

Reduced number of epithelial tuft cells and density of LGR5 expression in the myenteric plexus of the ageing human colon

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ABSTRACT

Background: Tuft cells are specialised chemosensory epithelial cells involved in mucosal immunity and epithelial homeostasis, while leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) regulates Wnt signalling, essential for post-mitotic neuronal maintenance. However, within the human colon the impact of ageing on tuft cell abundance in the mucosa and LGR5 distribution in the ganglionic area remains poorly characterised. The aims of this study were to quantify tuft cell populations in the adult human colonic epithelium and assess LGR5 presence within the ganglionic area.

Methods: Colonic tissue samples were obtained from younger adults (Ascending colon: AC, $n = 6$, aged 30–52 years; Descending colon: DC, $n = 10$, aged 42–60 years) and older adults (AC: $n = 12$, aged 70–89 years; DC: $n = 5$, aged 67–82 years). Immunolabelling was performed on formalin-fixed, paraffin-embedded sections using antibodies against cyclooxygenase-1 (COX-1) to identify tuft cells within epithelial crypts and LGR5 presence within the myenteric ganglia.

Results: Tuft cells exhibited a characteristic flask-shaped morphology with intense apical COX-1 staining. Their numbers per crypt and also per mucosal area were higher in younger adults compared with older adults (respectively, mean numbers per mm^2 mucosal area: AC, 109.7 ± 8.9 vs. 44.9 ± 2.1 ; DC, 103.2 ± 5.1 vs. 58.6 ± 3.7 ; $P < 0.05$ each), with a more pronounced decline in the AC. LGR5-positive structures were consistently observed within myenteric ganglia located between the circular and longitudinal muscle layers in both colonic regions. Compared with younger adults LGR5-positive structures within the ganglia was markedly reduced in the ageing AC (younger: $0.63 \pm 0.07 \times 10^{-3}$, older: $0.26 \pm 0.03 \times 10^{-3}$ per ganglionic area; $P < 0.05$) but not clearly in the DC (younger: $0.83 \pm 0.05 \times 10^{-3}$, older: $0.58 \pm 0.05 \times 10^{-3}$ per ganglionic area; $P > 0.05$).

Conclusion: In both regions of human colon, ageing reduced tuft cell abundance in the mucosal epithelium. Ageing also reduced LGR5-IR structures within the ganglionic area in the AC. These structural changes may contribute to age-dependent impairments of neuromodulatory functions of the epithelium and the mechanisms for maintaining myenteric ganglion homeostasis.

1. Introduction

In recent years, there has been a growing interest in understanding the structural and functional changes that occur within the adult human colon during the ageing process (Baidoo and Sanger, 2025a; Hofmann, Geiger, 2026). In terms of generating symptoms, these are thought to lower the threshold at which intestinal motility and mucosal functions are reduced by other age-associated changes (e.g., use of medications, reduced exercise, altered diet and microbiome, and bowel disorders),

which together (see Baidoo and Sanger, 2025b), increases the prevalence of disorders such as constipation, faecal impaction (Serrano Falcón et al., 2016), and incontinence (Pasricha and Staller, 2021). If left untreated, these conditions can lead to serious complications, including intestinal perforation (Sommers et al., 2019).

In the largest study to date, which has investigated age-related changes in functions of the adult human colon, a decline in myenteric cholinergic function was observed within the proximal but not distal colon (Broad et al., 2019). Subsequently, a loss of afferent innervation

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function was observed in human ascending and descending/sigmoid colon (Cibert-Goton et al., 2020). By contrast, no age-related changes were found in functional studies within the mucosa of the human small and large intestine (basal resistance and short-circuit current, and current evoked by neuronal stimulation; Krueger et al., 2016). However, several recent structural studies have found age-related structural changes within different sublayers of the adult human colon, including the mucosa (see Baidoo et al., 2025). For the latter, these include a loss of goblet cells throughout the colon (Baidoo and Sanger, 2024), a decline in specific gap junction proteins within the mucosal epithelium of the ageing ascending but not descending colon, and an increase in the amount of elastin fibres within the muscularis mucosae throughout the colon (Baidoo et al., 2025). Such changes could compromise the mucosal barrier and thereby expose the intestinal wall to potentially noxious or allergenic substances within the lumen (see Baidoo and Sanger, 2025a, for discussion).

Several different types of cells are embedded within the colonic epithelial layer. They include the tuft cells. First described seven decades ago (Rhodin and Dalhamn, 1956), tuft cells are post-mitotic epithelial cells derived from intestinal stem cells expressing leucine-rich repeat containing G protein-coupled receptor 5 (LGR5) (Gerbe et al., 2011). In addition to serving as a source of endogenous intestinal β -endorphin, tuft cells are currently recognised as the only epithelial cell type within the human colon that selectively expresses cyclooxygenase-1 (COX-1), making this enzyme a valuable marker for their identification (Kjærgaard et al., 2021). Through their expression of sensory receptors and production of biologically active mediators (Höfer et al., 1996; Hass et al., 2010; Howitt et al., 2020), tuft cells facilitate the detection of luminal stimuli and coordinate communication between the intestinal epithelium and the mucosal immune system. Consequently, impairment or loss of tuft cell function may compromise epithelial sensing of luminal contents, reduce interleukin-25-mediated activation of innate lymphoid cells, impair epithelial repair and regeneration, and weaken mucosal barrier integrity (see Sipos and Múzes, 2024). Such disturbances have the potential to disrupt immune surveillance, alter host-microbiota interactions, and diminish protective responses to environmental challenges. Consistent with these functions, alterations in tuft-cell abundance or activity have been implicated in a range of gastrointestinal disorders (see Hendel et al., 2022), including quiescent ulcerative colitis, where reduced tuft-cell numbers have been reported (Kjærgaard et al., 2021), as well as coeliac disease and duodenal ulceration (Huh et al., 2020). Furthermore, tuft cells have been identified as key early responders to parasitic infections, initiating type-2 immune responses that contribute to host defence and the maintenance of mucosal homeostasis (Howitt et al., 2016). However, the distribution of tuft cells in the human ascending and descending colon remain poorly characterised and it is unclear whether the natural ageing process similarly affects their abundance and function. Addressing this knowledge gap is critical for understanding the potential therapeutic value of modulating tuft cell populations to support epithelial barrier integrity and homeostasis.

LGR5 expression has been identified in several neuronal populations within the central nervous system (Sukhdeo et al., 2014; Müller et al., 2014), indicating that its biological functions may extend beyond its established role in epithelial stem cell maintenance. Of particular interest is the association between LGR5 and Wnt signalling pathways, which play essential roles in synaptic development, neuronal maturation, and the establishment and maintenance of neural connectivity (Yu et al., 2017). As mentioned earlier, tuft cells arise from LGR5-positive intestinal stem cell populations; consequently, any age-related alterations in LGR5-mediated signalling may affect epithelial lineage specification and the differentiation of specialised epithelial cell types, including tuft cells. Collectively, these findings suggest that LGR5 may participate in Wnt-dependent regulatory mechanisms within neural tissues, supporting the possibility that any age-associated changes in LGR5 signalling could exert parallel effects on epithelial homeostasis and the preservation of enteric neuronal integrity.

Despite these insights, the spatial distribution of LGR5 in the enteric nervous system (ENS) of the human colon remain unknown. Accordingly, the aim of this study is twofold. First, to characterise the distribution pattern of tuft cells within the epithelium. For this purpose, we used COX-1 as a validated marker of tuft cells (Gerbe et al., 2011; Schütz et al., 2019; Kjærgaard et al., 2021). The second aim was to investigate the spatial expression and localisation of LGR5 in the human ascending (AC) and descending (DC) colon. This comparative approach is particularly important due to the distinct physiological functions of the different regions and their differing vulnerabilities to increasing age (Phillips, 1984; see Baidoo and Sanger, 2025b for recent references and discussion). Such understanding could help inform the ageing-related pathology of the human colon (Short et al., 2025) and the development of targeted therapeutic strategies for lower bowel disorders.

2. Method

2.1. Human tissue specimens and subject selection

The colonic tissue samples used in this study have been previously described in detail (Baidoo et al., 2026). In brief, macroscopically normal, full-thickness specimens of ascending and descending colon were obtained from adult patients undergoing surgical resection for non-obstructive bowel cancer. Tissue samples were collected from regions located at least 5–10 cm away from the tumour margin. Data for tumour site and stage distributions were not available for all archival specimens and were therefore not included. The cohort comprised younger adults (ascending colon: $n = 6$, aged 30–52 years; descending colon: $n = 10$, aged 42–60 years) and older adults (ascending colon: $n = 12$, aged 70–89 years; descending colon: $n = 5$, aged 67–82 years; see Supplementary Table 1). All participants provided informed written consent prior to tissue collection. None of the individuals included in the study had a history of chemoradiotherapy, active inflammatory bowel disease, diverticulitis, or functional bowel disorders. Tissue samples were prospectively collected and processed in accordance with ethical guidelines and approval from the East London Research Ethics Committee (REC: 10/H0703/71), the London City Road and Hampstead Research Ethics Committee (REC: 15/LO/21/27), and subsequently from the University of Westminster Research Ethics Committee (Reference: ETH2324–1489).

2.1.1. Tissue processing and histological assessment

Tissues were fixed in formalin and paraffin-embedded transversely, as previously described (Broad et al., 2019; Baidoo et al., 2023a). Prior to immunohistochemical analysis, all sections were examined for structural integrity and potential abnormalities using haematoxylin and eosin (H&E) staining (Lamb et al., 2019). No structural abnormalities were observed in any of the assessed colonic tissues.

2.1.2. Immunofluorescence

Immunofluorescence was manually performed on thin sections as previously described (Baidoo and Sanger, 2024; Baidoo et al., 2025). Following antigen retrieval using heat-induced epitope retrieval in Universal HIER reagent (ab208572, Abcam, Cambridge, UK), non-specific binding was blocked with Protein Block (ab64226, Abcam, Cambridge, UK) for 30 min at room temperature. Primary antibodies (rabbit monoclonal COX-1 (ab109025) and rabbit polyclonal LGR5 (ab219107)) were diluted in universal antibody diluent (ab79995, Abcam, Cambridge, UK), applied to tissue sections, and incubated overnight at room temperature. Fluorescent detection was achieved using fluorophore-conjugated secondary antibodies (sheep anti-rabbit, ab50505; donkey anti-rabbit IgG, ab150075; diluted in 1:1000) in antibody diluent. Sections were counterstained with DAPI (ab104139, Abcam, Cambridge, UK). Positive and negative controls were included using colonic tissue with or without primary antibodies. Non-specific structures were counterstained with DAPI (4',

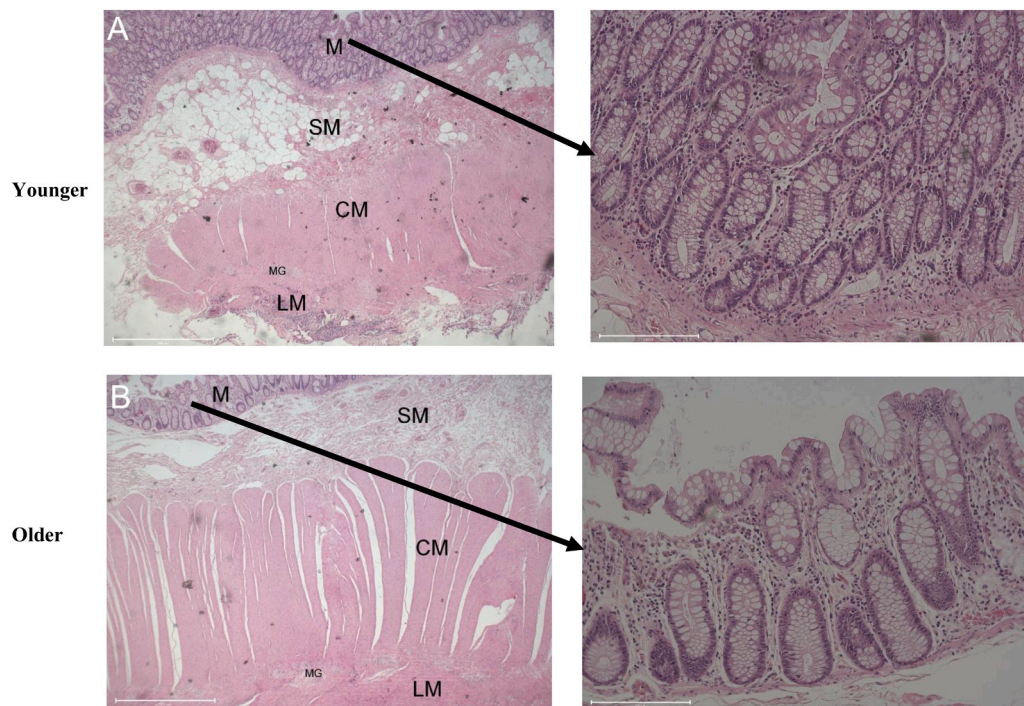


Fig. 1. Haematoxylin and eosin-stained sections of human colonic tissue from younger and older adults. Representative transverse sections illustrate normal histological architecture in younger (Panel A) and older (Panel B) adults. The mucosa (M) displays intact crypt organisation with a well-preserved epithelial lining, lamina propria and muscularis mucosae, with no overt abnormalities when assessed at a higher magnification (arrows; scale bar: 200 μ m). The submucosa (SM) and muscularis externa demonstrate normal connective tissue composition and smooth muscle arrangement, without evidence of inflammation, fibrosis, or other pathological change. The myenteric ganglion (MG) is located between the circular muscle layer (CM) and the longitudinal muscle layer (LM). Scale bar: 1000 μ m.

6-diamidino-2-phenylindole; ab104139, Abcam, Cambridge, UK).

2.1.3. Image acquisition and quantitative analysis

Images of immune-stained sections were acquired as previously described (Baidoo and Sanger, 2024) using an EVOS FL Auto 2 fluorescence microscope (Washington, USA) equipped with a digital camera. Briefly, sequential images were captured to encompass at least 80% of the tissue area, covering the mucosa from the apical epithelial surface to the crypt base, including the lamina propria and muscularis mucosae, across the full width of each tissue section from left to right. Individual COX-1-positive cells were counted directly under the microscope and also via digital analysis per crypt and per mm^2 of the mucosa. For each section, the entire available mucosal region was systematically examined; therefore, analyses were based on crypt counts rather than a predetermined number of microscopic fields. On average, a minimum of 15 crypts was analysed for each patient.

Colonic tuft cells were defined as COX-1-positive cells located within the crypt. Quantitative estimation adhered to stringent criteria: (1) Tuft cells exhibited distinct red fluorescence covering $\geq 50\%$ of the cell membrane and/or cytoplasm. (2) Overlapping or continuous regions of red staining containing two distinct nuclei were counted as two separate cells.

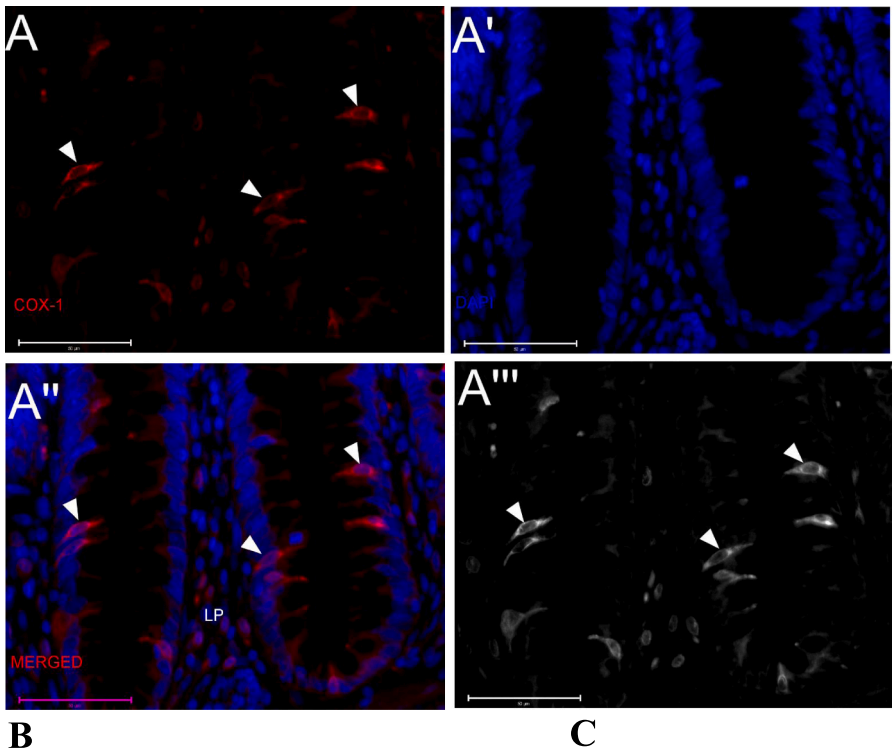
2.1.3.1. Analysis of LGR5-positive structures. LGR5-immunoreactive (IR) structures within myenteric ganglia were analysed following the presence and identification of the myenteric plexus (MP) using H&E-stained sections, as previously described (Veress et al., 2022). The MP was defined as the region located between the circular (CM) and longitudinal (LM) muscle layers. Fluorescent images of myenteric ganglia throughout each tissue section were acquired and analysed using ImageJ (version 1.54 g; Schneider et al., 2012). LGR5 immunoreactivity was quantified as the LGR5-immunoreactive area fraction, defined as the percentage of total ganglionic area occupied by LGR5-positive pixels. Individual ganglia were identified, and regions of interest (ROIs) were delineated

independently of the LGR5 signal using anatomical landmarks and tissue morphology. The LGR5 fluorescence channel was then thresholded in ImageJ using the Default thresholding algorithm to distinguish specific immunoreactive staining from background fluorescence. Thresholding was performed on an image-by-image basis to account for minor variations in background signal. The area occupied by thresholded LGR5-positive pixels within each ROI was measured and expressed relative to the total ganglionic area.

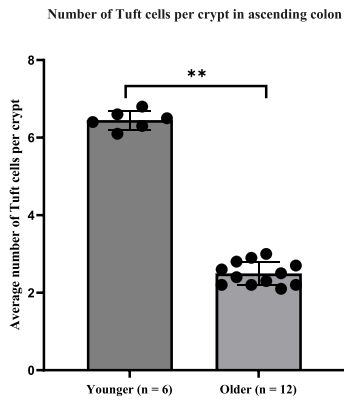
To maintain image integrity and clarity, all digital images were stored in uncompressed Tagged Image File Format (TIFF; RGB; 5.3 MB; 1348×1048 pixels) under identical conditions. Images were captured at $20\times$ magnification, with higher-resolution images acquired at $40\times$ or $60\times$ using automated focus and exposure settings. Sections exhibiting artefacts or poor staining were excluded. When staining artefacts occurred, replacement serial sections from the same tissue block were stained and analysed. No patient was excluded from the final dataset because of staining quality. Sequential imaging ensured systematic analysis of tissue sections, minimising bias from preferential evaluation of regions with low or high numbers of COX-1 positive cells or LGR5-IR structures within the ganglionic area. Each slide contained at least two tissue sections. Data were averaged and analysed separately for younger and older adults, and results were expressed as mean $\pm$ standard error of the mean (SEM).

2.2. Statistics and data analysis

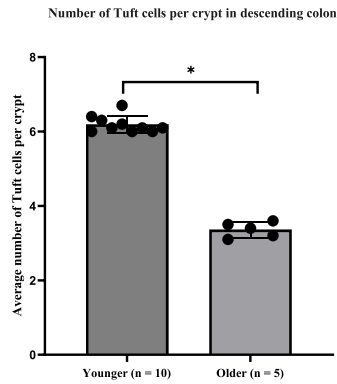
Data normality was assessed using the Shapiro-Wilk test, while homogeneity of variances was examined using Levene's test. The results confirmed that the data did not significantly deviate from a normal distribution and that variance was comparable between groups, thereby supporting the use of parametric statistical methods. The effect of ageing on the distribution of tuft cell populations within the colonic crypt and the area fraction of LGR5-IR structures in the ganglionic area was assessed using a two-tailed independent-samples Student's *t*-test to



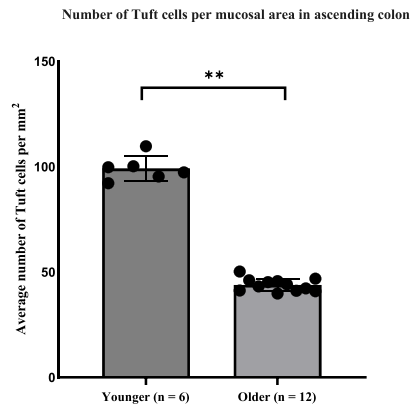
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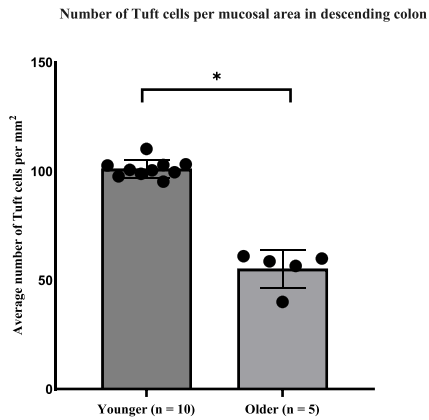
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Fig. 2. Ageing reduces the number of cyclooxygenase-1 (COX-1) immunoreactive (IR) cells in the crypt of human colon. Colonic mucosal sections were subjected to immunofluorescence staining using a rabbit monoclonal anti-COX-1 antibody (ab109025, Abcam, Cambridge, United Kingdom) to identify tuft cells followed by a fluorophore-conjugated secondary antibody (goat anti-rabbit IgG, Alexa Fluor 647; ab150079; Abcam, Cambridge, United Kingdom). Panel A displays COX-1-positive cells (red), while Panel A' shows nuclei counterstained with 4',6-diamidino-2-phenylindole (blue). Panel A'' represents the merged image, demonstrating the localisation of COX-1-IR (red) and nuclei (blue). Thresholding was applied to identify COX-1-positive structures using ImageJ (Panel A'''). Scale bar: 50 μm . Quantification of colonic tuft cells was performed using the Cell Counter tool in ImageJ (version 1.54 g). For each sample, at least 15 crypts were examined, and the mean number of tuft cells per crypt and per mucosal unit area (mm^2) was calculated. Graphs illustrate the effect of ageing on the number of tuft cells per crypt (B and C) and per mucosal area (D and E) in the ascending (AC) and descending (DC) colon. AC (younger adults, $n = 6$; older adults, $n = 12$); DC (younger adults, $n = 10$; older adults, $n = 5$). Data are presented as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$.

compare younger and older age groups. Where comparisons involved more than two groups, a one-way analysis of variance (ANOVA) was performed, followed by Bonferroni-adjusted post hoc testing to account for multiple comparisons. Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA), and graphical representations were generated using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Statistical significance was set at $P \leq 0.05$ for all analyses. Unless otherwise specified, n represents the number of patients included in each group.

3. Results

3.1. Histological assessment of colonic tissue integrity

Haematoxylin and eosin staining was performed on transverse sections of the colon from younger and older adult AC and DC to assess structural integrity and identify potential pathological changes. This revealed well-preserved tissue architecture in both regions across both age groups. The mucosal layer exhibited intact epithelial lining with clearly defined crypts and lamina propria, and the muscularis mucosae appeared continuous without evidence of disruption. No significant histopathological abnormalities were observed in any group. Specifically, there were no signs of active inflammation, cryptitis, ulceration, neoplasia, or increased lamina propria cellularity. The submucosa and muscularis externa were structurally normal, and ganglionic structures within the myenteric plexus were intact. Comparative analysis between younger and older adults showed no age-related differences in overall tissue morphology or organisation in either region of colon. Representative H&E-stained sections from both age groups are shown in Fig. 1, illustrating the absence of structural abnormalities and confirming the suitability of the samples for subsequent immunofluorescence analyses.

3.2. Distribution of Tuft cells

Tuft cells were identified by their distinct COX-1 immunoreactivity, appearing as slender, flask-shaped epithelial cells located predominantly at the base and mid-region of the crypts. They exhibited intense red fluorescence confined to the apical membrane, consistent with a characteristic microvillar tuft morphology. COX-1 positive cells were also found in the lamina propria in the younger and older adults, but these were not included in the quantification. Quantification of COX-1-positive tuft cells was performed both as the number of labelled cells per crypt and per mm^2 of mucosa from the AC and DC of, respectively, younger and older adults. In both the AC (6.6 ± 0.4 vs. 2.3 ± 0.1) and DC (6.3 ± 0.5 vs. 3.6 ± 0.2) of older adults, the number of COX-1-positive cells per crypt was markedly reduced compared with younger adults. A consistent pattern emerged when counts were normalised to mucosal area: older adults exhibited substantially fewer COX-1-positive cells, with reductions of approximately 59% in the AC and 43% in the DC (Fig. 2).

3.3. Expression of LGR5-IR in the myenteric plexus

In younger individuals, LGR5-IR structures were consistently observed within the myenteric ganglia across both regions of colon (Fig. 3). As determined from the area fraction measurements of LGR5-IR

structures within the ganglionic area, these were significantly reduced in the AC of older adults, compared with the younger adults (respectively, $0.26 \pm 0.03 \times 10^{-3}$ vs. $0.63 \pm 0.07 \times 10^{-3}$). In contrast, the DC did not exhibit a clear age-related decline in LGR5-IR area fraction and differences did not reach statistical significance (Fig. 3).

4. Discussion

Despite the clinical importance of understanding age-related factors contributing to lower bowel dysfunction, there remains a paucity of research investigating the impact of ageing on human colonic tuft cells and the presence and function of LGR5 in the myenteric plexus. The present study demonstrates a significant reduction in the number of epithelial tuft cells, identified by COX-1 expression, within the mucosal crypts of both the AC and DC of older adults. In addition, we identified an age-associated reduction in LGR5-IR within ganglionic area of the ascending colon, whereas a trend towards reduced LGR5-IR structures within the descending colon was not statistically significant.

Using immunofluorescence, we reliably identified human colonic tuft cells within crypts using COX-1 antibodies, a validated marker for human tuft cells (Jensen et al., 2018; Kjærgaard et al., 2021), in contrast to murine studies that primarily employ double cortin-like kinase 1 (Gerbe et al., 2012; Howitt et al., 2016). Our finding of fewer COX-1-positive tuft cells within crypts of both colonic regions in older adults is consistent with a decline in a *bona fide* human tuft cell population.

While several inflammatory and infectious conditions are associated with compensatory expansion of tuft-cell populations (e.g. during gastric metaplasia and dysplasia (Jang et al., 2024)), the reduced tuft-cell population observed in the present study may reflect age-related impairments in epithelial renewal, stem-cell function, or differentiation pathways. Consequently, the biological mechanisms underlying tuft-cell alterations in ageing may differ fundamentally from those driving disease-associated tuft-cell expansion.

The presence of COX-1-positive cells within the lamina propria is consistent with previous reports demonstrating constitutive COX-1 expression in resident stromal and immune cell populations (Jackson et al., 2000). These cells were excluded from tuft cell quantification based on their anatomical location and morphological characteristics. Their presence may reflect constitutive prostanoid-generating pathways that could contribute to mucosal homeostasis, including epithelial protection, vascular regulation, and local immune surveillance (Wei et al., 2023). However, as these COX-1 positive cells were not further characterised in the present study, their precise identity and functional significance in the lamina propria of human colon remain to be determined.

Mechanistically, age-related epithelial changes including the presence of inflammaging (Zhang et al., 2023), and shifts in epithelial lineage balance (Daly et al., 2025) may plausibly impair tuft cell differentiation or survival. However, these mechanisms are speculative and were not directly assessed in the current study. Further, single-cell atlases of the ageing mouse colon reveal changes in functionally-specific mucosal cell-types affecting epithelial composition and pro-inflammatory signalling (Sirvinskis et al., 2022), while organoid studies demonstrate age-driven TGF- β -Smad3-p16^{INK4a} activation with impaired epithelial regenerative capacity (Jo et al., 2023). Although these data were obtained from mouse tissues, they could

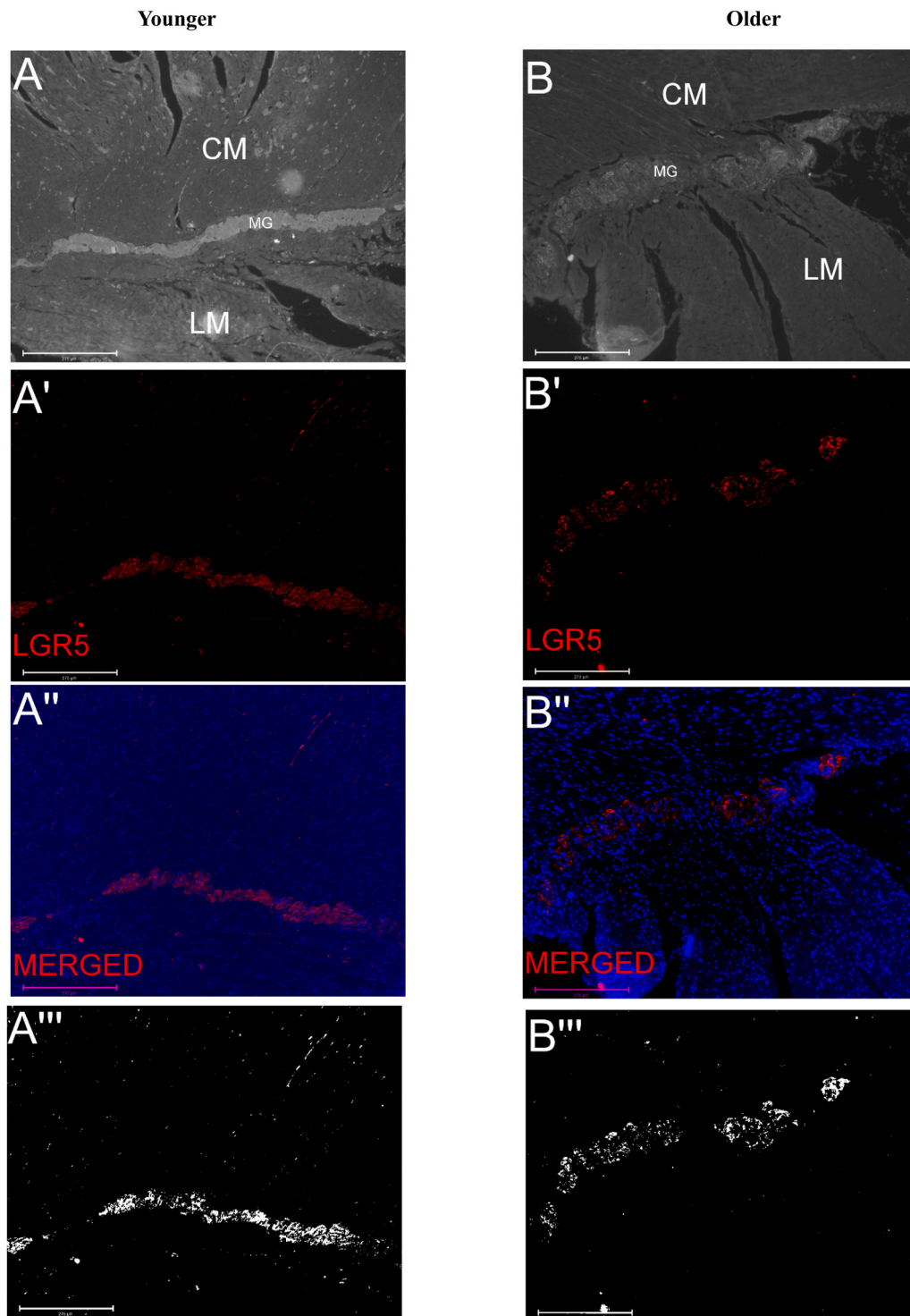


Fig. 3. Immunofluorescent localisation of leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5) within the myenteric plexus of younger and older adult human colon. Representative images from formalin-fixed, paraffin-embedded colonic tissue sections stained for LGR5 are shown for younger (Panels A, A', A'', A''') and older (Panels B, B', B'', B''') adults. Tissue sections were immunolabelled with a rabbit polyclonal anti-LGR5 antibody (ab219107; Abcam, Cambridge, UK), and fluorescent detection was achieved using a fluorophore-conjugated secondary antibody (donkey anti-rabbit IgG; ab150075; Abcam, Cambridge, UK). LGR5-immunoreactivity (IR) is visualised in red (Panels A' and B'), while nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; ab104139, Abcam, Cambridge, UK) and appear as blue. Merged images showing the localisation of LGR5-IR structures relative to nuclei are presented in Panels A'' and B''. Histological images were acquired using a fluorescence microscope (Evos FL Auto 2, Washington, USA) equipped with a digital camera, and subsequently processed using ImageJ (version 1.54 g). Myenteric ganglia (MG) were identified between the circular muscle layer (CM) and longitudinal muscle layer (LM). During analysis, images were thresholded and binarised to remove background fluorescence and isolate LGR5-IR structures within the ganglionic area (Panels A''' and B'''). Area fraction measurements of LGR5-IR within the myenteric ganglia of the ascending (AC) and descending (DC) colon were obtained as the ratio of LGR5-positive pixel area to total ganglionic area. Graphs (Panels C and D) show the effect of ageing on LGR5-IR area fraction within the ganglionic area. A significant reduction in LGR5-IR area fraction was observed in the AC of older adults ($p = 0.023$), whereas the reduction in the DC did not reach statistical significance ($p = 0.079$). AC (younger adults, $n = 6$; older adults, $n = 12$); DC (younger adults, $n = 10$; older adults, $n = 5$). Data are expressed as mean $\pm$ SEM. * $p \leq 0.05$; NS, non-significant. Scale bar: 275 μm .

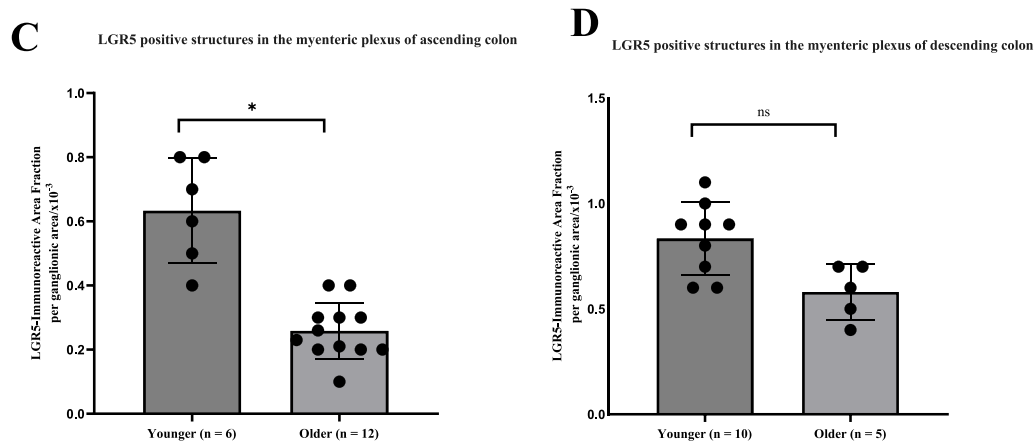


Fig. 3. (continued).

provide pathways through which ageing of the human colon diminishes chemosensory sentinel tuft cells. A reduction in tuft cells may blunt epithelial chemosensation and disrupt, for example the tuft cell–IL-25–innate lymphoid cell circuit (von Moltke et al., 2016), with downstream consequences for barrier integrity, mucosal immunity, and epithelial repair that are processes relevant to infection susceptibility, dysbiosis, and neoplasia risk in older adults. These interpretations align with contemporary reviews highlighting tuft cells as modulators of gut disorders and potential therapeutic targets (see Feng et al., 2024; Sipos and Müzes, 2024; Eshleman et al., 2026).

We also detected LGR5 expression within myenteric ganglia of both ascending and descending colon. Traditionally, LGR5 has been recognised as a marker of crypt base columnar epithelial stem cells in murine tissue (Barker et al., 2007), mediating R-spondin potentiation of canonical Wnt signalling. However, in previous studies, using formalin-fixed paraffin-embedded tissues, LGR5 expression was not found in the crypt base of normal healthy colonic epithelium (Ahmed et al., 2023; Baidoo et al., 2025). Notably, LGR5-IR structures within the intestinal epithelium has been reported predominantly in murine tissues (Barker et al., 2007; Nalapareddy et al., 2017), suggesting the presence of species-specific differences in LGR5 expression between mice and humans. Here, it should be noted that a substantial proportion of native intestinal stem cells are thought to exist in a slowly cycling or quiescent state, which may be associated with reduced translation of active receptors and, consequently, lower detectable levels of LGR5 protein expression (Yamazaki et al., 2015). In the present experiments the human colon was taken from patients with bowel cancer, albeit at a distance away from the tumour and ‘macroscopically normal’. However, the expression of several genes within the mucosa may be impacted by the tumour microenvironment (Sanz-Pamplona et al., 2014). Might the difference between the mouse and human studies relate to the biological state of intestinal stem cells? Further comparative studies are needed.

It has been reported that, Wnt ligands, Frizzled receptors, and Wnt modulators are present in adult murine ENS (Nothelfer et al., 2016; Scharr et al., 2024), suggesting a Wnt-rich milieu compatible with LGR5-related signalling. In addition, other studies revealed neurogenic glial/progenitor populations in human and murine myenteric ganglia (Mueller et al., 2024), supporting the concept of progenitor-like cells within ENS niches. Our findings extend the emerging picture of Wnt/R-spondin-sensitive programmes within myenteric circuits to include LGR5 immunoreactivity, although its functional identity (neuronal, glial, or progenitor) in human ENS requires further validation. If LGR5 in myenteric ganglia has a role in progenitor-like programmes or adaptive plasticity, age-dependent attenuation could translate into reduced regenerative capacity or altered neuromodulation, predisposing to dysmotility. Indeed, the detection of dysfunctional enteric cholinergic neurons and the existence of

post-mitotic cellular senescence-like activity within the myenteric plexus of the human ascending colon (Broad et al., 2019; Palmer et al., 2021) would be consistent with this hypothesis. Thus, therapeutic strategies that maintain or fine-tune Wnt signalling in ENS niches, while carefully avoiding pro-oncogenic risks may offer avenues to preserve motility in ageing colon (Scharr et al., 2024; Mueller et al., 2024).

The more pronounced reduction of LGR5-immunoreactive structures in the ascending colon is consistent with previous observations that ageing-related structural and functional alterations preferentially affect proximal regions of human colon (see Baidoo and Sanger, 2025a, for discussion). This regional vulnerability may reflect differences in microbial exposure, luminal environment, immune activity or enteric neural regulation between ascending and descending colon.

This study has several limitations that should be considered when interpreting the findings. First, although the sample size was sufficient for an exploratory analysis, it may limit the generalisability of the results to broader populations. Second, whilst tuft-cell numbers were normalised to mucosal area, neither epithelial cellularity nor epithelial nuclear density was quantified. Consequently, age-related alterations in epithelial composition may have contributed, at least in part, to the observed differences.

In addition, the identification of tuft cells relied on a single immunohistochemical marker. Although this marker is widely established and has been extensively validated in the literature, the use of a single marker may not fully account for cellular heterogeneity and carries a risk of misclassification. Validation using complementary markers such as POU2F3 or TRPM5, together with exclusion of intraepithelial leukocytes by CD45 co-labelling, independent methodological approaches, or functional assays would further strengthen the specificity and robustness of cell identification and should be incorporated in future studies. Finally, future investigations integrating epithelial and enteric nervous system molecular profiling with myenteric neuroimaging and physiological assessments would provide more comprehensive mechanistic insights into the biological relevance of the observed changes.

5. Conclusion

Adult human ageing is associated with a reduction in COX-1-positive epithelial tuft cells within the ascending and descending colon and a selective attenuation of LGR5-IR within myenteric ganglia of the ascending colon. These changes could implicate diminished luminal chemosensation and altered Wnt-related or progenitor-like programmes in the ENS. Together with other degenerative changes (e.g. Baidoo et al., 2023b; Baidoo et al., 2026) that have been observed within the aged adult human colon (see references cited within, Baidoo and Sanger, 2025a, and Hofmann, Geiger, 2026), these are likely to reduce functional reserves and increase the vulnerability of the colon to other

age-related changes which lead to symptoms such as chronic constipation and faecal incontinence (see Introduction for references). Further studies are now needed to understand the mechanisms of these changes so targeted interventions can be developed to mitigate age-related lower bowel dysfunction.

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CRediT authorship contribution statement

Nicholas Baidoo: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Investigation, Formal analysis, Conceptualization. **Gareth J. Sanger:** Writing – review & editing, Validation, Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Ethics approval

Approved by the East London Research Ethics Committee (REC: 10/HO703/71), the London City Road and Hampstead Research Ethics Committee (REC: 15/LO/21/27) and subsequently by the University of Westminster (ETH2324–1489).

Contributors

NB critically reviewed, designed, and conducted the experiments, analysed the data, cowrote the manuscript and supervised the overall project. GJS co-wrote the manuscript and facilitated the identification, collection, and governance of human tissue collection for this study, all authors participated in its construction and refinement.

Patient consent

All patients provided written informed consent for the donation of tissue (REC 10/HO703/71; East London ethics committee).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.acthis.2026.152371](https://doi.org/10.1016/j.acthis.2026.152371).

Data availability

Data will be made available on request.

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