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# GPR35 and mediators from platelets and mast cells in neutrophil migration and inflammation

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## Summary

Neutrophil recruitment from circulation to sites of inflammation is guided by multiple chemoattractant cues emanating from tissue cells, immune cells, and platelets. Here, we focus on the function of one G-protein coupled receptor, GPR35, in neutrophil recruitment. GPR35 has been challenging to study due the description of multiple ligands and G-protein couplings. Recently, we found that GPR35-expressing hematopoietic cells respond to the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA). We discuss distinct response profiles of GPR35 to 5-HIAA compared to other ligands. To place the functions of 5-HIAA in context, we summarize the actions of serotonin in vascular biology and leukocyte recruitment. Important sources of serotonin and 5-HIAA are platelets and mast cells. We discuss the dynamics of cell migration into inflamed tissues and how multiple platelet and mast cell-derived mediators, including 5-HIAA, cooperate to promote neutrophil recruitment. Additional actions of GPR35 in tissue physiology are reviewed. Finally, we discuss how clinically approved drugs that modulate serotonin uptake and metabolism may influence 5-HIAA-GPR35 function, and we speculate about broader influences of the GPR35 ligand-receptor system in immunity and disease.

## KEYWORDS

chemotaxis, GPCRs, lipid mediators, mast cells, neutrophils, platelets

## 1 | INTRODUCTION

The study of neutrophil chemoattraction has a rich history that has informed our understanding not only of how these highly motile cells are recruited but also of classes of molecules that are later found to have broader influences on physiology. Early reports on serum activities that could promote neutrophil recruitment led to the identification of chemoattractive components of complement that we now know engage C3a and C5a receptors on many cell types.<sup>1,2</sup> Additional efforts to define the neutrophil chemoattractant activity of serum implicated factors beyond complement components, including platelet derived factors.<sup>3,4</sup> Characterization

of a neutrophil chemoattractant made by activated monocytes led to the identification of IL-8 (CXCL8), the founding member of the chemokine superfamily.<sup>5</sup> The search for IL-8 receptors led to definition of the chemokine receptor family.<sup>6</sup> Moreover, studies on neutrophil recruitment to sites of inflammation were central in establishing the multistep selectin-chemokine-integrin cascade of cell movement from blood to tissue.<sup>7,8</sup> In this article, we review findings on a new ligand-receptor system, 5-HIAA-GPR35, that is involved in neutrophil recruitment to sites of inflammation. The unusual *in vitro* features of 5-HIAA activity on GPR35, including high sensitivity but low efficacy, and possible cell-type specific signaling will be discussed. Platelets and mast cells are important sources of multiple inflammatory cell recruitment mediators. We discuss how platelet

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and mast cell-derived 5-HIAA is likely to cooperate with additional mediators to promote neutrophil migration into inflamed tissue. We review how the approaches to selectively perturb 5-HIAA abundance in vivo have caveats, with each also altering the abundance of serotonin and possibly other serotonin metabolites. Based on the diverse findings regarding GPR35 function that are summarized in this review, we anticipate that GPR35, 5-HIAA, and possibly other GPR35 ligands will have broad roles in the immune system and in tissue physiology and disease.

## 2 | GPR35 BACKGROUND

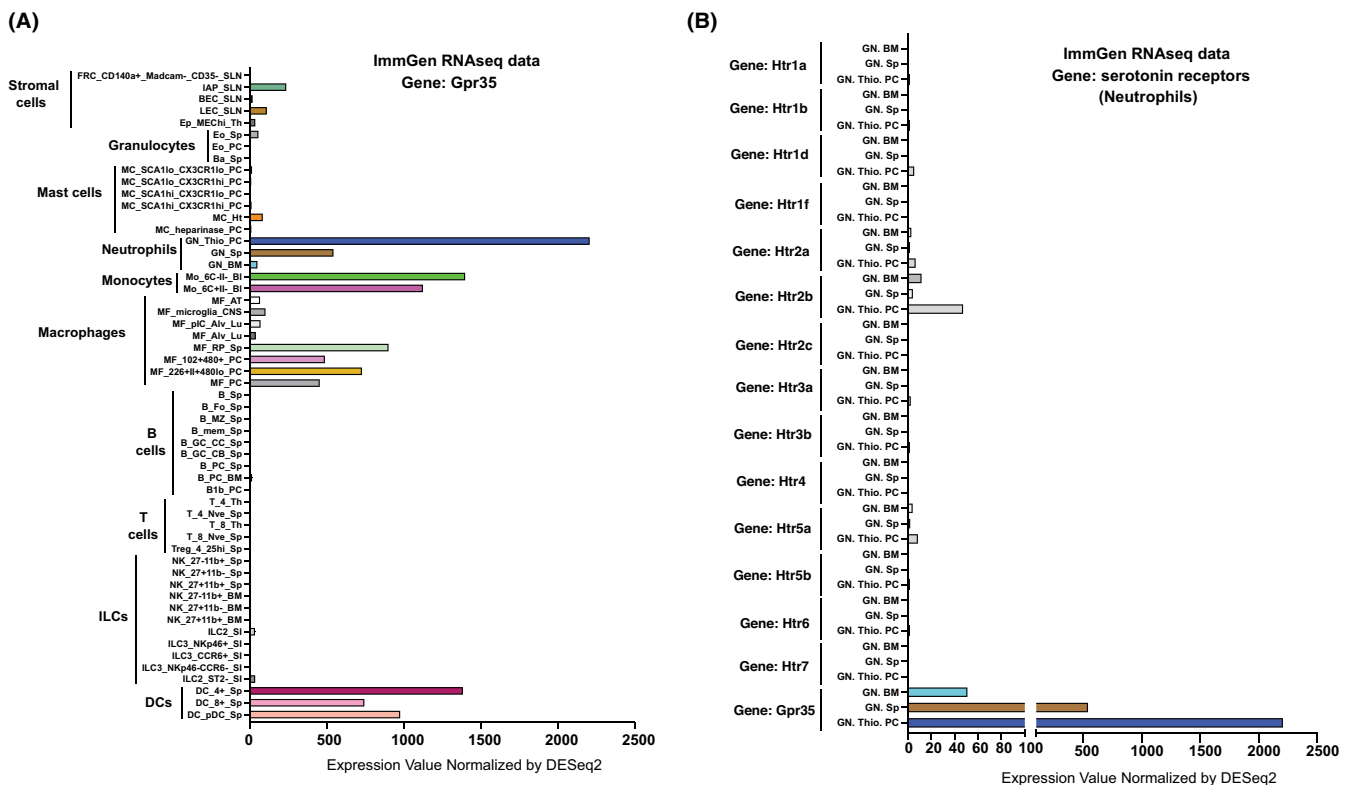
### 2.1 | GPR35 expression

GPR35 is expressed at low levels by immature neutrophils in the bone marrow (BM) and is more highly expressed by mature neutrophils in blood and spleen. Following exposure to an agent that elicits neutrophil mobilization from the BM, such as peritoneal thioglycolate injection or bacterial infection, GPR35 mRNA and protein is strongly upregulated.<sup>9</sup> GPR35 is expressed by additional myeloid cell types, including monocytes, dendritic cells, and eosinophils (Figure 1), and by intestinal epithelial cells, certain neurons, and cells in cardiovascular and adipose tissues.<sup>10–13</sup> Further work is needed to determine the relative expression level on different cell types. The

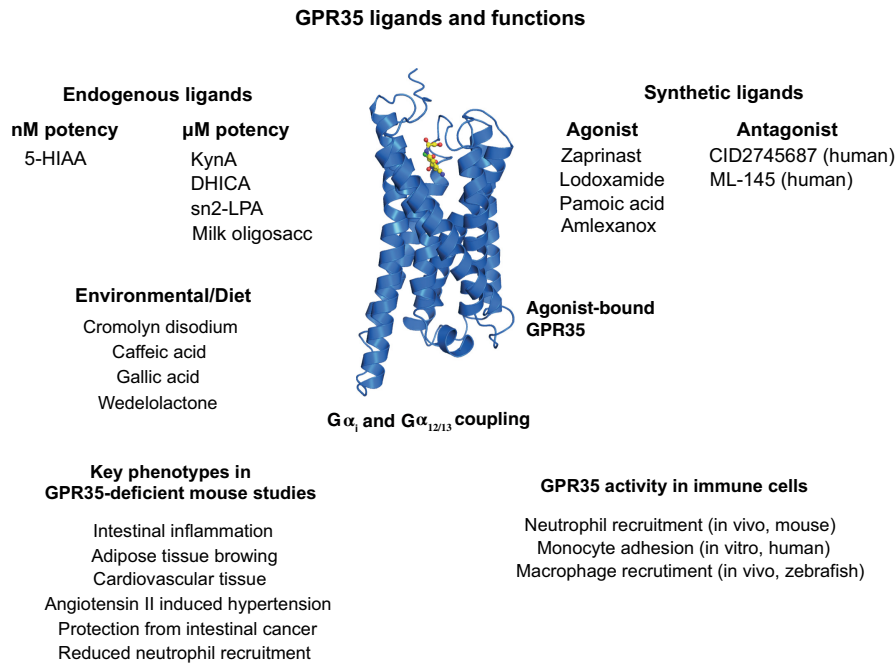
broad expression pattern reflects both immune and non-immune functions.

### 2.2 | GPR35 ligands

The natural agonists for GPR35 remain under intense investigation. In 2006, a team at Amgen screened biochemical intermediates against a panel of GPCRs in a  $Ca^{2+}$  mobilization assay and found that the tryptophan metabolite kynurenic acid (KynA) was a micromolar potent agonist for GPR35 but not for any of 50 other GPCRs<sup>10</sup> (Figure 2). A later report suggested that GPR35 was the receptor for CXCL17,<sup>14</sup> a putative chemokine family molecule, but this association was not supported in subsequent work<sup>12,15,16</sup> (unpublished obs.). Moreover, recent alignment and protein-folding prediction studies have indicated that CXCL17 most likely does not have a chemokine fold<sup>17</sup> (<https://alphafold.ebi.ac.uk>). GPR35 has been suggested to respond to lysophosphatidic acid (LPA)<sup>11,18</sup> and several other small molecules<sup>19–21</sup> but again only in the micromolar (or higher) range, and these molecules have not been validated as in vivo ligands.<sup>12</sup> For example, KynA concentrations in serum and plasma are 20–100 nM and not in the micromolar range.<sup>22,23</sup> Melanin synthesis intermediate 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and several analogs were also shown to be micromolar potent GPR35 agonists in vitro,<sup>24</sup> suggesting that a variety of indole acids may function as



**FIGURE 1** Mouse *Gpr35* transcript abundance in neutrophils and other immune cells and of serotonin receptors in neutrophils. Data are from [Immgen.org](https://immgen.org). The neutrophil (GN) data are for  $CD11b^+Ly6G^+$  immature cells in the BM, unstimulated cells from spleen, and cells recruited to the peritoneal cavity 18h after thioglycolate (1 mL, 3%) injection. See [Immgen.org](https://immgen.org) for detailed descriptions of the other cell types.



**FIGURE 2** Summary diagram of GPR35 ligands and functions. The overall structure of human GPR35 with the agonist Lodoxamide is shown (PDB:8H8J).<sup>25</sup> “Endogenous ligands” indicates molecules that can be generated by physiological pathways; evidence for reduced in vivo GPR35 function under conditions of reduced metabolite production has so far only been reported for 5-HIAA. In addition to DHICA, several other tyrosine metabolites have activity in vitro on GPR35 in the μM to mM range. “Environmental/Diet” refers to molecules produced by plants or microbes that have ligand activity. While we have tried to be comprehensive, the list of molecules with suggested agonist or antagonistic activity on GPR35 may not be complete. See the text for details and references.

GPR35 ligands. In our recent work, GPR35 was found to support pro-migratory responses to serum and to supernatants from activated platelets.<sup>9</sup> Serotonin is a tryptophan metabolite present in platelets, but it was not found to have GPR35 ligand activity.<sup>9,10</sup> However, platelets metabolize serotonin to 5-HIAA, and GPR35 was responsive to 5-HIAA in cell migration, adhesion, and receptor internalization assays in the nanomolar range.<sup>9</sup>

The pro-migratory and pro-adhesive activities of GPR35 were pertussis toxin (PTX) sensitive and thus Gα<sub>i</sub>-dependent<sup>9,26</sup> as was activity in a <sup>35</sup>S-GTPγS assay.<sup>10</sup> Cell culture experiments with low potency agonists revealed GPR35 could also couple to Gα<sub>12</sub>/Gα<sub>13</sub>.<sup>16,27–29</sup> Coupling to Gα<sub>13</sub> has been validated with the recent determination of a GPR35-Gα<sub>13</sub>βγ structure.<sup>25</sup> Further studies are needed to determine if these G-proteins contribute independently or cooperatively to GPR35 dependent responses of myeloid cells.

### 2.3 | GPR35 supports neutrophil recruitment in vivo

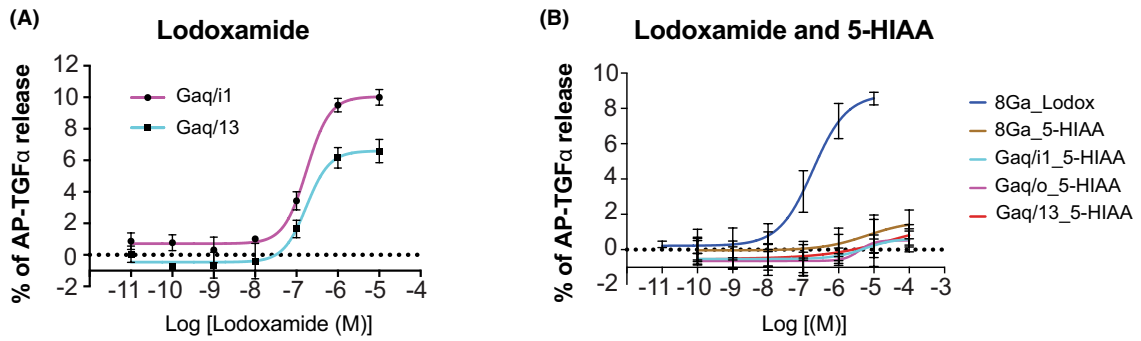
Neutrophil transfer experiments established that GPR35 contributed to the efficiency with which these cells were recruited to sites of inflammation.<sup>9</sup> This recruitment effect could be observed as early as 2 hours after elicitation of sterile or bacterial inflammation. Intravascular labeling and real-time imaging experiments showed a reduced frequency of GPR35-deficient neutrophils migrating from blood vessels into the tissue.<sup>9</sup> GPR35 promoted neutrophil

interaction with platelet clusters and subsequent extravasation. Both platelets and mast cells contributed to neutrophil recruitment through production of multiple factors, with pharmacological and genetic studies implicating a role for 5-HIAA. In the sections that follow, we review serotonin and 5-HIAA biology and key information about the platelet and mast cell contribution to inflammatory cell recruitment and we integrate information about GPR35 into this discussion. However, first, we consider the unusual properties of GPR35 signaling in response to 5-HIAA and other ligands.

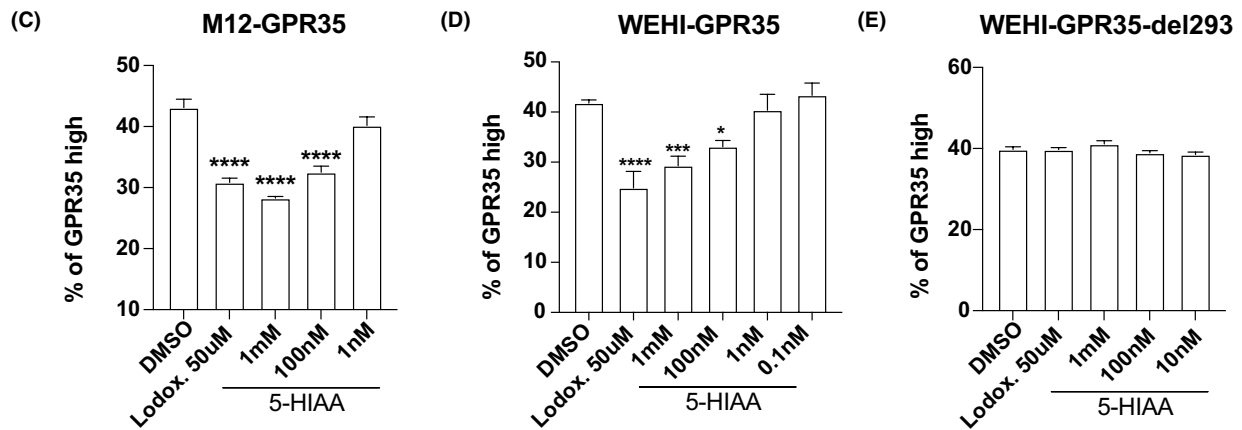
### 2.4 | GPR35 activation can be qualitatively distinct and cell type selective

The expression of GPR35 by a wide range of immune and non-immune cell types makes it likely that its function will be context dependent.<sup>10–13</sup> In addition, positively charged residues in the ligand pocket allow for promiscuous binding with many acidic molecules,<sup>12,13,21,25,30</sup> suggesting that GPR35 may respond to different agonists depending on the cell type and tissue niche. In agreement with this view, DHICA analogs show strong cell line and assay dependent relative efficacy and potency against GPR35, a feature referred to as “biased agonism”.<sup>24</sup> Other GPR35 ligands may also show biased agonism. Indeed, 5-HIAA induced little or no signal in TGFα-shedding assays<sup>31</sup> performed in HEK293T cells transiently transfected with GPR35 (Figure 3A,B). There was also minimal signal in β-arrestin and miniG12 recruitment assays performed in HT-29

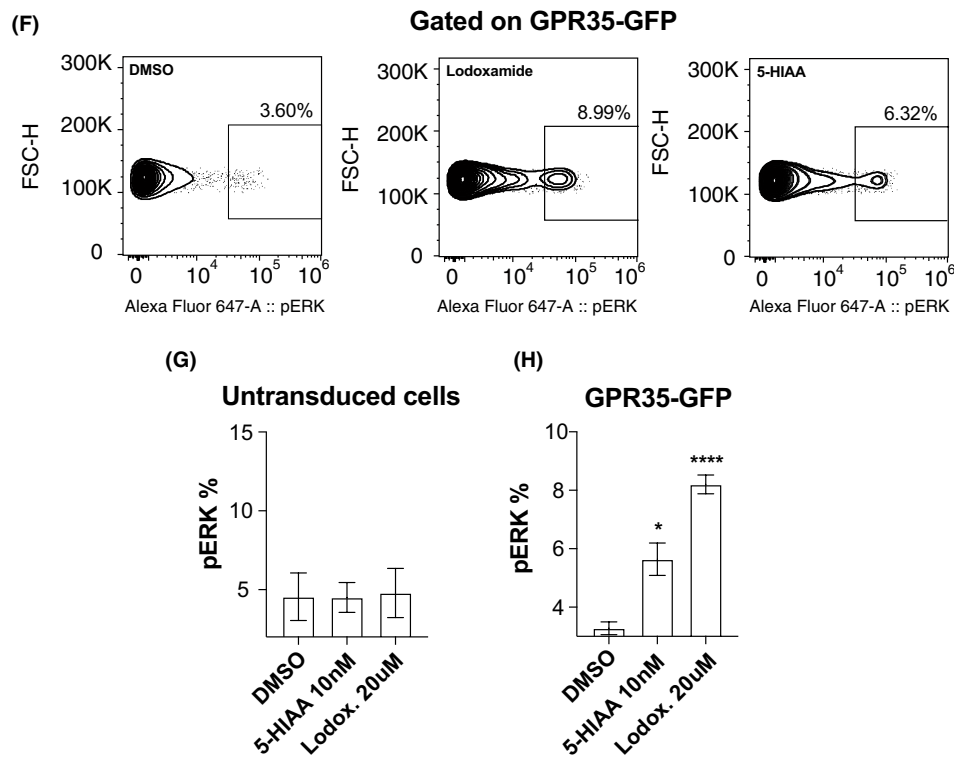
GPR35 activity in TGF $\alpha$  shedding assay (HEK293T cells)



GPR35 internalization assay in mouse B cell lymphoma lines



GPR35-dependent pERK induction in WEHI-231



**FIGURE 3** Selective GPR35 agonism by 5-HIAA. A, B, TGF $\alpha$  shedding assay with mGPR35 and chimeric G $\alpha$ q proteins in HEK293  $\Delta$ G $\alpha$ q/11/12/13 cells that express the alkaline phosphatase (AP)-TGF $\alpha$  membrane construct. Gq activation leads to AP-TGF $\alpha$  shedding and allows GPCR activation to be measured based on AP activity in the culture supernatant.<sup>31</sup> G $\alpha$ q/i1 reports G $\alpha$ i coupling, G $\alpha$ q/13 G $\alpha$ 13 coupling, and G $\alpha$ q/o G $\alpha$ o coupling. 8G $\alpha$  refers to cells receiving a mixture of G $\alpha$ q/s, G $\alpha$ q/i1, G $\alpha$ q/i3, G $\alpha$ q/o, G $\alpha$ q/z, G $\alpha$ q/12, G $\alpha$ q/13, G $\alpha$ q/16. A, Response to Lodoxamide, a synthetic GPR35 agonist. B, Response to 5-HIAA compared to Lodoxamide. Data are mean  $\pm$  SD (n = 3). Experiments were repeated at least twice with similar results. C-E, Internalization of N-terminally OX56 epitope-tagged WT (C, D) or C-terminally truncated ( $\Delta$ 293, E) mGPR35 in M12 cells (C) or WEHI-231 cells (D, E) in response to the indicated stimuli for 30 min at 37°C. M12 and WEHI-231 cell transductions and internalization assays were performed as previously described.<sup>9</sup> Both are mouse B lymphoma cell lines. The  $\Delta$ 293 mutant lacks the last 13 amino acids of the receptor, including Ser and Thr residues required for  $\beta$ -arrestin recruitment.<sup>37</sup> F-H, Intracellular FACS for pERK in WEHI-231 B lymphoma cells transduced with mGPR35. Staining of permeabilized cells was with Cell Signaling antibody 197G2 against phospho-ERK1/2 (Thr202/204). Example FACS plots for GPR35-IRES-GFP transduced cells (F) and summary data for untransduced (G) and GPR35-IRES-GFP transduced cells (H) stimulated with diluted carrier (DMSO), 5-HIAA (10 nM) or Lodoxamide (20  $\mu$ M) for 10 min.

cells (Daniel Graham, personal communication). Yet, 5-HIAA stimulation resulted in consistent internalization of wild-type GPR35 (but not of a desensitization-resistant C-terminal truncation mutant), in transduced M12 and WEHI-231 lymphoid cell lines (Figure 3C-E). 5-HIAA also triggered increased ERK phosphorylation in WEHI-231 cells in a GPR35-dependent manner (Figure 3F-H). In another complex case, 2-acyl LPA (but not 1-acyl LPA) induced a Ca<sup>2+</sup> response in HEK293T cells stably expressing GPR35,<sup>18</sup> and LPA stimulated in vitro migration of GPR35-expressing macrophages.<sup>11</sup> However, 1-acyl and 2-acyl LPA did not induce receptor internalization or GPR35-dependent migration in WEHI-231 cells,<sup>9</sup> and in contrast to a synthetic GPR35 agonist, LPA had little effect on GPR35<sup>+</sup> goblet cells.<sup>32</sup> An added challenge in making definitive statements about new LPA ligand-receptor relationships is the widespread expression of six established LPA receptors and the need to rule out their involvement.<sup>33</sup> Returning to KynA, early work showed it induced a robust Ca<sup>2+</sup> response and [<sup>35</sup>S] GTP $\gamma$ S incorporation in membrane preparations from CHO-GPR35 cells<sup>10</sup> and later work showed induction of cell adhesion and migration in vitro.<sup>9,26</sup> However, it induced only a weak or no response in cAMP-based assays,  $\beta$ -arrestin recruitment assays and internalization assays with transiently GPR35 transfected cells.<sup>11,13,34,35</sup> Also, KynA did not trigger measurable Ca<sup>2+</sup> flux or receptor internalization in HEK293T cells that stably express GPR35.<sup>18</sup> To further increase the complexity, many GPR35 agonists show species ortholog selectivity,<sup>36</sup> and different isoforms of GPR35 have distinct extracellular N-termini that allosterically modify receptor coupling and mediate intracellular pathway bias.<sup>35</sup> Additionally, increased extracellular concentrations of divalent cations (eg, Ca<sup>2+</sup>) can augment the activity of some GPR35 agonists.<sup>25</sup>

GPR35 can function as a pro-migratory and pro-adhesive receptor in response to several agonists,<sup>9,26</sup> and the absence of this receptor can result in 50% reduced neutrophil recruitment in vivo.<sup>9</sup> However, the overall migration activity obtained in vitro with GPR35 ligands is low compared to other pro-migratory GPCRs (eg, CXCR4).<sup>9,11</sup> We speculate that ligand association to carrier protein(s), simultaneous receptor stimulation by multiple ligands, perturbations in divalent ion concentration, receptor modifications and/or receptor interacting proteins may influence the magnitude of pro-migratory activity induced by in vivo GPR35 stimulation in the inflammatory microenvironment. It is also possible that the pro-adhesive functions

of GPR35 play a more dominant role during cell recruitment in vivo than the pro-migratory functions.<sup>9,26</sup> Future studies are needed to characterize the factors determining the context dependent activity of GPR35 and which endogenous agonists are more important in different tissue microenvironments.

### 3 | SEROTONIN BIOLOGY IN BRIEF

To place the actions of 5-HIAA as a GPR35 ligand in context, it is important to consider the functions of its precursor, serotonin. Also known as 5-hydroxytryptamine (5-HT), serotonin is a pleiotropic mediator that is perhaps most widely recognized for its actions as a neurotransmitter in the central nervous system (CNS). It influences many behaviors including mood, appetite, attention, and fear.<sup>38</sup> Multiple classes of anti-depressant, including SSRIs (selective serotonin reuptake inhibitors) and MAOIs (monoamine oxidase inhibitors), interfere with the reabsorption or degradation of serotonin, respectively, thereby augmenting serotonin receptor signaling in the nervous system. Despite important roles in the CNS, the majority of serotonin in the body is made in the intestine by neuroendocrine cells known as enterochromaffin cells (ECCs).<sup>38,39</sup> ECCs are chemosensory cells that communicate multiple properties about the gut environment to the nervous system. In the gut, serotonin has many actions and most of these are thought to be mediated through effects on neurons, including affecting peristalsis, secretion, vasodilation, and nutrient absorption.<sup>38</sup> The diverse actions of serotonin are mediated by a large family of receptors that includes 13 GPCRs and one ligand-gated cation channel.<sup>40</sup>

Serotonin that reaches circulation from the intestine is taken up via the serotonin-selective reuptake transporter (SERT, encoded by Slc6a4) into platelets where it is stored in dense granules.<sup>38</sup> Platelet activation leads to release of serotonin along with many other mediators, as discussed further in the next section. Actions of platelet-derived serotonin include vasoconstriction or vasodilation depending on the vessel bed, and platelet aggregation (by receptor-independent transglutaminase-dependent covalent linkage of proteins).<sup>38</sup> Indeed, serotonin's ability to modulate vascular tone was one of the activities that led to its discovery, and it being named serotonin. Early work showed that treatment with serotonin led to

similar increases in vascular permeability to those occurring after histamine exposure.<sup>41</sup>

Serotonin is synthesized from tryptophan by Tryptophan Hydroxylases (Tph), of which there are two types (Figure 4). Tph2 is expressed in neurons in the brain as well as some enteric neurons, whereas Tph1 is expressed in non-neuronal peripheral cells, particularly by intestinal ECCs but also by mast cells.<sup>39,42</sup>

Duerschmied and coworkers found that Tph1-deficient mice (that lack peripheral serotonin) have reduced frequencies of rolling neutrophils in acutely inflamed mesenteric vessels, most likely due to reduced serotonin-mediated Weibel-Palade Body (WPB) mobilization by endothelial cells.<sup>43</sup> WPBs are a key source of P-selectin in endothelial cells. Chronically blocking serotonin uptake with fluoxetine (Prozac™) is a method to reduce platelet serotonin content and this treatment also caused reduced neutrophil rolling frequencies.<sup>43</sup> This study observed reduced neutrophil homing to lung, peritoneum, and skin wounds in Tph1-deficient mice. This reduction was attributed to the reduced rolling-mediated attachment of neutrophils as a consequence of the lack of serotonin but as we will discuss further below, the recruitment data may also reflect reduced 5-HIAA availability and loss of GPR35 function. Subsequent work by this group showed that acute (2 hours) fluoxetine treatment induced slow rolling of leukocytes on surgically exposed mesenteric endothelium.<sup>44</sup> Plasma serotonin was elevated after the acute SERT inhibition (in contrast to its depletion after chronic treatment), and this may have triggered WPB mobilization and increased P-selectin availability. Acute fluoxetine was without effect in Tph1-deficient mice in accord with a serotonin-dependent mode of action.

High dose peritoneal zymosan treatment causes multiple organ failure, and this was prevented in Tph1-deficient mice.<sup>45</sup> The protective effect of Tph1-deficiency was associated with reduced neutrophil infiltration and suggested to reflect a role for serotonin in neutrophil recruitment. Myocardial reperfusion injury was dampened in Tph1-deficient mice, and this was associated with reduced neutrophil accumulation in the heart. Fluoxetine-mediated serotonin

depletion was also protective from myocardial ischemia-reperfusion injury.<sup>46</sup>

These studies have led to the suggestion that in addition to actions on the vasculature, serotonin has direct actions on neutrophils. However, there is a lack of convincing data showing expression of any of the serotonin receptors in neutrophils (eg, [Immgen.org](http://Immgen.org), Figure 1). By contrast, GPR35 is highly expressed by activated neutrophils. Moreover, the treatments used to block serotonin production (Tph1-deficiency or inhibition of serotonin uptake by platelets) also block production of 5-HIAA. While direct actions of 5-HT on neutrophils remain possible, we suggest that past findings implicating direct actions of serotonin on neutrophils could also fit with a mechanism involving the serotonin metabolite, 5-HIAA, engaging neutrophil GPR35.

## 4 | 5-HIAA BIOLOGY

The rate limiting step in serotonin catabolism is catalyzed by the mitochondrial membrane-associated monoamine oxidases (MAO) A and B.<sup>47</sup> MAO catalyzes the oxidative deamination of serotonin by converting it to 5-hydroxy-3-indolacetaldehyde (5-HIAL) which is further processed into 5-HIAA by aldehyde dehydrogenase (Figure 4). 5-HIAA is excreted from the body via the kidneys. As a major metabolite of serotonin, 5-HIAA is measured in the blood and urine as an indicator of serotonin levels. This is important in several clinical situations, including in the context of neuroendocrine tumors. 5-HIAA is elevated in the cerebrospinal fluid of stroke patients<sup>48</sup> and in the plasma after myocardial infarction.<sup>49</sup>

Platelets express MAO-B and as well as storing serotonin, they harbor 5-HIAA.<sup>50</sup> Serum contains serotonin and 5-HIAA in about a 10:1 ratio, likely reflecting the relative abundance of these mediators in platelets.<sup>51</sup> 5-HIAA is released with serotonin during platelet activation.<sup>52</sup> However, one study reported that the 5-HT:5-HIAA content of platelets was close to 1:1 perhaps indicating there can be differential release of these small molecules from activated platelets

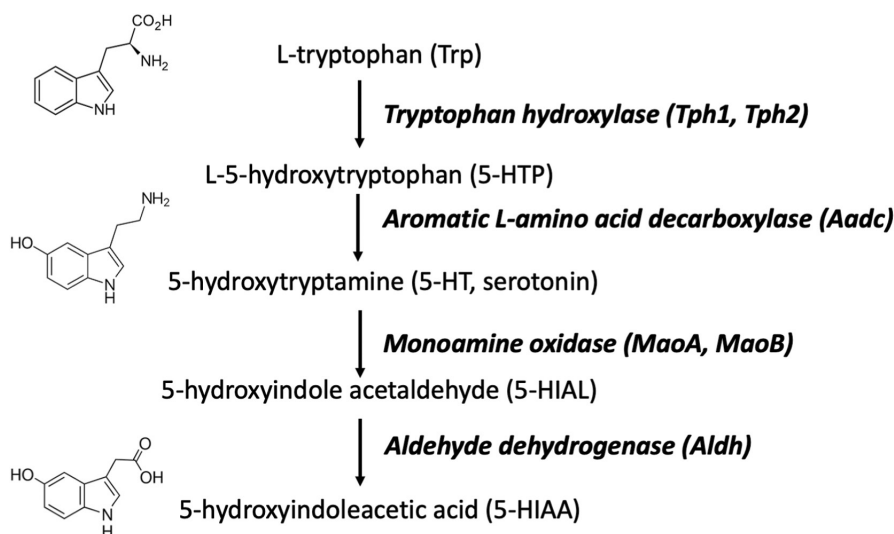


FIGURE 4 Biosynthetic pathway of serotonin and 5-HIAA. Chemical structure diagrams of L-tryptophan, serotonin and 5-HIAA are shown. Enzyme names are in italics.



during serum preparation.<sup>53</sup> They may also have different clearance rates from circulation.

In contrast to the multitude of functions ascribed to serotonin, 5-HIAA has largely been considered a “waste” metabolite that is released into the blood to be excreted from the body in the urine. The action of 5-HIAA as a GPR35 ligand indicates that this is an oversimplified view and that 5-HIAA, like many other metabolites, is used by the body for important signaling functions. In addition to acting on GPR35, another recently defined function of 5-HIAA is as an arylhydrocarbon receptor (AHR) agonist.<sup>54</sup> Microbiota-derived butyrate suppressed arthritis in a mouse model, and this was associated with an increase in tryptophan-metabolizing bacteria, and 5-HIAA generation. 5-HIAA induced AHR activity in B cells, promoted activation of IL10 producing regulatory B cells.<sup>54</sup> We speculate that microbiome-derived 5-HIAA engages epithelial and myeloid GPR35 to modulate gut physiology, though whether 5-HIAA can signal via GPR35 in epithelial cells has not yet been determined. Enteric neuron-derived 5-HIAA may also interface with gut-associated GPR35 pathways.

Studies in an invertebrate have pointed to additional roles for 5-HIAA. A search for genetic modifiers of RAS/MAPK signaling in *Caenorhabditis elegans* identified MAO (AMX-2) as a negative regulator. Systemic treatment of the nematode with 5-HIAA inhibited RAS/MAPK signaling in several organs. The authors suggested that 5-HIAA may act via SER-1, a serotonin receptor, in a manner opposing the function of serotonin.<sup>55</sup> A subsequent report found that 5-HIAA inhibited *C. elegans* egg laying by antagonizing serotonin actions on SER-1.<sup>56</sup> It remains to be seen whether 5-HIAA antagonizes or modulates the function of any of the 14 vertebrate serotonin receptors.

While ECCs have been given the most attention as peripheral sources of serotonin, mast cells also express Tph1 and contain serotonin.<sup>57</sup> Mast-cell activation promotes increased vascular permeability<sup>58</sup> and the early phase depends on serotonin (likely acting in the endothelium and smooth muscle cells) and histamine while later permeability depends on neutrophil recruitment by chemoattractants such as platelet activating factor (PAF) and chemokines.<sup>59</sup> As well as expressing Tph1, mast cells express high amounts of SERT and take up serotonin from their microenvironment. This may include uptake from plasma as some studies have suggested mast cells can extend processes into the lumen of blood vessels.<sup>60–62</sup> Mast cells also highly express MAO-B<sup>63</sup> and express MAO-A ([Immgen.org](http://Immgen.org)) and they contain 5-HIAA.<sup>64</sup> Indeed, early work provided in vivo evidence that mast cells can be a source of 5-HIAA.<sup>65</sup>

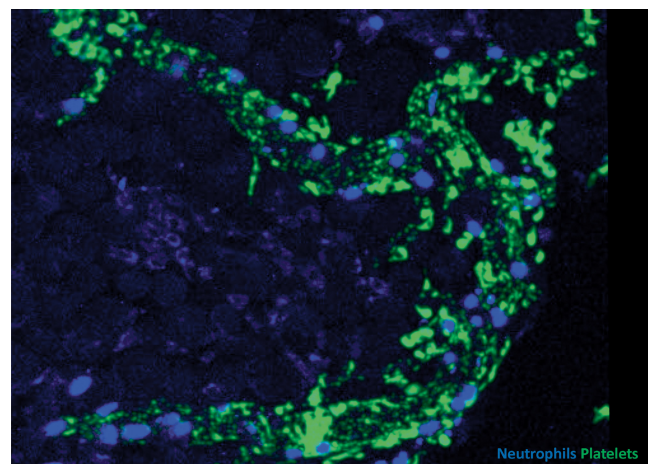
## 5 | PLATELET AND MAST CELL ACTIONS IN NEUTROPHIL RECRUITMENT

Platelets are derived from megakaryocyte membrane and cytoplasm and are biconvex discs with a diameter of ~2 μm in humans and ~0.5 μm in mouse. They are very numerous in blood, though about 10-fold less frequent than red blood cells. Their lifespan is about 9 days in humans and 5 days in mice. Their most widely recognized

role is to respond to vascular injury by aggregating and thereby contributing to the blood clot. However, it has long been recognized that platelets are active participants in inflammatory processes due to their adhesion properties and the hundreds of bioactive mediators that they release following activation.

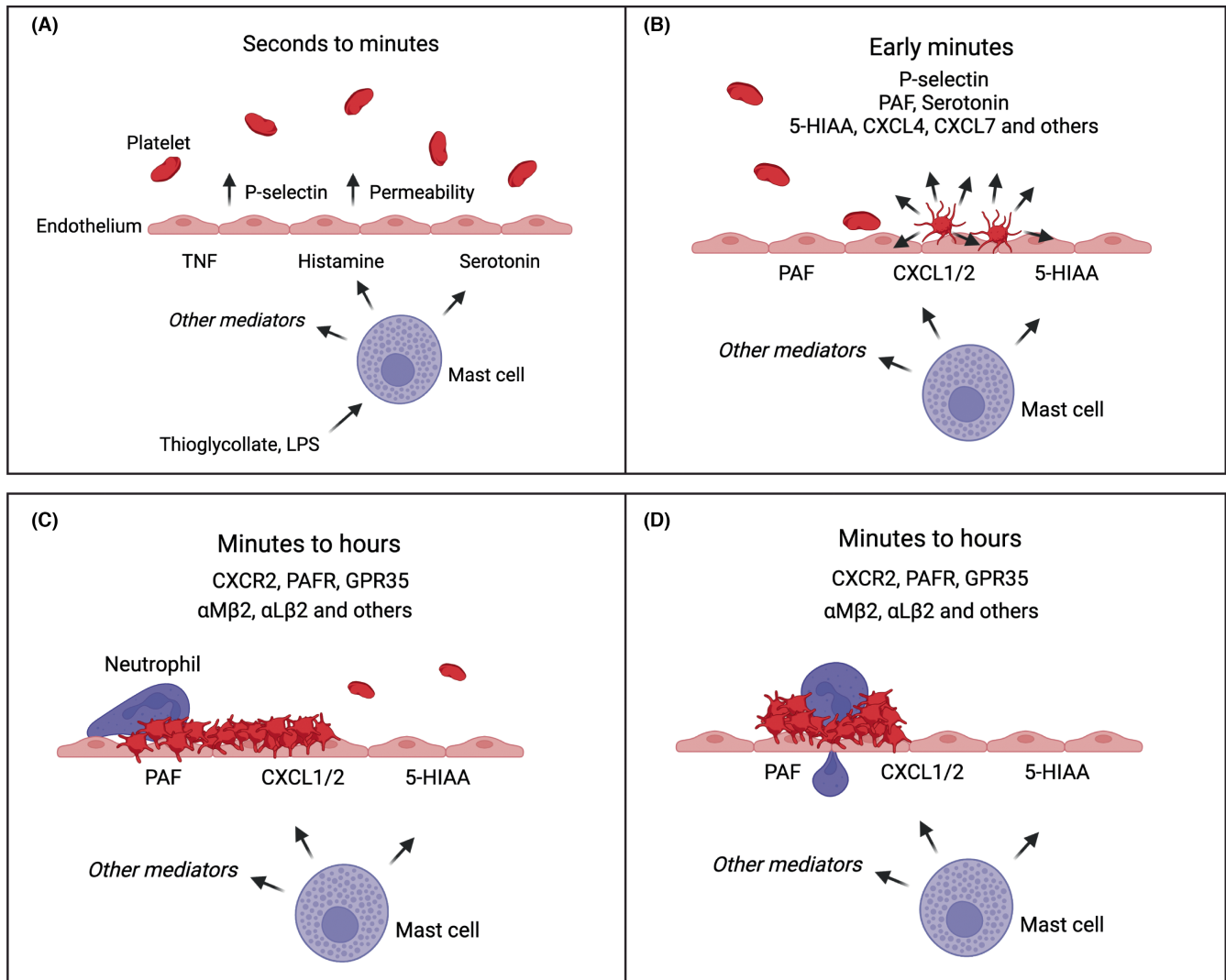
A role for platelets in neutrophil recruitment to sites of inflammation was recognized over two decades ago<sup>66–68</sup> (Figure 5). Multiple studies have demonstrated that platelets augment neutrophil recruitment, including to inflamed peritoneum, kidney, lung, skin, lymph nodes and brain,<sup>68–74</sup> and early tumor metastatic niches.<sup>75</sup> This can involve activated platelets that are adherent to the endothelium expressing P-selectin to help tether neutrophils via P-selectin glycoprotein ligand-1 (PSGL1). Platelet-derived factors including PAF can contribute to promoting neutrophil integrin activation and firm adhesion to both the platelets and the endothelium (Figure 6). As well as activated platelets, resting platelets can become attached to inflamed endothelium by binding endothelial P-selectin via platelet PSGL1 or GP1b-V-IX. Platelets in circulation can also attach to adherent neutrophils that are displaying PSGL1.<sup>76</sup> Additional mechanisms of platelet endothelial attachment have been described.<sup>68</sup> Platelet adhesion through the integrin GPIIb/IIIa can promote platelet activation and display of molecules such as CD40L that can in turn cause activation of the endothelium, promoting increased expression of P- and E-selectin, VCAM1 and ICAM1.

Platelets dense bodies or d-granules contain multiple mediators including ATP, ADP, histamine, and serotonin.<sup>68</sup> Platelet a-granules contain many proteins that have activities in cell adhesion and recruitment including von Willebrand factor (vWf), thrombospondin-1, fibronectin, vitronectin, various chemokines—including multiple neutrophil chemoattractants (CXCL1, 5, 8, 12 and βTG that gives rise



**FIGURE 5** Neutrophil association with platelet coated endothelium in the inflamed mouse omentum. Neutrophils (Cell trace violet labeled, blue) in the inflamed omentum of a mouse that has all its platelets labeled green (GFP<sup>+</sup>). Image was acquired by intravital 2-photon microscopy 2 h after intraperitoneal treatment with thioglycolate. The platelets outline the inflamed endothelium, and many intravascular neutrophils are observed in contact with platelet clusters. Neutrophil and platelet labeling and imaging was performed as in a previous work.<sup>9</sup>





**FIGURE 6** Model of early events in neutrophil recruitment to a site of inflammation where mast cell and platelet activation is occurring. Panels highlight some of the key molecular mediators acting in the first minutes to hours of recruitment. A, Mast cells are highlighted as an early source of mediators acting on the endothelium. The extent of their involvement will depend on the tissue and type of stimulus. Other cell types that are not shown such as macrophages will also usually be involved. Serotonin and histamine from mast cells cooperate to promote vascular permeability and Weibel-Palade body mobilization and thus P-selectin display. Other preformed inflammatory mediators such as TNF are also rapidly released. B, These events are quickly followed by platelet activation, attachment to the endothelium, and release of a range of platelet-derived mediators. C, Neutrophils that first associate with the endothelium and platelets via P-selectin-supported rolling are triggered via chemokines to undergo integrin ( $\alpha\text{M}\beta 2$  and  $\alpha\text{L}\beta 2$ ) mediated adhesion to the endothelium. D, Association of adherent, migrating neutrophils with activated platelet clusters is promoted by further  $\text{G}\alpha\text{i}$ -coupled receptors including GPR35 responding to platelet-derived 5-HIAA. Subsequent extravasation is promoted by the concerted action of multiple chemoattractants including chemokines, PAF and 5-HIAA as well as other mediators and adhesion molecules that are not shown. For simplicity the vascular wall is represented by the endothelium; the associated pericytes, smooth muscle cells and extracellular matrix (ECM) are not shown and nor are additional mediators such as IL-17A that act on these cells and proteases that act on the ECM. Image generated using BioRender.

to CXCL7)—and platelet factor 4 (CXCL4), a molecule that can contribute to adhesive processes but does not act as a chemokine.<sup>68,77</sup> Platelets synthesize lipid mediators rapidly following activation, including PAF and thromboxane A2 (Tbxa2) that promotes endothelial activation and platelet aggregation.

Mast cell activation has a long-established role in promoting endothelial permeability and leukocyte recruitment. Mast cells are found in most tissues but are particularly rich in barrier tissues such as the skin and intestine where they reside in close proximity with

blood vessels. As well as their widely known expression of the high affinity IgE receptor and response to IgE-engaging allergens, mast cells can be activated by a range of additional stimuli including C3a, C5a, TLR ligands, ATP, TNF, PAF, endothelin 1, IL-33, cationic drugs, and neurotransmitters.<sup>78,79</sup> Mast cells contain large vesicular reservoirs (granules) of bioactive mediators, including many that are shared with platelets.<sup>80</sup> Mast cell-derived histamine is important in promoting increased endothelial permeability within minutes of mast cell activation (Figure 6). Histamine also mobilizes WPBs and

thereby increases P-selectin levels on the endothelium. Mast cell derived PAF drives later vessel permeability through its actions in promoting neutrophil adhesion and transmigration and via direct actions on the endothelium.<sup>59,81</sup> Leukotrienes, the slow-reacting substances of anaphylaxis, are produced by mast cells and prolong the histamine-induced vascular changes, including mobilization of WBPs.<sup>82</sup> Mast cell-derived CXCL1 and CXCL2 can contribute directly to neutrophil recruitment<sup>83–85</sup> while mast cell TNF can act locally on the endothelium but may operate more prominently in a systemic manner to augment CD11b expression and activation in circulating neutrophils.<sup>62</sup> Excessive levels of mast cell derived CXCL1 in aged tissues has been associated with less efficient neutrophil extravasation due to increased reverse transendothelial migration.<sup>85</sup>

Studies in mast cell deficient or depleted mice have provided evidence that mast cells contribute to neutrophil recruitment to the skin during contact sensitization,<sup>86,87</sup> and to the peritoneum during thioglycolate-induced inflammation and following cecal ligation and puncture.<sup>9,84,88</sup> Most recently, mast cell-derived IL-17A was shown to promote neutrophil breaching of the pericyte layer of TNF-exposed cremaster muscle endothelium through local enrichment of ICAM1 and CXCL1.<sup>89</sup> As well as using mast cell deficient bone marrow chimeric mice, this work used mice with conditional deletion of IL-17A in mast cells (Mcp5-Cre x IL-17A<sup>fl/fl</sup>). While the transmigration step was suggested to be unaltered in this study, the authors reported a non-statistically significant 40% reduction of transmigrated neutrophils in the absence of mast cells.<sup>89</sup> These data suggest that mast cells can promote both neutrophil transmigration and subsequent pericyte layer breaching in TNF-inflamed cremaster muscle vessels.

Mast cells are the hematopoietic cell type expressing the highest amounts of *Tph1* in mice (Immgen.org) and they have long been known to make serotonin.<sup>57,65,90</sup> However, the relative importance of mast cell derived serotonin has not been well-defined. A microscopy study of the delayed type hypersensitivity (DTH) reaction showed that skin mast cells released their serotonin in an early phase of the response.<sup>58</sup> Some studies have indicated that rodent and human mast cells can achieve differential release of serotonin and certain other mediators depending on the type of activating stimulus<sup>91–93</sup> and connective tissue (eg, skin) mast cells have higher stores of serotonin than mucosal (eg, intestinal) mast cells.<sup>79</sup> While serotonin has prominent effects on endothelium as noted above, in particular promoting vascular permeability, it has not been straightforward to resolve between mast cell-derived and platelet-derived serotonin in the context of vascular actions, though contributions by both have been suggested.<sup>94</sup> Treatment with methysergide, a serotonin and  $\alpha$ -adrenergic receptor antagonist, inhibited early vascular permeability increases following skin treatment with a mast cell activator.<sup>59</sup> In an oral allergen-induced diarrhea model, serotonin and PAF both appeared to function downstream of mast cells to promote the intestinal response.<sup>95</sup> The propensity of mast cells to take up serotonin via SERT adds to the complexity of the system.<sup>96</sup> In a study where *Tph1* was conditionally removed from mast cells using *carboxypeptidase-3* driven Cre recombinase, there was a reduction in serotonin

in adipose tissue without a change in plasma serotonin suggesting minimal mast cell uptake of serotonin from plasma in this setting.<sup>97</sup>

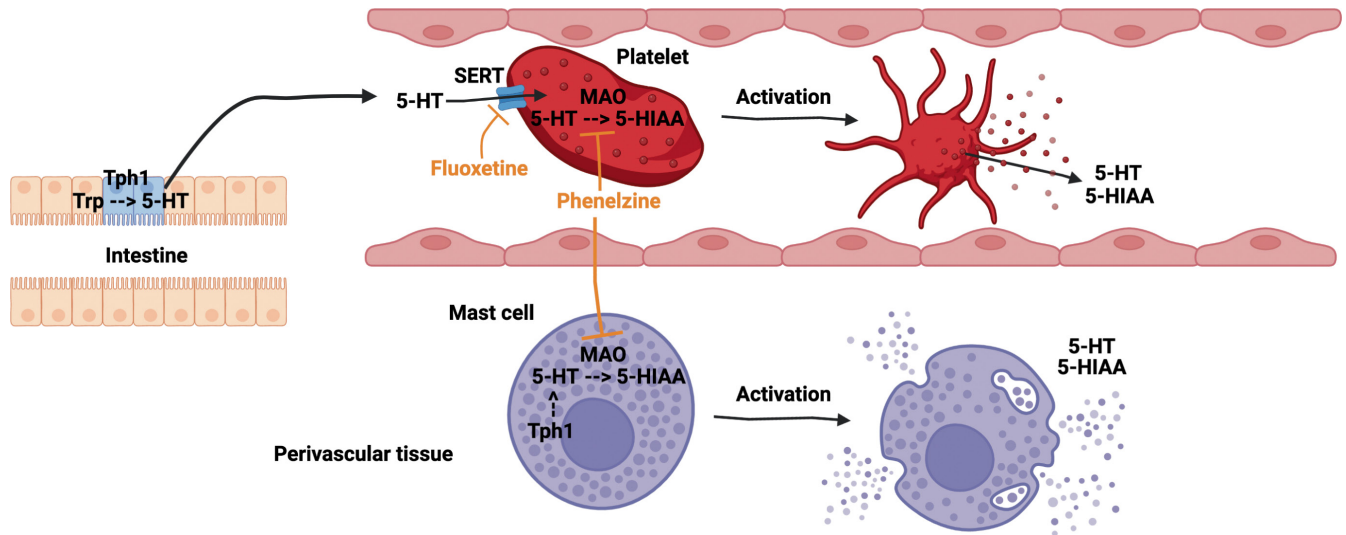
While serotonin production by murine mast cells is well-established, production by human mast cells was initially questioned.<sup>65</sup> However, later work showed serotonin in the supernatant of human mast cells in amounts within 5-fold of those produced by mouse mast cells.<sup>78,98,99</sup> A proteomic study revealed that MAO-B was one of the proteins most enriched in human and mouse mast cells compared with non-mast cells.<sup>63</sup> Moreover, many patients with mastocytosis had elevated serum serotonin, and patients with urticaria pigmentosa (a condition of elevated mast cell activity) had increased circulating 5-HIAA.<sup>99,100</sup> Based on these findings we consider it likely that human mast cells will be physiologically relevant sources of serotonin and 5-HIAA at sites of inflammation.

Communication between mast cells and platelets has been demonstrated in several studies. In recent work it was found that microvascular platelet retention following a surgical procedure led to activation of perivascular mast cells. Mast cell-deficient mice were protected from a platelet activation-mediated drop in body temperature. Platelet aggregates formed near perivascular mast cells and platelet derived PAF mediated mast cell activation.<sup>101</sup> Platelet-mast cell crosstalk at sites of inflammation may lead to stronger recruitment of circulating immune cells.

## 6 | GPR35 FUNCTION IN NEUTROPHILS MAY BE LINKED TO PLATELETS AND MAST CELLS VIA 5-HIAA

Platelets contribute to the GPR35-dependent recruitment of neutrophils to sites of inflammation.<sup>9</sup> These data indicated that, despite the very large number of neutrophil stimulating and attracting agents that can be released by activated platelets, platelet-derived GPR35 ligand(s) such as 5-HIAA make a major non-redundant contribution in some inflammatory settings and likely in certain time windows during the inflammatory process. A loss of GPR35 function during neutrophil recruitment was also observed in mice chronically treated with the SERT inhibitor fluoxetine (Figure 7) or genetically deficient in SERT, findings consistent with platelets being a required source of 5-HIAA since they are thought to be the only cell type fully dependent on SERT to generate 5-HIAA.<sup>38</sup> However, it must be acknowledged that treatment with SERT inhibitors or deficiency in SERT leads to major changes in serotonin abundance and these changes may contribute to the loss of GPR35-dependent recruitment.

In agreement with studies showing mast cells can be a source of 5-HIAA, peritoneal mast cells released 5-HIAA on activation and they could promote GPR35-dependent chemoattraction.<sup>9</sup> Inhibition of MAO by treatment with phenelzine led to reduced GPR35-mediated recruitment of neutrophils, consistent with a contribution by both platelets and mast cells to production of inflammatory 5-HIAA and possibly other undefined GPR35 ligands. BM chimeras lacking *Tph1* in hematopoietic cells also showed reduced GPR35-dependent recruitment.<sup>9</sup> Since platelet serotonin



**FIGURE 7** Diagram highlighting sites of action of serotonin and 5-HIAA modulating drugs. Tph1-expressing enterochromaffin cells in the intestine (shaded blue) are a major source of serotonin (5-HT). Gut-derived serotonin that reaches circulation is taken up by platelets via the serotonin-selective reuptake transporter (SERT). Serotonin is stored in platelet dense granules but also metabolized via MAO to 5-HIAA. Mast cells express Tph1 and produce serotonin and they also metabolize it to 5-HIAA. Activated platelets and mast cells release both serotonin and 5-HIAA (along with many other metabolites, proteins, and lipids that are not shown). Fluoxetine (Prozac™) inhibits SERT, and chronic treatment depletes platelets of serotonin and 5-HIAA. Phenelzine inhibits MAO and thus prevents conversion of serotonin into 5-HIAA in platelets and mast cells. Image generated using BioRender.

(and thus 5-HIAA) is derived from radiation resistant ECCs, the effect of hematopoietic Tph1-deficiency may reflect the loss of 5-HIAA from mast cells. Future studies in mice conditionally lacking Tph1 in mast cells will be needed to reach more definitive conclusions regarding hematopoietic sources of 5-HIAA. However, it must again be recognized that loss of Tph1 will additionally cause a loss of serotonin and its associated actions on the vasculature, tissue and possibly immune cells.

Keeping the limitations of *in vivo* tools to manipulate 5-HIAA abundance in mind, our current working model is that platelet derived 5-HIAA promotes integrin-mediated adhesion of migrating neutrophils in association with platelet clusters, while mast cell-derived 5-HIAA cooperates with other factors to promote directed migration of neutrophils from the vessel into the tissue (Figures 6 and 7). As we discussed above, several studies have established direct crosstalk between platelets and mast cells at sites of inflammation, with PAF being one molecule of significance in this cross talk.<sup>59,101</sup> Such platelet-mast cell bi-directional communication may contribute to forming pro-recruitment gradients of multiple mediators including PAF and 5-HIAA. Since mast cells express SERT as well as Tph1 and MAO, it seems possible that part of this communication could be delivery of platelet-derived serotonin to mast cells for generation of additional 5-HIAA.

Kaya et al<sup>11</sup> reported that LPA injection in zebrafish could mediate recruitment of macrophages in a GPR35-dependent manner. However, whether this involved direct action of LPA on GPR35 was unclear as it is possible that the LPA injection led to changes in other cell types, such as activation of LPAR-expressing mast cells.<sup>102</sup>

The strong expression of GPR35 by monocytes ([Immgen.org](http://Immgen.org)) and the ability of KynA to promote GPR35-dependent monocyte adhesion *in vitro*<sup>26</sup> makes it likely that GPR35 and 5-HIAA will contribute to monocyte as well as neutrophil recruitment *in vivo*. Deletion of GPR35 in CX3CR1<sup>+</sup> macrophages was associated with more severe dextran sodium sulfate (DSS)-induced colitis and this was suggested to reflect a role for the receptor in promoting TNF expression in macrophages<sup>11</sup>; whether it had a role in their migration within the intestine was not determined though the reduced macrophage recruitment to an injection site in GPR35-deficient Zebrafish is consistent with a pro-migratory role.<sup>11</sup> Like neutrophils, monocytes have been observed to interact with platelets during vascular adhesion and transmigration.<sup>68</sup>

Beyond platelets and mast cells, additional immune cell types may contribute to 5-HIAA production at sites of inflammation although the data here are sparse. Macrophages can express SERT and MAO-A ([Immgen.org](http://Immgen.org)) and they can take up serotonin and generate 5-HIAA.<sup>103</sup> Several other immune cell types including T cells, ILC2s and DCs have been suggested to have the machinery to generate serotonin but further study is needed to establish if these cells are a relevant source of 5-HIAA.<sup>104,105</sup> We also note that GPR35 is expressed in immune cell types beyond neutrophils and monocytes, including DCs and eosinophils ([Immgen.org](http://Immgen.org)). It will be of interest in future studies to define the contexts in which 5-HIAA and GPR35 act to direct the migration and function of these and other immune cell types. A complete understanding of how this ligand-receptor system acts to regulate immune cell behavior can be expected to advance the understanding of the disease conditions that are impacted by GPR35 expression.

## 7 | GPR35 FUNCTIONS IN OTHER TISSUES

A study on adipose tissue and energy expenditure found that GPR35-deficiency compromises exercise-induced adipose tissue browning in mice.<sup>106</sup> The study suggested GPR35 was acting in adipocytes though the expression of GPR35 by adipocytes was not reported. An alternative possibility is that GPR35 was acting in adipocyte-associated immune cells such as macrophages. The work showed that KynA increased energy expenditure and it was suggested that KynA was acting via GPR35.<sup>106</sup> However, no experiments were performed where KynA production in vivo was prevented to definitively establish that the GPR35-dependent events were KynA dependent.

Genetic studies have provided evidence for a linkage between GPR35-associated single nucleotide polymorphisms (SNPs) and both inflammatory bowel disease (IBD) and primary sclerosing cholangitis (PSC, bile duct inflammation).<sup>107-110</sup> A study in mice showed that GPR35-deficiency exacerbated DSS-induced colitis.<sup>111</sup> One mechanism by which GPR35 might protect from colitis is by promoting mucosal repair. In vitro, GPR35 agonists promoted mouse colonic epithelial cell migration and more rapid repair of a damaged monolayer.<sup>112</sup> This response appeared to be Gαi-mediated, consistent with a direct pro-migratory role for GPR35 in epithelial cells. However, it is unclear if the small molecules used in this study are agonists for mouse GPR35,<sup>12</sup> or what range of off-target effects they may have, making it important to revisit the findings with GPR35-deficient epithelial cells.

Contrary to the above findings, Zheng et al<sup>113</sup> found GPR35-deficiency led to lower susceptibility to DSS colitis and in another report<sup>114</sup> GPR35-deficiency had no effect on the DSS response. The strong expression of GPR35 by intestinal epithelium and myeloid cells and the genetic association of GPR35 SNPs with IBD make it highly likely that GPR35 will influence gut physiology. However, the basis for the discrepant findings in the DSS studies is unclear, though it is known that the DSS colitis model is variable across facilities. One possibility is that properties of the microbiome influence gut GPR35 function. In this regard, it is intriguing that GPR35 was suggested to be a target of enterotoxigenic *Bacteroides fragilis* toxin (BFT), with GPR35-deficient mice being more resistant to toxin-induced colitis.<sup>115</sup> As noted above, the microbiome may also be a diet-influenced source of 5-HIAA.<sup>54</sup>

Most recently, Melhem et al<sup>32</sup> found that epithelial GPR35 expression in the intestine was highest in goblet cells in the proximal colon. GPR35-deficiency in epithelial cells led to increased pyroptosis of proximal colon goblet cells, reduced mucin production and more proximal association of bacteria with the colon. Moreover, there was an increased bacterial burden in the colon and a shift in the microbiome. These alterations were associated with a reduced ability to restrain growth of the colonic pathogen *Citrobacter rodentium* and spread to mesenteric lymph nodes and liver.<sup>32</sup> Mice lacking GPR35 in epithelium also suffered worse DSS-induced colitis. The source of ligand and the nature of the ligand

needed to protect proximal colon goblet cells from pyroptosis has not yet been defined.

An association between GPR35 SNPs and coronary artery calcification has been reported.<sup>116</sup> GPR35 is expressed by some types of endothelium in mammals and fish<sup>117-119</sup> and by vascular smooth muscle cells.<sup>118</sup> In vitro, GPR35 synthetic agonists promoted more rapid healing in the scratch-wound assay, and this was blocked by GPR35 antagonists.<sup>118</sup> A study in GPR35-deficient mice showed that GPR35 contributes to angiotensin-II (AngII) induced hypertension and cardiac dysfunction.<sup>117</sup> The mechanism for this effect was unclear but it was suggested that GPR35 signaling via Gα13 and Rho in endothelial and myocardial cells was involved. Since the hypertension and cardiac dysfunction emerge over 2 weeks of AngII treatment, it was also possible that GPR35 contributed to disease development indirectly through actions on immune cells. Notably, studies in the brain have shown that AngII can act via the AT1 receptor to increase 5-HIAA as well as dopamine metabolites.<sup>120</sup> Thus, serotonin turnover may be under the influence of AngII, providing a possible connection between AngII and GPR35.

GPR35 expression has been reported in rat TRPV1<sup>+</sup> sensory neurons in the dorsal root ganglion (DRG).<sup>121,122</sup> Analysis of recently published scRNAseq data shows GPR35 is broadly expressed in peripheral DRG sensory neurons while being minimally expressed in the CNS<sup>123</sup> though expression in cortical astrocytes and in hippocampal neurons has been reported.<sup>124,125</sup> GPR35 agonists (KynA, Zaprinast) were able to antagonize prostaglandin E2-induced depolarization of resting membrane potential in cultured rat DRG neurons, and in vivo treatment with the agonists induced analgesia.<sup>122</sup> GPR35 signaling in DRGs was Gαi-mediated.<sup>121</sup> Future work needs to determine whether GPR35-regulates pain sensitivity in response to 5-HIAA at sites of inflammation.

Beyond conventional signaling via heterotrimeric G-proteins, GPR35 may associate with and signal via the Na/K-ATPase pump.<sup>114</sup> In the absence of GPR35, bone marrow-derived macrophages and epithelial cell lines appeared to have a disrupted electrochemical gradient and elevated basal Ca<sup>2+</sup>. Further study will be needed to understand how universal this finding is, though the observation that the response of GPR35-deficient cells to other GPCR ligands was defective due to the altered electrochemical gradient<sup>114</sup> did not extend to studies of GPR35-deficient neutrophils.<sup>9</sup> Schneditz et al also showed that GPR35-deficiency led to a reduced incidence of colorectal cancer in mouse models, and GPR35 was suggested to have an intrinsic role in promoting epithelial cell turnover. A subsequent study by this group showed a similar reduction in tumor burden when GPR35 was removed from macrophages and neutrophils using LysM-Cre.<sup>126</sup> The tumor-promoting mechanism in this case was suggested to be through induction of angiogenesis, with GPR35-deficient macrophages having less angiogenic activity. The ability of GPR35 to influence macrophage production of angiogenic factors was thought to be occurring in the absence of ligand, perhaps due to tonic effects on the Na/K-ATPase. However, the culture studies were performed in 10% fetal bovine serum, a source of 5-HIAA,<sup>9</sup> and

thus in the presence of amounts of GPR35 ligand(s) that may have influenced the outcome of the assays. In another work, GPR35 promoted monocyte transendothelial migration in response to macrophage conditioned medium containing 10% fetal bovine serum.<sup>126</sup> However, whether GPR35 modulates transmigration and parenchymal positioning of tumor-promoting myeloid cells in vivo remains to be determined. Intriguingly, pharmacological inhibition of serotonin has recently been associated with enhanced immune checkpoint blockade therapy,<sup>127</sup> raising the question of whether the GPR35-5-HIAA axis plays a role in the success of such therapies.

In another example of non-conventional GPCR signaling, GPR35 was found to account for KynA-mediated protection of cardiac tissue from ischemia. KynA engagement of GPR35 caused its trafficking to intracellular membranes including the outer mitochondria membrane, and it altered ATP synthase inhibitory factor subunit 1 (ATPIF1) activity, to prevent ATP loss upon ischemia.<sup>13</sup> While this study elegantly showed the effect of GPR35 on ATPIF1 function, it was unclear whether this G $\alpha$ i-mediated effect depended on GPR35 signaling at the plasma membrane vs the mitochondrial membrane. Impressively, this report used purified GPR35 protein to show direct binding of radiolabeled KynA to the receptor. However, the work aimed to show the protective action of exogenously administered KynA, and it did not demonstrate a protective effect of GPR35 against ischemia-mediated tissue damage in the absence of administered GPR35 agonists.<sup>13</sup> Thus, it is unclear whether GPR35 ligands are produced in sufficient amounts physiologically to have a protective effect against ischemia-induced injury.

Defining the in vivo sources of functionally relevant GPR35 ligand(s) remains an important challenge for the field if we are to understand how GPR35 activity is modulated in each of the functional contexts discussed above, including during tumorigenesis.

## 8 | CONCLUDING REMARKS

The role of GPR35 in neutrophil recruitment to sites of inflammation reveals a new example of how activated platelets and mast cells contribute to inflammatory cell recruitment. More work is needed to understand how the multiple mediators produced by these cell types cooperate to promote extravasation of neutrophils and other inflammatory cell types. New techniques of spatial metabolite detection will be needed to examine 5-HIAA distribution and to determine if gradients form across the endothelium. As well as promoting extravasation of GPR35-expressing cells, we anticipate that GPR35 will contribute to inflammatory cell migration within tissues. The possible role of GPR35 in priming neutrophils for activation needs investigation. Moreover, we anticipate that additional GPR35 expressing cell types will be recruited in response to 5-HIAA. As we have noted, while 5-HIAA is the most potent endogenous GPR35 ligand so far identified, it has low efficacy in vitro and this together with the ability of GPR35 to respond to

high concentrations of multiple metabolites makes it important to consider the possibility that further physiological ligands will be identified, perhaps including diet- and microbiome-derived metabolites. Moreover, novel approaches will be needed to definitively establish the selective contribution of 5-HIAA to GPR35 function in vivo. Should an enzyme that selectively metabolizes 5-HIAA be identified, over-expression of this enzyme at sites of inflammation could be one such approach.

The discoveries that 5-HIAA can signal via GPR35 and AHR points to a need to revisit some findings regarding serotonin actions. This need arises because most perturbations used to study serotonin biology—analysis of Tph1-deficient mice or treatment with SERT inhibitors—lead to reductions in 5-HIAA as well as serotonin. Treatment with MAO inhibitors leads to both increased serotonin and reduced 5-HIAA levels. With this complexity in mind, many questions arise. As already detailed, the altered recruitment of neutrophils in Tph1-deficient and fluoxetine treated mice<sup>9,43</sup> may be a consequence of reduced 5-HIAA engagement of neutrophil GPR35 as well as reduced serotonin engagement of endothelial serotonin receptors. The microbiome plays a prominent role in colitis, and it can produce 5-HIAA. Given the abundant GPR35 expression in the intestinal epithelium and the association of GPR35 SNPs with colitis, perhaps 5-HIAA-GPR35 crosstalk has a direct role in maintaining intestinal homeostasis. Could it be that some of the pleiotropic intestinal functions ascribed to serotonin are secondary to its conversion to 5-HIAA and action via GPR35? Serotonin also has pleiotropic actions on neurons. It seems likely that 5-HIAA will contribute to a subset of these ascribed actions, especially in GPR35<sup>+</sup> peripheral DRG sensory neurons. In this regard it is notable that 5-HIAA was found to modulate pain sensitivity in an adjuvant-induced inflammation model.<sup>128</sup>

Finally, we suggest that the deep understanding of therapeutic strategies to target the serotonin pathway can be leveraged to target the 5-HIAA-GPR35 system. For example, by taking advantage of MAO inhibitors that are preferentially active in the periphery,<sup>129</sup> it may be possible to manipulate GPR35<sup>+</sup> myeloid cell recruitment in various disease contexts including peritonitis, septic shock, arthritis, lung infection, and cancer.

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## CONFLICT OF INTEREST STATEMENT

To the best of their knowledge, the authors have no financial or personal relationships that could be viewed as a conflict of interest.



## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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