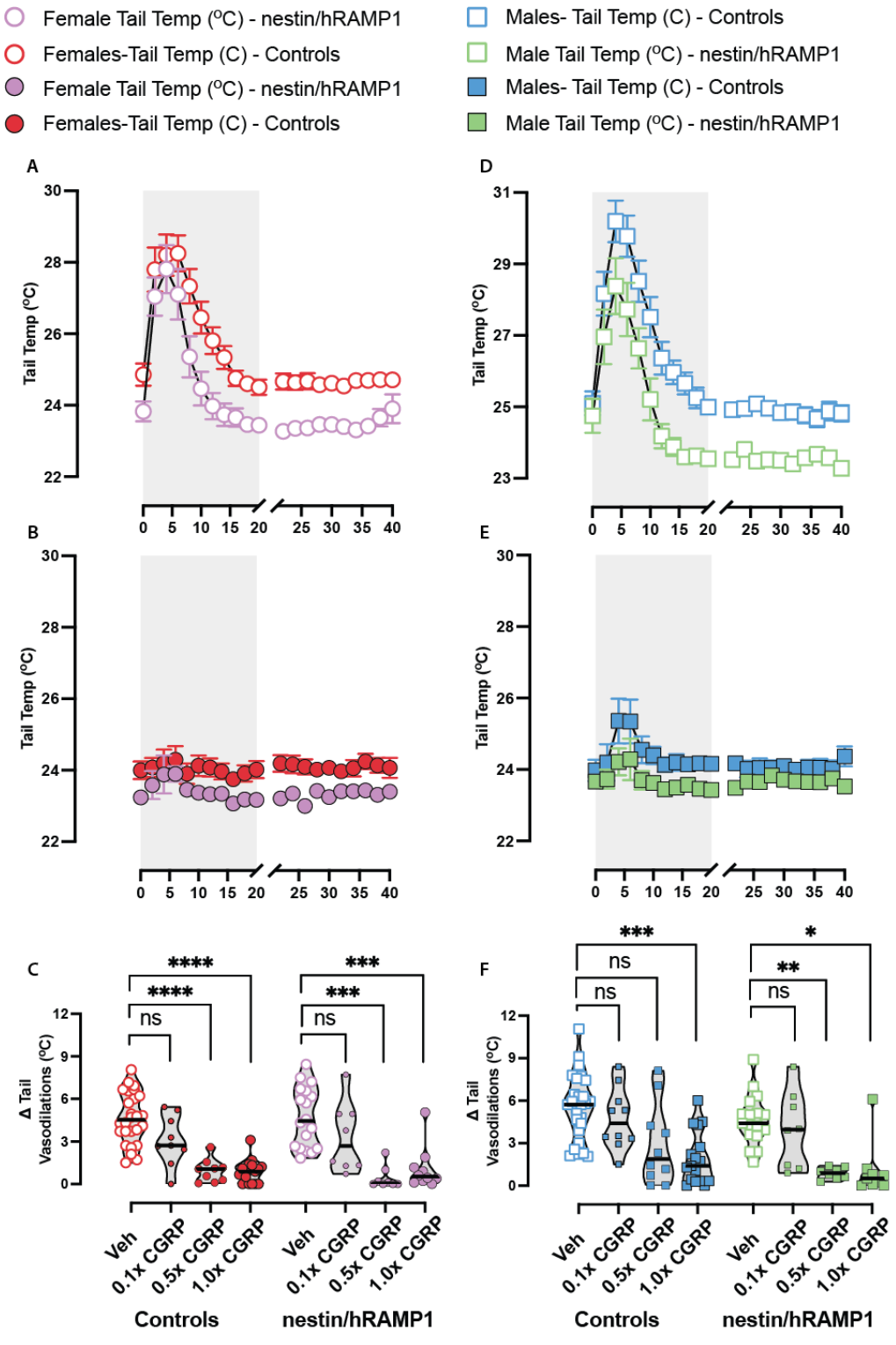
Fig. 2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Females-Tail Temp (C) - Controls
Female Tail Temp (ºC) - nestin/hRAMP1
Females-Tail Temp (C) - Controls
Males- Tail Temp (C) - Controls
Male Tail Temp (ºC) - nestin/hRAMP1
Males- Tail Temp (C) - Controls
Male Tail Temp (ºC) - nestin/hRAMP1
A
B
C
D
E
F
Tail Temp (ºC)
Δ Tail Vasodilations (ºC)
****
***
ns
*
**
Veh
0.1x CGRP
0.5x CGRP
1.0x CGRP
Controls
nestin/hRAMP1

493 Fig. 3

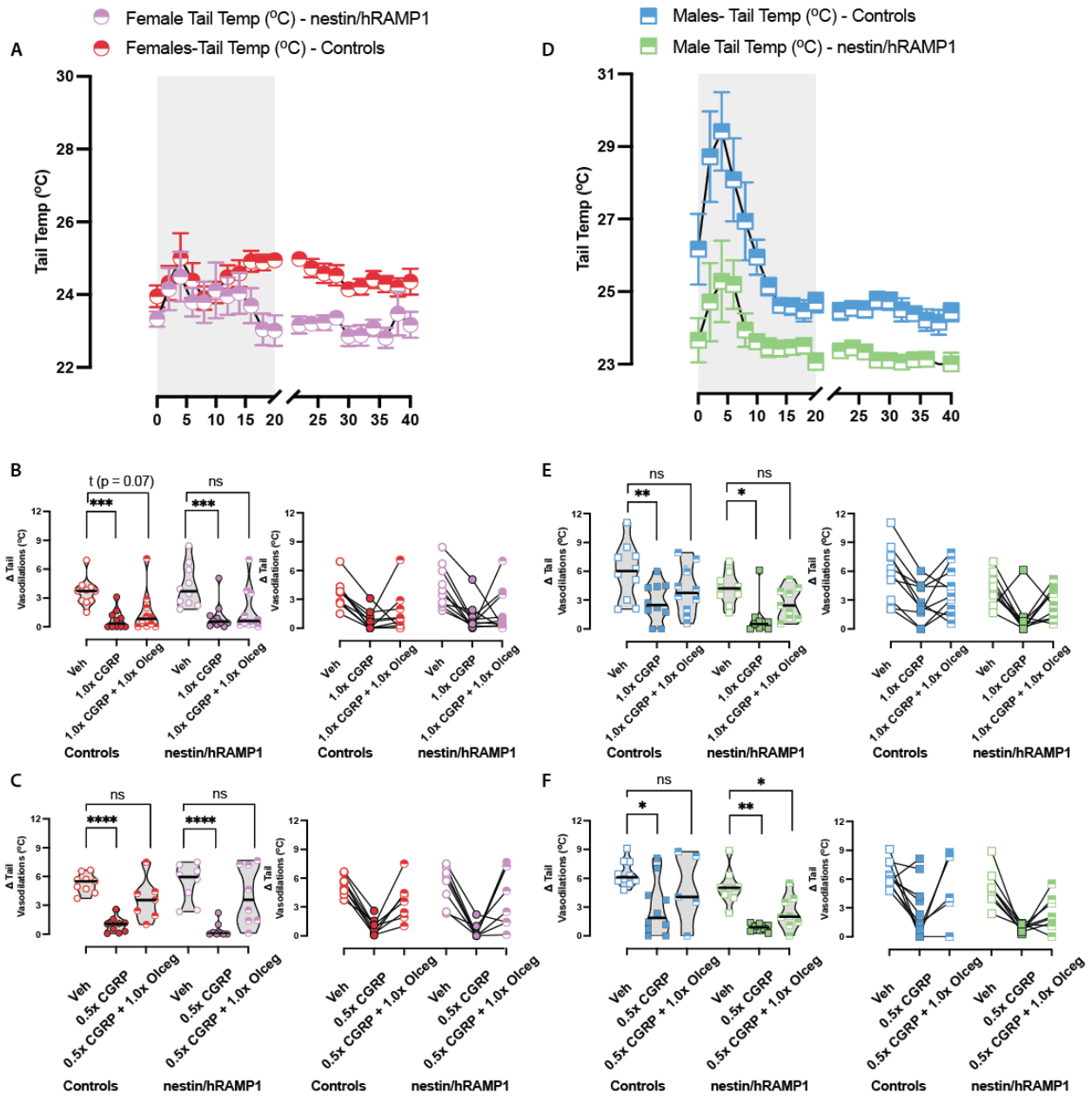

A
Female Tail Temp (ºC) - nestin/hRAMP1
Females-Tail Temp (ºC) - Controls
Tail Temp (ºC)
D
Males- Tail Temp (ºC) - Controls
Male Tail Temp (ºC) - nestin/hRAMP1
Tail Temp (ºC)
B
t (p = 0.07)
ns
Δ Tail Vasodilations (ºC)
Veh
1.0x CGRP
1.0x CGRP + 1.0x Olceg
Controls
nestin/hRAMP1
E
ns
C
ns
0.5x CGRP
0.5x CGRP + 1.0x Olceg
F
ns

Fig. 4

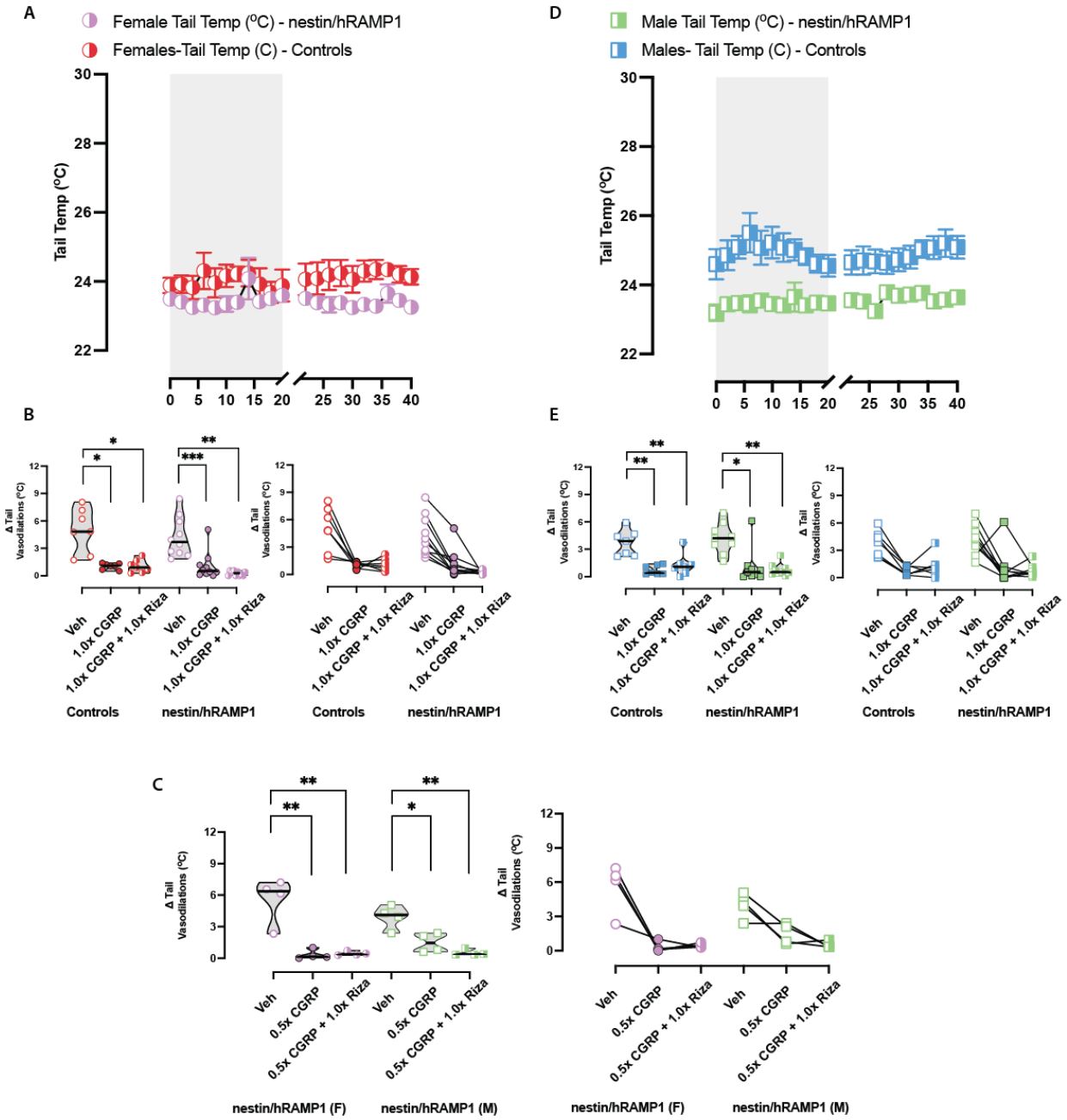
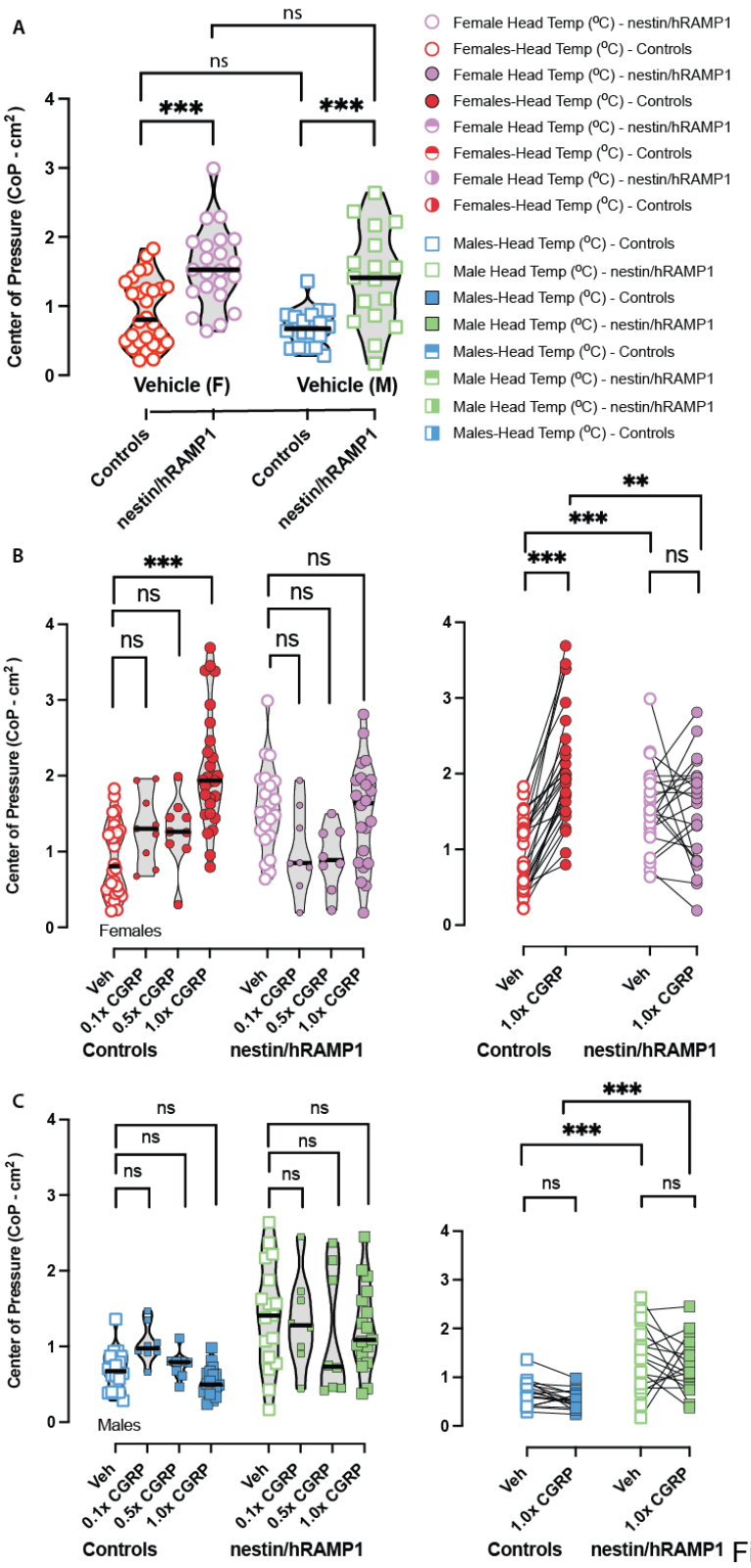
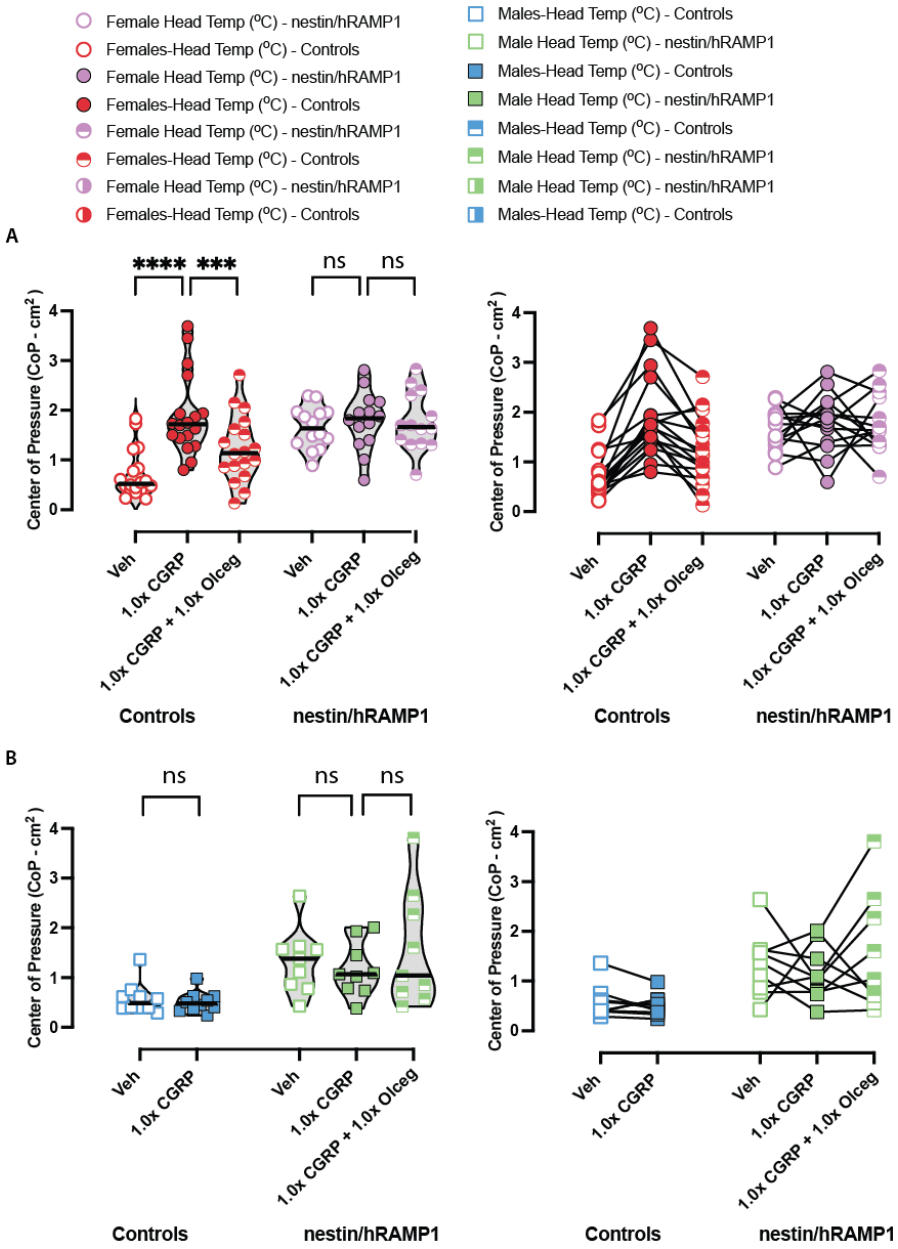

A
Female Tail Temp (ºC) - nestin/hRAMP1
Females-Tail Temp (C) - Controls
Tail Temp (ºC)
D
Male Tail Temp (ºC) - nestin/hRAMP1
Males- Tail Temp (C) - Controls
B
E
Δ Tail Vasodilations (ºC)
Veh
1.0x CGRP
1.0x CGRP + 1.0x Riza
Controls
nestin/hRAMP1
C
0.5x CGRP
0.5x CGRP + 1.0x Riza
nestin/hRAMP1 (F)
nestin/hRAMP1 (M)

Fig. 5


A
Center of Pressure (CoP - cm²)
ns
ns
***
***
Vehicle (F)
Vehicle (M)
Controls
nestin/hRAMP1
Controls
nestin/hRAMP1
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Males-Head Temp (ºC) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (ºC) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (ºC) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (ºC) - Controls
B
Females
Veh
0.1x CGRP
0.5x CGRP
1.0x CGRP
Controls
nestin/hRAMP1
C
Males

498 Fig. 6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Females-Head Temp (ºC) - Controls
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Males-Head Temp (ºC) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (ºC) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (ºC) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (ºC) - Controls
A
B
Center of Pressure (CoP - cm²)
****
***
ns
Veh
1.0x CGRP
1.0x CGRP + 1.0x Olceg
Controls
nestin/hRAMP1

Fig. 7

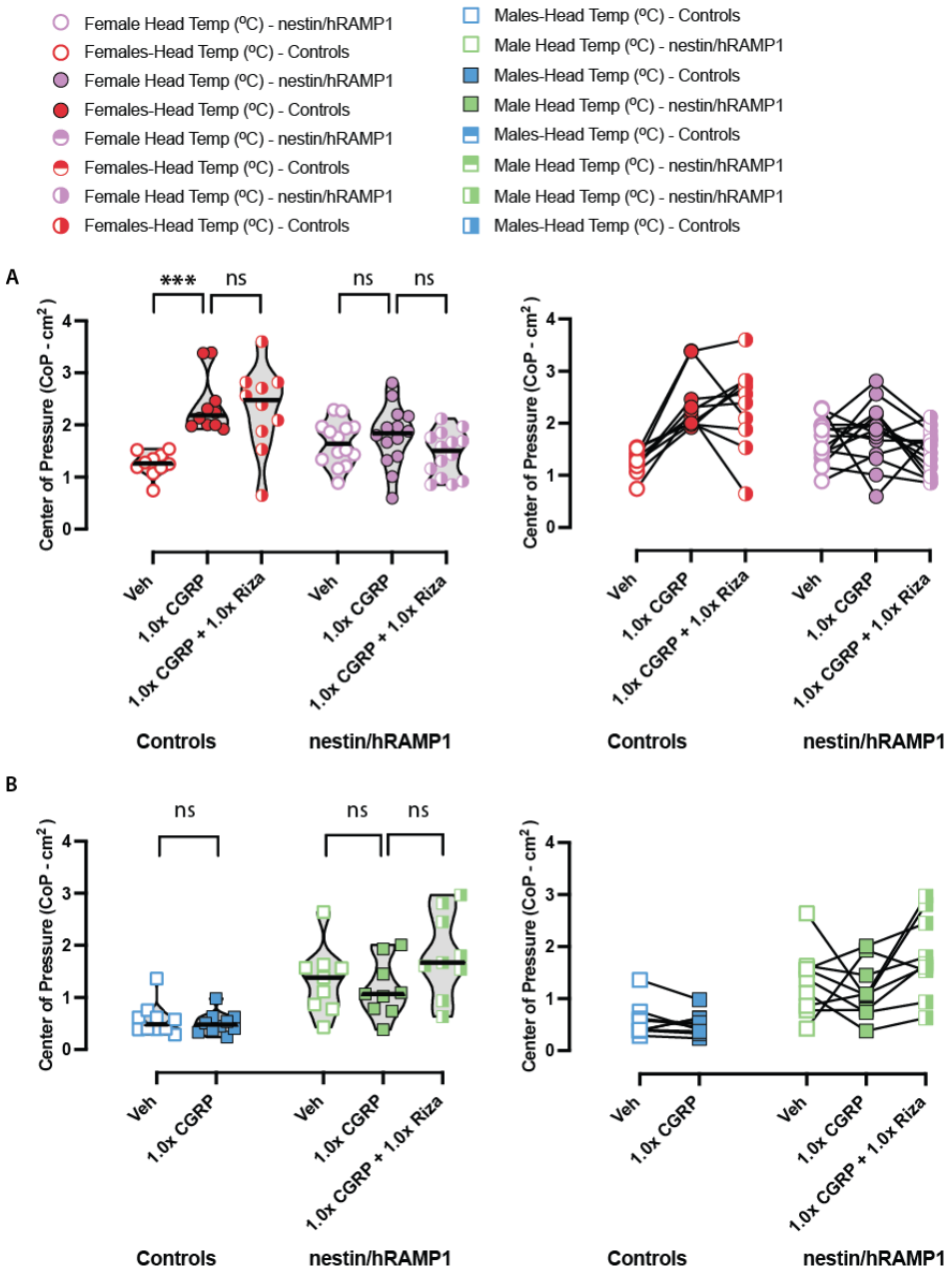
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Female Head Temp (ºC) - nestin/hRAMP1
Females-Head Temp (ºC) - Controls
Males-Head Temp (ºC) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (ºC) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (ºC) - Controls
Male Head Temp (ºC) - nestin/hRAMP1
Male Head Temp (ºC) - nestin/hRAMP1
Males-Head Temp (ºC) - Controls
A
***
ns
ns
ns
Center of Pressure (CoP - cm²)
Veh
1.0x CGRP
1.0x CGRP + 1.0x Riza
Controls
nestin/hRAMP1
B
ns
ns
ns

Fig. 8

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506 **Table 1**

Recovery from Hypothermia - controls vs nestin/hRAMP1								
Sex	Treatment	nestin/hRAMP1		controls		Recovery Difference	F(DFn, DFd)	p-val
		Recovery	Fit	Recovery	Fit			
Male	vehicle	40.10	0.90	39.80	0.88	0.30	0.08056 (1, 36)	0.78
	1.0x CGRP	42.00	0.73	40.63	0.82	1.37	0.2664 (1, 36)	0.61
	1.0x CGRP + 1.0x Olceg	44.00	0.50	39.60	0.50	4.40	1.598 (1, 36)	0.21
	1.0x CGRP + 1.0x Riza	35.94	0.55	36.33	0.36	-0.39	0.01133 (1, 36)	0.92
Female	vehicle	39.30	0.79	38.28	0.90	1.02	0.7515 (1, 36)	0.39
	1.0x CGRP	40.70	0.67	40.80	0.83	-0.10	0.001123 (1, 36)	0.97
	1.0x CGRP + 1.0x Olceg	39.39	0.39	31.50	0.32	7.89	3.294 (1, 36)	0.08
	1.0x CGRP + 1.0x Riza	38.79	0.74	41.44	0.34	-2.65	0.7528 (1, 36)	0.39

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511 **Table 2**

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Diminished Δ tail vasodilations (< 1.5 ^{o} C)				
Treatment	**Controls (F)**	**nestin/hRAMP1(F)**	**Controls (M)**	**nestin/hRAMP1 (M)**
Vehicle	0%	0%	0%	0%
0.1x CGRP	22%	38%	0%	38%
0.5x CGRP	78%	88%	50%	100%
1.0x CGRP	88%	80%	53%	89%
0.5x CGRP + 1.0x Olceg	14%	38%	20%	38%
1.0x CGRP + 1.0x Olceg	60%	70%	20%	33%
1.0x CGRP + 1.0x Riza	71%	100%	86%	89%
0.5x CGRP + 1.0x Riza	NA	100%	NA	100%

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515 **Table 3**

CGRP Dose Response on delta tail vasodilations and center of pressure (CoP) ellipse areas						
Dose Response analysis	**Factors**	**p-value**	**F (DFn, DFd)**	**Comparisons (Dunnett)**	**controls | adj. p val**	**nestinhRAMP1 | adj. p val**
tail vasodilations (females) - 2 way RM ANOVA	CGRP dose	<0.0001	F (1.484, 27.20) = 43.67	Vehicle vs. 0.1x CGRP	0.082	0.557
	controls vs nestin/hRAMP1	0.9785	F (1, 42) = 0.0007370	Vehicle vs. 0.5x CGRP	<0.0001	0.001
				Vehicle vs. 1.0x CGRP	<0.0001	0.000
tail vasodilations (males) - 2 way RM-ANOVA	CGRP dose	<0.001	F (3.000, 56.00) = 20.74	Vehicle vs. 0.1x CGRP	>0.99	>0.99
	controls vs nestin/hRAMP1	0.02	F (1, 42) = 5.495	Vehicle vs. 0.5x CGRP	0.100	0.004
				Vehicle vs. 1.0x CGRP	<0.001	0.020
CoP (females) - 2way RM-ANOVA	CGRP dose	<0.001	F (2.548, 66.24) = 16.03	Vehicle vs. 0.1x CGRP	0.070	0.170
	controls vs nestin/hRAMP1	0.74	F (1, 48) = 0.1078	Vehicle vs. 0.5x CGRP	0.100	0.080
				Vehicle vs. 1.0x CGRP	<0.001	0.980
CoP (males) - 2way RM-ANOVA	CGRP dose	0.13	F (3, 61) = 1.96	Vehicle vs. 0.1x CGRP	0.220	0.870
	controls vs nestin/hRAMP1	<0.001	F (1, 33) = 15.77	Vehicle vs. 0.5x CGRP	>0.99	0.240
				Vehicle vs. 1.0x CGRP	0.560	0.520
***Olcegepant efficacy* on delta tail vasodilations and center of pressure (CoP) ellipse areas**						
Olcegepant analysis	**Factors**	**p-value**	**F (DFn, DFd)**	**Comparisons (Tukey)**	**controls | adj. p val**	**nestinhRAMP1 | adj. p val**
tail vasodilations (females) - 2 way RM ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x olcegepant	<0.001	F (1.330, 23.95) = 15.68	Vehicle vs. 1.0x CGRP	<0.001	<0.001
	controls vs nestin/hRAMP1	0.460	F (1, 18) = 0.5770	Vehicle vs 1.0x CGRP + 1.0x Olceg	0.070	0.110
				1.0x CGRP vs 1.0x CGRP + 1.0x Olceg	0.570	0.790
tail vasodilations (males) - 2 way RM-ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x olcegepant	< 0.001	F (1.626, 27.64) = 11.39	Vehicle vs. 1.0x CGRP	0.001	0.030
	controls vs nestin/hRAMP1	0.030	F (1, 17) = 5.885	Vehicle vs 1.0x CGRP + 1.0x Olceg	0.320	0.310
				1.0x CGRP vs 1.0x CGRP + 1.0x Olceg	0.300	0.230
CoP (females) - 2way RM-ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x olcegepant	< 0.0001	F (1.98, 59.4) = 15.1	Vehicle vs. 1.0x CGRP	<0.0001	0.752
	controls vs nestin/hRAMP1	0.023	F (1, 30) = 5.77	Vehicle vs 1.0x CGRP + 1.0x Olceg	0.015	0.830
				1.0x CGRP vs 1.0x CGRP + 1.0x Olceg	0.000	0.990
CoP (males) - 1 way ANOVA on nestin/hRAMP1	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x olcegepant	0.561	F (1.62, 13.0) = 0.536	Vehicle vs. 1.0x CGRP	NA	0.841
				Vehicle vs 1.0x CGRP + 1.0x Olceg	NA	0.878
				1.0x CGRP vs 1.0x CGRP + 1.0x Olceg	NA	0.562
tail vasodilations (females) - 2 way RM ANOVA	vehicle vs 0.5x CGRP vs 0.5x CGRP + 1.0x olcegepant	<0.001	F (1.812, 24.46) = 15.61	Vehicle vs. 0.5x CGRP	0.020	0.002
	controls vs nestin/hRAMP1	0.006	F (1, 16) = 10.05	Vehicle vs 0.5x CGRP + 1.0x Olceg	0.580	0.020
				0.5x CGRP vs 0.5x CGRP + 1.0x Olceg	0.520	0.150
tail vasodilations (males) - 2 way RM-ANOVA	vehicle vs 0.5x CGRP vs 0.5x CGRP + 1.0x olcegepant	<0.001	F (1.498, 20.97) = 33.47	Vehicle vs. 0.5x CGRP	<0.001	<0.001
	controls vs nestin/hRAMP1	0.950	F (1, 15) = 0.004	Vehicle vs 0.5x CGRP + 1.0x Olceg	0.120	0.520
				0.5x CGRP vs 0.5x CGRP + 1.0x Olceg	0.020	0.040
***Rizatriptan efficacy* on delta tail vasodilations and center of pressure (CoP) ellipse areas**						
Rizatriptan analysis	**Factors**	**p-value**	**F (DFn, DFd)**	**Comparisons (Tukey)**	**controls | adj. p val**	**nestinhRAMP1 | adj. p val**
tail vasodilations (females) - 2 way RM ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x rizatriptan	< 0.001	F (1.541, 23.11) = 39.84	Vehicle vs. 1.0x CGRP	0.020	<0.001
	controls vs nestin/hRAMP1	0.320	F (1, 15) = 1.076	Vehicle vs 1.0x CGRP + 1.0x Riza	0.010	0.001
				1.0x CGRP vs 1.0x CGRP + 1.0x Riza	>0.99	0.290
tail vasodilations (males) - 2 way RM-ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x rizatriptan	< 0.001	F (1.659, 23.23) = 25.14	Vehicle vs. 1.0x CGRP	0.001	0.030
	controls vs nestin/hRAMP1	0.800	F (1, 14) = 0.06604	Vehicle vs 1.0x CGRP + 1.0x Riza	0.003	0.003
				1.0x CGRP vs 1.0x CGRP + 1.0x Riza	0.500	0.880
CoP (females) - 2way RM-ANOVA	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x rizatriptan	< 0.001	F (1.99, 43.7) = 9.90	Vehicle vs. 1.0x CGRP	<0.001	0.750
	controls vs nestin/hRAMP1	0.020	F (1, 22) = 6.28	Vehicle vs 1.0x CGRP + 1.0x Riza	0.020	0.260
				1.0x CGRP vs 1.0x CGRP + 1.0x Riza	0.950	0.160
CoP (males) - 1 way ANOVA on nestin/hRAMP1	vehicle vs 1.0x CGRP vs 1.0x CGRP + 1.0x rizatriptan	0.057	F (1.83, 14.6) = 3.59	Vehicle vs. 1.0x CGRP	NA	0.841
				Vehicle vs 1.0x CGRP + 1.0x Olceg	NA	0.130
				1.0x CGRP vs 1.0x CGRP + 1.0x Olceg	NA	0.069
CoP (females) - 1 way ANOVA on nestin/hRAMP1	vehicle vs 0.5x CGRP vs 0.5x CGRP + 1.0x rizatriptan	0.003	F (2, 6) = 18.3	Vehicle vs. 0.5x CGRP	NA	0.004
				Vehicle vs 0.5x CGRP + 1.0x Olceg	NA	0.005
				0.5x CGRP vs 0.5x CGRP + 1.0x Olceg	NA	0.991
CoP (males) - 1 way ANOVA on nestin/hRAMP1	vehicle vs 0.5x CGRP vs 0.5x CGRP + 1.0x rizatriptan	0.005	F (2, 6) = 14.5	Vehicle vs. 0.5x CGRP	NA	0.023
				Vehicle vs 0.5x CGRP + 1.0x Olceg	NA	0.005
				0.5x CGRP vs 0.5x CGRP + 1.0x Olceg	NA	0.362

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525 **Figure Legends**

526 **Fig.1:** (A) Calcitonin gene-related peptide (CGRP) receptor structure consists of receptor
527 activity modifying protein 1 (RAMP1), calcitonin-receptor like receptor (CLR), and receptor
528 component protein (RCP). The nestin/hRAMP1 mice are designed to have enhanced
529 CGRP signaling due to overexpression of human RAMP1 (hRAMP1). **(B-C)** Methods for
530 motion-induced thermoregulation and center of pressure (CoP) testing as surrogate
531 behaviors for motion-induced nausea and postural sway.

532

533 **Fig. 2:** Head temperatures during motion-induced nausea test after intraperitoneal (IP)
534 injections of vehicle, CGRP, CGRP + olcegepant, or CGRP + rizatriptan in female **(A – D)**
535 and male **(E-H)** nestin/hRAMP1 vs controls**.** **(I-J)** Hypothermia magnitudes (Δ heads)
536 were not different between nestin/hRAMP1 and controls after each treatment in either sex.
537 Sample sizes are listed for male (M) and female (F): vehicle = 17M/18F nestin/hRAMP1
538 and 27M/26F controls; CGRP - 9M/10F nestin/hRAMP1 and 17M/17F controls; CGRP +

539 Olceg - 9M/10F nestin/hRAMP1 and 10M/10F controls; CGRP + Riza - 9M/10F
540 nestin/hRAMP1 and 7M/7F controls. A legend is provided to indicate color code and
541 symbol shape across treatment, mouse strain, and sex.

542 **Fig. 3:** Tail temperatures during motion-induced nausea test and dose response analyses
543 of Δ tail vasodilations are depicted in females (left) and males (right). **(A, D)** Mice exhibit a
544 transient tail vasodilation approximately 5 to 10 minutes after the rotation starts. However,
545 this response is diminished after 1.0x CGRP as seen in **(B, E**). Dunnett multiple
546 comparison's test is used to compare Δ tail vasodilations at each CGRP dose to vehicle.
547 **(C)** In female nestin/hRAMP1 and controls, a significant effect on Δ tail vasodilations is
548 observed due to 0.5x CGRP and 1.0x CGRP. **(F)** Male controls were affected by 1.0x
549 CGRP but not 0.5x CGRP, whereas male nestin/hRAMP1 mice were affected by 0.05x
550 CGRP and 1.0x CGRP, highlighting a potential sensitivity to CGRP in the male
551 nestin/hRAMP1. Sample sizes are listed for male (M) and female (F): vehicle = 17M/18F
552 nestin/hRAMP1 and 27M/26F controls; 0.1x and 0.5x CGRP = 8M/8F nestin/hRAMP1 and
553 10M/9F controls, 1.0x CGRP - 9M/10F nestin/hRAMP1 and 17M/17F controls. A legend is
554 provided, and significance levels are indicated as follows: $p < 0.05$*, $p < 0.01$**, $p <$
555 0.001***.

556

557 **Fig. 4:** The CGRP-receptor antagonist olcegepant was assessed for its efficacy in motion-
558 induced nausea. Drugs were injected intraperitoneally at the following concentrations:
559 0.5x CGRP – 0.05 mg/kg, 1.0x CGRP – 0.1 mg/kg, 1.0x olcegepant – 1 mg/kg. **(A, D)**
560 Raw temperatures after intraperitoneally delivered CGRP + olcegepant treatment are
561 depicted. Post-hoc analysis was conducted using Tukey's multiple comparisons test. **(B)**
562 In female controls, 1.0x CGRP caused reduced Δ tail vasodilations, but co-administration
563 of olcegepant helped some female controls regain a similar response to their vehicle test.
564 This observation was also seen in nestin/hRAMP1 mice, where some females showed
565 restored Δ tail vasodilations with olcegepant. (E) Olcegepant was more effective in
566 blocking CGRP-induced changes in male controls and male nestin/hRAMP1 mice. A
567 separate group of mice were tested at 0.5x CGRP, and mice with Δ tail vasodilations < 1.5
568 ^{o}C were further tested with 0.5x CGRP + 1.0x olcegepant. **(C)** A majority of CGRP-
569 affected female controls and nestin/hRAMP1 regained their tail vasodilation response with
570 olcegepant blocking this sub-maximal dose. **(F)** While a majority of 0.5x CGRP-affected
571 male controls regained their tail vasodilation with olcegepant, many male nestin/hRAMP1
572 still presented with diminished Δ tail vasodilations compared to their vehicle response.
573 Before/after plots are provided alongside violin plots to visualize treatment differences
574 within animals. For detailed F-statistics and p-values, please refer to Table 2. Sample
575 sizes are listed: vehicle, 1.0x CGRP, and 1.0x CGRP + 1.0x olcegepant - 9M/10F
576 nestin/hRAMP1 and 10M/10F controls; 0.5x CGRP = 8M/8F nestin/hRAMP1 and 10M/9F

577 controls; 0.5x CGRP + 1.0x olcegepant = 8M/7F nestin/hRAMP1 and 5M/7F controls. A
578 legend is provided, and significance levels are indicated as follows: $p < 0.05$*, $p < 0.01$**,
579 $p < 0.001$***, $p < 0.0001$****

580 **Fig. 5:** The selective serotonin receptor agonist rizatriptan – a clinically prescribed
581 migraine drug – was assessed for its efficacy in motion-induced nausea. Drug
582 concentrations are listed: 0.5x CGRP – 0.05 mg/kg, 1.0x CGRP – 0.1 mg/kg, 1.0x
583 rizatriptan – 0.6 mg/kg. **(A-B)** Raw temperatures after CGRP + rizatriptan treatment are
584 depicted. **(C - D)** In female and male controls, CGRP caused diminished Δ tail
585 vasodilations and CGRP+rizatriptan did not block these effects. The nestin/hRAMP1 mice
586 resembled control mice, as rizatriptan was not effective in blocking CGRP-induced
587 changes. **(E)** A small subset of nestin/hRAMP1 mice assessed rizatriptan's efficacy at half
588 the maximum CGRP dose, but no blocker effects were seen. F-statistics and p-values can
589 be found in bottom half of Table 2. Sample sizes are as follows: vehicle, 1.0x CGRP, and
590 1.0x CGRP + 1.0x rizatriptan – 9/10 Nestin/hRAMP1 and 7/7 Controls; vehicle, 0.5x
591 CGRP, and 0.5x CGRP + 1.0x rizatriptan – 4F/4M nestin/hRAMP1. Significance levels are
592 listed: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***

593

594 **Fig. 6: (A)** 2-way ANOVA computed the factors i) male vs female and ii) controls versus
595 nestin/hRAMP1 on sway obtained after vehicle injections (saline). While biological sex

596 had no effect on sway ($F(1, 81) = 2.609$, $p = 0.11$), a significant difference was observed
597 between controls and nestin/hRAMP1 ($F(1, 81) = 35.45$, $p < 0.001$). Tukey's multiple
598 comparisons indicated nestin/hRAMP1 males and females have a higher sway baseline
599 than their complement controls. **(B)** CGRP induces higher CoP ellipse areas in female
600 controls at 1.0x CGRP but not in female nestin/hRAMP1. **(C)** While male nestin/hRAMP1
601 sway is significantly higher than male controls at all doses of CGRP, there is no
602 modulation in their sway area with increasing CGRP dose. Before/after plots are placed
603 next to violin plots and show animals that were specifically tested with 1.0x CGRP.
604 Sample sizes are listed: vehicle and 1.0x CGRP– 18M/22F nestin/hRAMP1 and 17M/28F
605 controls; 0.1x and 0.5x CGRP -8M/8F nestin/hRAMP1 and 8M/9F controls. Dose
606 response statistics for sway can be found in top half of Table 2. $p < 0.05$*, $p < 0.01$**, $p <$
607 0.001***

608

609 **Fig. 7**: Changes in sway were observed after vehicle, CGRP, and CGRP + olcegepant
610 treatment in controls and nestin/hRAMP1, using same concentrations from motion-
611 induced thermoregulation studies. **(A)** In female controls, CGRP causes increases in
612 postural sway but co-administration of olcegepant blocks CGRP-induced changes. In
613 contrast, female nestin/hRAMP1 mice do not appear affected by CGRP, and olcegepant is
614 not effective either. **(B)** Male nestin/hRAMP1 mice do not respond to CGRP or

615 CGRP+olcegepant. For male controls, a paired t-test was used to compare vehicle vs
616 CGRP effects but no differences were observed. The lack of a CGRP-induced change in
617 male control sway provided us the rationale to discontinue testing these mice with
618 blockers. Before/after plots show intra-animal changes to treatment. Sample sizes
619 include 9M/14F nestin/hRAMP1 and 10M/18F controls repeatedly tested. Full list of F and
620 p-values for these sway experiments are found in the middle portion of Table 2.
621 Significance levels are indicated as $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, $p < 0.0001$****

622

623 **Fig. 8:** Sway changes after vehicle, CGRP, or CGRP + rizatriptan in nestin/hRAMP1
624 versus controls using concentrations from motion-induced thermoregulation studies. **(A)**
625 While rizatriptan blocked CGRP-induced changes in sway for a subset of female controls,
626 a majority of female controls were not responsive to rizatriptan in this study. **(A – B)**
627 Female and male nestin/hRAMP1 were not responsive to CGRP + rizatriptan. Sample
628 sizes include 9M/14F nestinRCP (-/-) and 10M/10F controls repeatedly tested. Full list of F
629 and p-values are found in the bottom portion of Table 2. Significance levels are set at $p <$
630 0.05*, $p < 0.01$**, $p < 0.001$***, $p < 0.0001$****

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634 **Table Legends**

635 **Table 1.** Time to recover from hypothermia (minutes) was analyzed between tamoxifen-
636 treated controls and nestinRCP (-/-) mice using an x-intercept quadratic model. Males and
637 females were separated. A constraint to the model was applied so that the x-intercept
638 analyzed occurred after time = 20 minutes. While F-statistics and p-values are listed,
639 comparisons are made more confidently in curve fits with $R^2 \geq 0.60$. No significant
640 differences were observed in hypothermia recovery between nestin/hRAMP1 and
641 controls. While 1.0x CGRP + 1.0x olcegepant testing appeared significant between female
642 nestin/hRAMP1 and controls, curve fits were poor so an interpretation is not made.
643 Sample sizes are listed for male (M) and female (F): vehicle = 17M/18F nestin/hRAMP1
644 and 27M/26F controls; CGRP - 9M/10F nestin/hRAMP1 and 17M/17F controls; CGRP +
645 Olceg - 9M/10F nestin/hRAMP1 and 10M/10F controls; CGRP + Riza - 9M/10F
646 nestin/hRAMP1 and 7M/7F controls/

647 **Table 2.** Percentage of mice with diminished delta tail vasodilations during motion-induced
648 thermoregulation test after each treatment (row). Columns are categorized by
649 nestin/hRAMP1 vs controls and male vs female.

650 **Table 3.** Multivariate statistics are listed per study with corresponding F and p values.
651 Dose responses were analyzed using 2-way repeated measures (RM) mixed effects
652 models to handle missing values since not all animals were repeatedly tested with every

653 CGRP dose, whereas 1.0x CGRP + 1.0x blocker studies used 2-way RM ANOVAs since
654 animals were repeatedly tested. 1-way ANOVAs were used in case strain differences
655 could not be made.