

Dio2+Il11+ fibroblasts constitute a local stromal-endocrine niche that promotes intestinal stem cell regeneration after immune-mediated injury

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Dio2⁺Il11⁺ fibroblasts constitute a local stromal-endocrine niche that promotes intestinal stem cell regeneration after immune-mediated injury

Running Title: Endocrine fibroblast niche control of ISC repair following immune injury

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ABSTRACT

Immune-mediated damage depletes intestinal stem cells (ISCs) and their homeostatic niche. Whether inflammatory injury induces an alternative niche that promotes regeneration remains unknown. Using single-cell spatial transcriptomics in murine gastrointestinal graft-versus-host disease, we identified cytotoxic CD8⁺ T cells accumulated near intestinal crypts coinciding with ISC, Paneth cell depletion, and epithelial death pathway activation. A distinct *Dio2*⁺*Il11*⁺ fibroblast population emerged in regions of maximal intestinal damage between the epithelial progenitors and stromal cells. These fibroblasts expressed cAMP-response and immune-recruitment programs, suggesting niche reorganization. Thyroid hormone receptor antagonism reduced organoid budding, whereas enhancing thyroid hormone signaling promoted morphogenesis, particularly with IL-11, linking local T3 and IL-11 secretion by these *Dio2*⁺*Il11*⁺ cells to ISC regeneration. Similar niches were observed in DSS-induced colitis model and in biopsies from patients with Crohn's disease. Our data identify *Dio2*⁺*Il11*⁺ fibroblasts as a stromal-endocrine niche at the site of immune injury that supports ISC regeneration.

39 **One-sentence summary:** Spatial transcriptomics identifies a novel *Dio2*⁺ *Il11*⁺ fibroblast-enriched
40 niche in damaged crypts as regulators of intestinal stem cell regeneration through local thyroid
41 hormone activation after immune injury.

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INTRODUCTION

The intestinal epithelium undergoes continuous renewal at homeostasis, a process that depends on a precisely organized crypt niche. Lgr5⁺ intestinal stem cells (ISCs) at the crypt base generate both absorptive and secretory lineages; transit-amplifying progenitors expand the precursor pool; Paneth cells supply trophic and antimicrobial factors; and subepithelial stromal cells calibrate Wnt, BMP, EGF, and R-spondin signals.¹⁻⁵ This system maintains the intestinal barrier despite constant exposure to dietary antigens, microbial products, and immune surveillance. When pathological immune responses target the ISCs and their homeostatic niche, epithelial repair and renewal depend not only on the intrinsic resilience of the injured ISC but also on the niche's capacity to enable repair.^{6,7} However, whether injured tissues develop a new niche, whether that niche is critical for repairing the homeostatic niche, and whether it promotes ISC-intrinsic capacity for regeneration remain unknown.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) can cure refractory hematologic malignancies and nonmalignant diseases, but gastrointestinal graft-versus-host disease (GI-GVHD) limits its efficacy.⁸ Severe GI-GVHD carries the highest clinical risk: it impairs barrier integrity, drives secretory diarrhea, enables microbial translocation, and resists therapy once crypt regeneration fails.⁹ The cytotoxic donor T cells directly target the ISC and its niche, causing ISC attrition, inducing long-term qualitative defects in the ISCs that survive the attack, and leading to loss of Paneth cells and destruction of the homeostatic niche.^{10,11} However, whether such destruction generates a new niche that compensates for and promotes a reparative response during the immune assault remains unclear. The comprehensive, spatially discrete, transcriptome-scale account that links immune effector position, epithelial death state, stromal support, homeostatic niche architecture, development of alternate sources of trophic factors and niche as reparative responses, and regenerative competence within the same crypt lesion remains unknown.

A gap that has been difficult to close with conventional tools. Dissociated single-cell sequencing labels each cell but ignores its original location, a crucial feature in crypt biology.¹² Conversely, histology preserves spatial context but sacrifices molecular detail. For instance, multiplexed ion-beam imaging in human gut GVHD revealed the loss of ISCs and Paneth cells, with an increase in CD8⁺ T cells at the crypt. Still, it detects proteins rather than transcriptional activity.¹³ To understand which niche signals are maintained or lost at the crypt interface and which ligand-receptor interactions are involved, we used spatial transcriptomics at single-cell resolution to circumvent these limitations and address gaps in our understanding of the role of the ISC niche in repair after immune injury. We used Xenium in situ transcriptomics to localize more than 5,000 gene targets at subcellular resolution in an uncut tissue section. We profiled the mouse small intestine after syngeneic or allogeneic bone-marrow transplantation (BMT) across 10 animals, decoded 3.1 billion high-confidence transcripts, segmented 6.2 million cells, and mapped 5,056 targets across intact Swiss-roll sections. The data redefined GI-GVHD beyond ISC, Paneth cells, and crypt loss and apoptosis: as a crypt-centered failure of immune, epithelial, and stromal cells. Cytotoxic CD8⁺ T cells crowded the ISC and TA compartments. The epithelium exhibited apoptosis, ferroptosis, necroptosis, and pyroptosis. The homeostatic stromal trophic circuits that supply healthy crypts were lost. In their place, a fibroblast state, absent from the normal gut at homeostasis and marked by *Dio2* and *Il11*, defined the zones of maximal injury as a stromal-endocrine niche that promotes a reparative response.

The gene *Dio2* encodes the enzyme type-2 deiodinase, which converts thyroxine (T4) into the active hormone T3. Its induction in fibroblasts, rather than neurons, suggested an endocrine source locally produced in the damaged crypt. These fibroblasts also expressed *Il11*. Functional and organoid assays showed that thyroid hormone receptor signaling, boosted by IL-11, promotes the morphogenesis of intestinal stem cells (ISCs) and progenitors. Therefore, a stromal endocrine niche emerges, where *Dio2*⁺ *Il11*⁺ fibroblasts produce T3 locally, acting as a key stromal factor in repairing a damaged ISC niche.

96 Dissection of the cellular and spatial composition of GI-GVHD at the single-cell level

97 To delineate single-cell gene expression within its spatial context in immune-mediated intestinal
98 damage, we transplanted CD3⁺ T cells and T-cell-depleted (TCD) bone marrow (BM) from
99 BALB/c or C57BL/6 donors into lethally irradiated (10 Gy) C57BL/6 recipients. By day 7 after
100 transplantation, allogeneic recipients (Allo) showed significant weight loss and clinical signs of
101 GVHD.¹⁵; syngeneic recipients (Syn) remained clinically normal (Supplementary Table 1). We
102 harvested small intestines, prepared Swiss-roll sections, and analyzed each section using Xenium
103 in situ transcriptomics with a probe panel comprising 5,000 pre-designed and 56 custom genes
104 targeting 5,056 transcripts (Fig. 1A-B and Supplementary Table 2). Across 10 mice (2 Control, 4
105 Syn, 4 Allo), the analysis recovered 3.1 billion high-confidence decoded transcripts and
106 segmented 6.2 million individual cells. Donor T cells were identified, mapped, and assessed in
107 both Syn and Allo groups using congenic and haplotype markers. Unsupervised clustering
108 assigned all cells to seven parental lineages: epithelial, immune, fibroblast, smooth muscle,
109 endothelial, enteric neuron, and adipocyte, with minimal marker cross-contamination between
110 clusters (Supplementary Fig. 1, Fig. 1C, and Supplementary Table 3). Secondary clustering
111 established the small-intestinal epithelial subsets: nine enterocyte subsets (IEC1-9), goblet cells,
112 Paneth cells, ISCs, transit-amplifying cells (TAs), enteroendocrine cells (EECs), and tuft cells (Fig.
113 1D).

114 Allo-tissue shifted the epithelial compartment toward stress-adapted states. IEC4, IEC7, and TA
115 cells expanded; Paneth cells and EECs contracted (Fig. 1E). Paneth cells in Syn-tissue showed
116 homeostatic metabolic signatures that were reduced in Allo-tissue while the microbial-sensing
117 and inflammatory programs emerged in them (Fig. 1F). TA cells showed a similar shift between
118 the syngeneic and allogeneic tissues (Fig. 1G). IEC4 showed enrichment of metabolic pathways,
119 and the membrane protein localization signals were consistent with inflammation-driven epithelial

adaptation. IEC7 co-expressed the necroptosis effectors *Ripk3* and *Mkl1*, along with lipid metabolism and endocytic programs, demonstrating that intracellular trafficking and necroptotic cell death were also activated in the same cells in allogeneic tissues (Fig. 1H).

Immune subclustering identified 43 distinct populations (Fig. 1I). T cells and macrophages dominated the Allo-immune compartment. Interestingly, B cells and plasma cells were prominent in Control and Syn-tissues but largely absent in the Allo-tissues, an observation that has not been documented to date. Three T-cell subsets expanded selectively in Allo-tissue. CD4-2 co-expressed TCR signaling genes (*Zap70*, *Lat*) and *Tigit*, indicating active TCR engagement with expression of co-inhibitory/exhaustion markers. CD8-6 expressed high *Mki67* and *Top2a*, demonstrating the proliferating effector pool. CD8-7 lacked proliferative markers but expressed *Gzma* at high levels, consistent with a cytotoxic effector phenotype (Fig. 1J-M). Within the stromal compartment, fibroblast subsets expressing *Cxcl9* and *Cxcl13* expanded specifically in the Allo group. Perivascular cells adopted a hybrid fibroblast-venous transcriptional state. Two glial subpopulations with partial fibroblast and macrophage programs emerged, and the smooth muscle subset SMC3 appeared exclusively in Allo animals (Supplementary Fig. 2A-H). Taken together, these stromal and immune shifts defined the cellular landscape within which crypt-specific events unfolded, a focus we next pursued.

Immune injury reorganizes the crypt, villus, lamina propria, and muscularis niches.

Computational niche analysis partitioned the small intestine into four anatomically distinct regions: villus epithelium, crypt epithelium, lamina propria, and muscularis (Fig. 2A). The villus niche in Syn-tissue contained IEC1-3, IEC5, and IEC6. By contrast, the Allo-tissue was enriched for IEC4 and IEC7 and showed an epithelium-proximal CD8-4 population that was absent in the Syn villi (Supplementary Fig. 3A). Interaction analysis, both composition-adjusted and composition-independent, ranked CD8-4 as the dominant Allo villus interaction partner, a shift demonstrating

that immune infiltration has reorganized epithelial communication at the villus surface (Supplementary Fig. 3B-D).

The crypt niche showed more significant changes: some were described previously, and others were not previously reported. Specifically, Syn crypts were dominated by ISCs, Paneth cells, and TAs. As suggested by prior studies, the Allo crypts showed TA expansion, ISC and Paneth cell depletion, and accumulation of proliferating CD8-6 cells (Fig. 2B).^{4,5} Given experimental evidence that upper-crypt progenitors sustain epithelial renewal during ISC attrition,¹⁶ TA expansion in the Allo group likely reflects a compensatory progenitor response to ISC loss. In Syn crypts, cell-cell interaction networks centered on ISCs; by contrast, in Allo crypts, this shifted to TA cells. Notably, CD8⁺ T-cell-crypt epithelial interactions were observed exclusively in Allo tissue, indicating direct immune T-cell contact with the progenitor compartment that was not observed in the Syn controls (Fig. 2C-E).

The lamina propria showed parallel stromal remodeling. Fibroblast 14, a basement membrane-associated subset beneath the epithelial layer, predominated in Control and Syn tissue alongside Macrophage 1 and Macrophage 3, which expressed programs for extracellular matrix organization and antigen presentation. In contrast to the naïve controls and Syn, the Allo-tissue enriched for Fibroblast 2 and Fibroblast 6, both marked by high Cxcl13 expression, is consistent with an immunologically active stromal state that recruits circulating immune cells. Fibroblast 2 mediated extensive multicellular interactions in the Allo group. The Ki67⁺ Fibroblast 11 gained broad connectivity in Allo tissue but was largely quiescent in the Syn-tissues (Fig. 2F-I).

We next used the Cellchat database (Supplementary Table 4) to perform ligand-receptor analysis across niches, which documented inflammation-driven rewiring of adhesion, permeability, and trophic circuits throughout the intestinal wall. CDH1 and CDH5 interactions were enriched in the Allo-epithelial niche, while the OCLN-mediated junctional interactions predominated in Syn. Within the lamina propria, BAFF-related signaling associated with B cells was prominent in Syn tissue. In the Allo group, this was replaced by vascular permeability pathways (MADCAM, VEGF,

VCAM, ICAM), TRAIL signaling, PD-L1, and collagen-related networks (Fig. 2J-K), demonstrating a marked shift from a homeostatic to an inflammatory and profibrotic state.¹⁷ Furthermore, within the smooth muscle layer, a niche-like signaling network was organized among cells adjacent to the muscularis. The IGF signaling network, which supports smooth muscle proliferation and survival, originated predominantly from arterial cells and targeted multiple enteric neuronal populations in Syn mice. In Allo mice, however, this network was remodeled to establish new interactions with smooth muscle cells, with interstitial cells of Cajal (ICCs) serving as a new source of IGF signaling (Supplementary Fig. 3I-J).¹⁸ These data suggest that GI-GVHD inflammation not only added immune cells but also systematically reprogrammed the interaction circuits between the cells in each anatomical compartment of the intestine. It re-programs the interaction circuits.

Donor cytotoxic CD8⁺ T cells and Tregs occupy distinct niches, and the host epithelial cells show pleiotropic cell-death programs.

Cytopathic alloreactive donor T cells are known to drive ISC loss and change their functions in murine GI-GVHD.^{5,10,17} MIBI-TOF studies of human GI-GVHD documented ISC and Paneth cell depletion with CD8⁺ T cell apposition in crypts.¹³ However, the single-cell resolution of these molecular programs executing injury was not resolved spatially, which caused the severity of the injury to be unresolved spatially.¹³ We therefore next assessed the spatial and transcriptomic changes that are critical for T-cell-mediated ISC cell loss and the associated functional changes. When the distances between ISCs or TA cells and all CD8⁺, CD4⁺ T-cell, and Treg populations were plotted together with their corresponding cell densities, all CD8⁺ T-cell clusters were markedly enriched in close proximity to both ISCs and TA cells in the Allo group compared with the Syn group. In contrast, CD4⁺ T cells and Tregs exhibited an increased frequency in the vicinity of TA cells in the Allo group, whereas their distribution around ISCs showed only minimal differences between the two groups (Supplementary Fig. 4A).

To further delineate immune-cell positioning across the full intestinal wall thickness, we developed the Immune Allocation Map (IMAP): a two-dimensional coordinate system in which the x-axis encodes distance from the epithelial surface and the y-axis encodes distance from the smooth muscle layer (Supplementary Fig. 4B-C).¹⁹ Cells with $x < 50 \mu\text{m}$ and $y < 50\mu\text{m}$ occupy crypt-adjacent positions. In Allo-tissues, CD4⁺ and CD8⁺ T cells accumulated preferentially at those crypt-adjacent coordinates. By contrast, Tregs showed no significant difference in crypt proximity between Syn and Allo groups. Furthermore, cytotoxic, but not regulatory T cells (Tregs), were located at the progenitor interface (Supplementary Fig. 4D-E). These data provide new insights into the previously unrecognized differences in the compartmentalization between donor Tregs and cytopathic T cells in Allo-tissues.

Next, to characterize the full crypt microenvironment, we defined an ISC/TA-centered niche encompassing all cells within 30 μm of the ISC and TA populations (Fig. 3A). Within this niche, the Allo group showed increased CD8⁺ T-cell frequency, TA expansion, ISC and Paneth cell depletion, and macrophage accumulation (Fig. 3B, Supplementary Fig. 5A-E). The dominant crypt-proximal CD8⁺ subset, CD8-3, expressed partial intestinal epithelial gene signatures and upregulated *Gzma*, *Lag3*, *Tigit*, and *Pdcd1* in the Allo group compared with the Syn tissues, indicating cytotoxic potential and co-inhibitory receptor engagement at the same crypt-proximal site (Fig. 3C). Macrophage-2, an M2-polarized subset with high *Arg1* expression, expanded within this same niche in Allo mice. These data point to an unrecognized concurrent tissue-repair macrophage activity in spatial proximity to the effector T-cell infiltrate. In addition, Fibroblast 2, which was significantly enriched in Allo, showed increased expression of *Cxcl13*, a chemokine previously reported to be upregulated in the gastrointestinal tissues of Allo recipients.²⁰ These findings suggest that crypt-associated fibroblasts contribute to the increased tissue levels of *Cxcl13* in Allo recipients.

We next assessed responses within epithelial cells in proximity to cytotoxic donor T cells, because epithelial cell apoptosis in the crypt is the pathognomonic clinical feature of GI-GVHD. In addition to apoptosis pathways, the epithelial compartment in the Allo-tissues showed simultaneous activation of multiple, pleiotropic programmed cell death pathways. TA cells upregulated apoptosis effectors (*Bcl2a1a*, *Casp3*, *Casp8*), the ferroptosis regulator *Acsf4*, necroptosis effectors (*Ripk1*, *Ripk3*, *Mkl1*), and pyroptosis machinery (*Gsdmd*, *Casp1*) (Fig. 3D). The ISCs, goblet cells, EECs, and Paneth cells also showed all related pathways, albeit with quantitatively variable patterns (Supplementary Fig. 6A-F). The simultaneous engagement of four mechanistically distinct death pathways (apoptosis, ferroptosis, necroptosis, and pyroptosis) in the same progenitor population implies that pathogenic immune pressure at the crypt operates through multiple parallel cytotoxic cell-death mechanisms rather than a single pathway. This multiplicity of pleiotropic engagement of cell-death pathways predicts that blockade of any single death effector will likely provide only partial protection, a conclusion consistent with the limited efficacy of pathway-specific interventions in GI-GVHD^{21,22-24}.

We next performed the neighborhood network analysis. The analysis confirmed that ISC/TA cells were in spatial proximity to donor CD8-3 and CD8-7 subsets, while Tregs were localized within the Allo ISC/TA-centered niche and exhibited close spatial proximity to TA cells and multiple fibroblast subsets (Fig. 3E-F, Supplementary Fig. 7A-B). These findings suggest that Treg cells contribute to intestinal stem cell regeneration following immune-mediated injury.²⁵ Ligand-receptor analysis of the crypt niche identified *Gzma-F2rl* and *Gzma-Pard3* pairs between CD8⁺ T cells and ISC/TA cells, again exclusively in the Allo group, representing direct cytotoxic molecular contact at the crypt epithelial interface (Fig. 3G). Macrophage-derived *Spp1* signaling toward TA cells was amplified in the Allo crypt niche, consistent with a reparative macrophage response directed at progenitor survival (Fig. 3H). In addition to the above, we made a surprising new observation. Fibroblast-derived *midkine* and *NRG*(Neuregulin)-*ERBB2/ERBB3* signaling

exhibited distinct enrichment in Syn and Allo. (Fig. 3I-K). Midkine has been implicated in mucosal wound healing in DSS-induced colitis, suggesting that the loss of midkine signaling in Allo may contribute to defective epithelial repair..^{26,27} HER2 acquires signaling activity through heterodimerization with HER3, with neuregulin serving as its ligand to promote intestinal epithelial proliferation. This NRG–HER2/HER3 interaction was lost in Allo, suggesting impaired maintenance of epithelial homeostasis through proliferative signaling..^{28,29} The GVHD crypt niche thus combined active immune cytotoxicity with the concomitant loss of the homeostatic stromal signals that ordinarily buffer epithelial injury. These data collectively show that cytopathic T cells and Tregs do not co-localize in the same spatial compartment, that epithelial target cells undergo multiple cell death programs, and that direct ISC loss is further exacerbated by the concomitant loss of the stromal niche-derived trophic support system.

Dio2⁺IL11⁺ fibroblasts form a stromal-endocrine niche in the severe crypt lesions

Given the identification of cytotoxic immune programs and lost trophic signals within the crypt, we next determined whether distinct stromal states characterize and potentially define the sites of loss of intestinal regeneration. To identify such microenvironmental features, we defined spatial regions of interest (ROIs) in proximal (jejunum) and distal (ileum) segments of the small intestine and stratified Allo samples into moderate and severe injury zones based on the integrity of the villus-crypt structure (Fig. 4A). Severe regions, occurring predominantly in the distal small intestine, showed significantly greater epithelial cell depletion, fibroblast expansion, and accumulation of proliferating cytotoxic CD8⁺ T cells compared with Syns, and moderate Allo regions (Fig. 4B, Supplementary Fig. 8A-C). These severe zones diverged substantially in cellular composition from both Syn tissue and moderate Allo regions, underscoring them as a pathologically distinct microenvironment.

Unbiased transcript discovery analysis of the microenvironments in severe lesions identified *Dio2* as one of the transcripts most selectively enriched at these sites (Fig. 4C). *Dio2* encodes type 2 deiodinase, which converts inactive T4 to biologically active T3.¹⁴ In Syn and moderate Allo tissue, *Dio2* expression was confined to a subset of enteric neurons. By contrast, in severe lesions, *Dio2* expression was observed in the fibroblast populations, identifying a non-neuronal stromal source of local T3 activation within the injured ISC niche (Fig. 4D). This redistribution was spatially restricted to zones of maximal crypt disruption and was not a general transcriptional response to systemic inflammation, indicating that the injured local microenvironment specifically induces *Dio2* expression in fibroblasts at the sites where ISC-driven regeneration has stalled and where crypt tissues have been maximally altered.

We next performed comparative transcriptomics of *Dio2*-positive versus *Dio2*-negative fibroblasts within severe regions. The analysis revealed a distinctive and internally consistent gene expression program. *IL11* was markedly elevated in *Dio2*-positive fibroblasts, linking this state to IL-11 production and identifying a distinct population of *Dio2*⁺*IL11*⁺ fibroblasts. In addition, these *Dio2*⁺*IL11*⁺ fibroblasts expressed IL-6, and also the chemokines Cxcl5 and Cxcl14, which recruit neutrophils, NK cells, and dendritic cells, were upregulated.^{30,31} *Adcy2*, encoding adenylate cyclase 2, and *Nptx2*, implicated in synapse organization, increased together, indicating activation of a cAMP-linked signaling program with neural connectivity features within this fibroblast subset (Fig. 4E). Beta-adrenergic receptor signaling and cAMP elevation are well characterized to drive *Dio2* transcription in adipose tissue³²; the co-upregulation of *Adcy2* and *Nptx2* in these fibroblasts suggests that an analogous neuron-to-fibroblast cAMP relay drives *Dio2* in the injured intestinal stroma.

Across individual ROIs, we examined the expression of the sympathetic neuron-associated genes *Dbh* and *Slc18a2*. Enteric neurons within the Severe lesions exhibited higher expression of both genes than those in the other ROIs, suggesting an enrichment of neurons with a sympathetic-like

transcriptional program in the Severe regions (Fig. 4F). Neighborhood network analysis showed that, in Syn tissues, cell populations sharing the same parental cluster preferentially localized together, indicating that compartment-specific tissue architecture was largely preserved. In contrast, these spatial networks were markedly disrupted in Allo tissues, particularly within Severe lesions. Consistent with the ISC/TA-centered niche analysis, Allo tissues displayed prominent network connections between CD8-3 cells and TA cells, supporting the notion that GVHD profoundly disrupts the intestinal stem cell niche.

In Severe lesions, in addition to the close association between CD8-3 cells and TA cells and the depletion of Paneth cells, *Dio2*-expressing Fibroblast14 cells were also closely associated with TA cells. Furthermore, a distinct Fibroblast12 population expressing *Grem1*, a secreted BMP antagonist, together with *Dio2*, emerged specifically in Severe lesions. These fibroblasts established extensive neighborhood networks with CD4 T cells, CD8 T cells, Tregs, B cells, ILC2s, and ILC3s, suggesting that stromal compartment remodeling and local T3-associated signaling broadly influence the surrounding immune microenvironment (Fig. 4G).^{33,34}

Finally, we performed a comprehensive analysis of cell–cell communication across the different ROI-defined microenvironments. Canonical Wnt signaling interactions were enriched in both Syn and Allo tissues but were markedly diminished in Allo_Severe lesions, suggesting impaired self-renewal signaling within the intestinal stem cell niche. Consistent with the ISC/TA-centered niche analysis, Spp1- and nectin-mediated interactions were selectively enriched in Allo tissues. Reflecting the inflammatory milieu of GVHD, IL-6- and TNF-associated signaling interactions were increased in both Allo and Allo_Severe lesions compared with Syn tissues. Notably, Allo_Severe lesions exhibited unique enrichment of IL-2- and MAdCAM-mediated signaling, suggesting enhanced immune-cell activation together with increased vascular permeability and immune-cell recruitment. Collectively, these findings demonstrate that progressive GVHD is accompanied by a profound rewiring of intercellular communication, characterized by attenuation of regenerative

Wnt signaling and the emergence of inflammatory and immune-recruiting signaling networks (Fig. 4H).

Simultaneous analysis of the proximal small intestine recapitulated the major spatial relationships observed in the distal intestine while additionally identifying proximal-specific noradrenaline and GABA-B signaling interactions that were absent in the distal region (Supplementary Fig. 9A-F). Previous studies have shown that adrenergic receptor activation promotes intestinal wound healing and alters the gut microbiota by modulating antimicrobial peptide production, whereas GABA-B activation suppresses experimental colitis through interleukin-1 receptor-associated kinase M (IRAK-M)-mediated anti-inflammatory and antioxidant effects.^{35,36} These findings suggest that such neuroimmune signaling pathways are preferentially engaged in the proximal small intestine, reflecting anatomically distinct regulatory mechanisms in this region.

***Dio2⁺IL11⁺* fibroblasts form a thyroid-hormone niche promoting ISC regeneration**

We next assessed the functional relevance of this novel stromal-endocrine niche characterized by the *Dio2⁺IL11⁺* fibroblasts. Because the *Dio2⁺IL11⁺* fibroblasts spatially concentrated at sites of ISC/TA depletion and crypt failure, we determined whether the niche served as a site where locally generated thyroid hormone regulates ISC-driven progenitor morphogenesis. Specifically, we determined whether the injured tissue actually makes the hormone there, and whether that hormone drives repair. We first determined local hormone production at the stromal-endocrine niche in the allo-tissues. We reasoned that if the niche at severe distal lesions has functional fibroblast *Dio2*, then the injured ileum should have higher active T3 levels than the less-injured proximal gut. To this end, we extracted the matched segments and measured T3 using competitive ELISA. The allo ileum, which contains the *Dio2⁺IL11⁺* fibroblast, showed higher T3

levels than the matched syn ileum (Fig. 5A). This demonstrated that the increase in T3 was local and not from systemic changes.

Next, we asked whether our spatial transcriptomic findings could be independently validated using RNAscope, a multiplex fluorescence *in situ* hybridization platform that detects multiple transcripts simultaneously. In Swiss-roll sections of the small intestine, Syn samples exhibited only sparse punctate *Pdgfra* (fibroblast marker) signals. In contrast, Allo samples showed a striking accumulation of *Dio2*, *Il11*, and *Pdgfra* transcripts, which were spatially restricted to regions of severe epithelial destruction (Fig. 5B-C).

We examined the mechanism for induction of these fibroblasts by determining whether cAMP alone can induce the *Dio2*⁺*Il11*⁺ state in the resting fibroblasts. The spatial program linked *Dio2* to *Adcy2*, *Nptx2*, and a neuron-to-fibroblast relay, all upstream of cAMP.³² We therefore treated primary mouse intestinal fibroblasts with forskolin, a direct activator of adenylate cyclase.³⁷ Administering forskolin (10 μM for 8 hours) increased *Dio2* and *Il11* expression and transformed resting fibroblasts into the *Dio2*⁺*Il11*⁺ state observed in vivo (Fig. 5D). A single cAMP signal effectively recreated the injury-related fibroblast profile, suggesting that the sympathetic-cAMP pathway is the likely physiological trigger.

The same cAMP pathway influenced the responding epithelium. In Allo organoids, T3 (400 nM) increased the expression of its known target, *Ppargc1a* (PGC-1α), at 4, 8, and 24 hours (Fig. 5E), indicating a mitochondrial biogenesis response that links local thyroid signaling to the metabolic needs of crypt regeneration.

Next, we investigated whether localized T3 production by the *Dio2*⁺*Il11*⁺ stromal-endocrine niche directly influences ISC function and regeneration. We isolated crypts enriched for ISC, cultured them as organoids, and treated them with T3 in the presence of the thyroid hormone receptor

antagonist NH-3 (Fig. 5F). NH-3 inhibited organoid budding and reduced organoid size, indicating that active thyroid hormone receptor signaling is essential for ISC-driven morphogenesis (Fig. 5G). Conversely, addition of T3 promoted budding, while NH-3 diminished this effect. These data demonstrate that thyroid hormone receptor signaling directly regulates ISC's regenerative functions.³⁸

Because these fibroblasts also express *IL11*, we tested whether IL-11 enhances the effect of T3. Recent studies suggest that IL-11 contributes to intestinal epithelial repair and proliferation through STAT3 signaling.³⁹ Consistent with the data, IL-11 alone increased organoid formation, but it further potentiated the regenerative effects of T3 (Fig. 5H). To further confirm the impact on ISC regenerative potential in the context of inflammation, we assessed the effects of IL-11 and T3 following IFN- γ exposure. Under IFN- γ stress, both IL-11 and T3 individually helped preserve budding and organoid size, and their combination provided the best protection (Fig. 5I). Thus, the local presence of T3 and IL-11 promoted the regenerative potential of ISCs under inflammatory duress.

We next explored whether the T3 and IL-11 produced by the *Dio2*⁺*IL11*⁺ fibroblasts constitute a niche unit that determines the regenerative ability of the injured crypt. To test the niche as a unit, we co-cultured intestinal organoids with either unstimulated or forskolin-treated fibroblasts.

In fibroblast–intestinal organoid cocultures, spheroids were more frequently observed than budding organoids. Moreover, coculture with forskolin-treated fibroblasts significantly increased spheroid size (Fig. 5J-K). *Dio2*-high fibroblasts exhibited elevated expression of the *EGF* family member epiregulin (Ereg) (Fig. 4E), suggesting that these stem cell niche-associated factors contribute to the regulation of organoid morphology.

Conserved *Dio2*⁺*Il11*⁺ niche in murine and human IBD

Finally, we investigated whether the emergence of *Dio2*⁺*Il11*⁺ fibroblasts and the establishment of an ISC neighborhood network observed in the GVHD mouse model were also broadly germane to other forms of immune injury in the GI tract. To this end, we used the well-characterized chemically induced mouse model of DSS colitis to study inflammatory bowel disease (IBD). To address this, we collected colonic tissues from mice after 7 days of administration of 3% DSS in the drinking water and performed RNAscope analysis. Regions containing cells co-expressing *Dio2*, *Il11*, and *Pdgfra* were detected throughout the colonic mucosa (Figures 5L–M). These findings suggest that the emergence of *Dio2*⁺*Il11*⁺ fibroblasts is a conserved feature of colitis-associated immune-mediated tissue injury.

Next, we determined whether this endocrine-stromal niche is germane to human immune-mediated injury of the GI tract. To confirm clinical relevance, we searched for a human equivalent of the mouse niche by analyzing the spatial transcriptomics dataset from human patients with Crohn's disease, using the original cell annotations.⁴⁰ This Xenium spatial transcriptomics dataset comprises healthy controls (n=7), alongside diverse Crohn's disease-associated fistulae (n=5, including both epithelialized and non-epithelialized tissues) and non-fistulating Crohn's disease ulcer samples (n=7). *DIO2* and *IL11* were enriched in the stromal part of lesional tissue, with Stromal 2 cells carrying this signal (Fig. 5N–O).

Spatial mapping showed *DIO2*⁺*IL11*⁺ Stromal 2 cells near transit-amplifying epithelium in the ulcerated ileum (Fig. 5P). We next assessed the niche-network of Stromal cell 2. The niche-network analysis demonstrated that Stromal 2 cells primarily connect to TA cells in Crohn's disease, consistent with the stromal-to-progenitor relationship observed in mice (Fig. 5Q). This *DIO2*⁺*IL11*⁺ fibroblast niche, which forms at sites of the most severe lesions in the immune-

424 mediated intestinal disease, is thus observed in both alloimmune murine and idiopathic human
425 intestinal injuries.

426

427

DISCUSSION

The ISC niche and its functions were largely defined at homeostasis. Paneth cells provide ISCs with Wnt and EGF; subepithelial fibroblasts supply a BMP counterbalance and R-spondin; and the local immune milieu remains tolerant.¹⁻⁵ Immune attack, such as in GI GVHD and IBD, has shown that ISCs are direct targets and that the homeostatic niche is perturbed. Niche failure compounds the direct killing of ISCs, but the mechanisms of disruption and whether an alternative niche develops at the site of attack to provide regenerative signals to ISCs remain unknown. In this study, we addressed these questions. We show that the effector lesion concentrates at the crypt, eliminates ISCs and Paneth cells, and disrupts the homeostatic niche at the same site. In its place, the tissue assembles a new stromal-endocrine niche, marked by the *Dio2⁺ Il11⁺* fibroblasts, at the zones of regenerative failure. Organoid, forskolin, and hormone quantification experiments show that locally produced T3 and IL-11 promote ISC- and progenitor-mediated regeneration. Thus, our data show that severe intestinal injury is driven by unrestrained crypt cytotoxicity and the loss of the normal trophic niche, and that this can be potentially offset by an emergent *Dio2⁺ Il11⁺* fibroblast niche that supports the surviving ISCs.

The cytopathic lesions demonstrated a clear spatial structure. CD8-3 and CD8-7 T cells, which are high in *Gzma* and express co-inhibitory receptors *Lag3*, *Tigit*, and *Pdcd1*, were located within 30 μ m of ISC and TA cells only in Allo tissue. Their proximity aligned with *Gzma*-F2rl and *Gzma*-Pard3 contacts at the crypt surface, indicating simultaneous activation of apoptotic, ferroptotic, necroptotic, and pyroptotic pathways in TA cells. The presence of four distinct cell death routes in a single target population suggests that cytotoxicity involves multiple mechanisms rather than a single cell death pathway. Recent studies support this, showing different death modes in isolation; our spatial data now unify these findings at the crypt level. This has two important implications: blocking one downstream effector will only partially protect, necessitating interventions that target the immune-epithelial interface upstream or simultaneously inhibit all four

pathways. Furthermore, crypt apoptosis, currently the sole diagnostic marker for gut GVHD, may underestimate severity, and exploring other death modes as diagnostic tools could improve assessment.

Our data show several novel aspects of the putative repair process of the ISC niche. Specifically, the crypt's repair side reveals new insights that have heretofore gone unrecognized. The macrophage and stromal responses expected to promote ISC-driven regeneration in the Allo crypt are either absent, partial, or too weak. Specifically, macrophages labeled Macrophage-2 and those secreting Spp1 supported the Allo crypt, with an increase in macrophage osteopontin aimed at TA cells, indicating a genuine repair attempt. However, the trophic signals from fibroblasts and glia, such as midkine and Nrg1-ErbB2/ErbB3, that nourish Syn crypts, are absent in Allo tissue.²⁷⁻

²⁹ This leaves the ISCs vulnerable and deprived of their usual support, a gap that macrophages cannot fully fill. Whether restoring midkine-ErbB signaling alone can ensure ISC survival remains an open question that requires further testing.

The *Dio2*^{+/H11} fibroblast niche within damaged tissue is an unexpected finding that could lead to new therapeutic approaches. T3 promotes intestinal stem cell activity by inducing *Olfm4* and *Lgr5* expressions in crypts, leading to enhanced organoid budding.³⁸ In a healthy intestine, *Dio2* is typically expressed in enteric neurons.⁴¹ By contrast, our data show that in severely immune-damaged lesions, *Dio2* was expressed in fibroblasts together with *Adcy2* and *Nptx2*. In addition, EN4 neurons exhibited markedly increased expression of sympathetic neuron-associated transcripts compared with other regions, suggesting the establishment of a neuron-to-fibroblast signaling axis that locally supplies the active thyroid hormone T3 to the most severely damaged epithelial regions. The β -adrenergic-cAMP pathway activates *Dio2* in fat tissue³²; the forskolin experiment shows that this pathway is also directly activated in fibroblasts, and increased T3 in the injured ileum demonstrates local hormone synthesis. Promoting this pathway in damaged

crypts might augment ISC regenerative capacity through local T3 production. The presence of IL-11 adds complexity: MERFISH analysis in DSS colitis found that IL-11 facilitated epithelial repair under mild inflammation but also hindered it during severe conditions.⁴² In severe GVHD lesions, *Dio2*⁺ fibroblast IL-11 works with T3 to promote repair, implying that the stromal-endocrine niche influences whether IL-11 supports or inhibits ISC recovery. Our studies nonetheless point to a critical functional role of this pathway: blocking thyroid hormone receptors halted organoid budding, while T3 reactivated it, and the addition of IL-11 further enhanced it. The findings demonstrate that an ISC-autonomous response to thyroid hormone receptors, downstream of stromal *Dio2*-dependent T3, is a key factor in progenitor differentiation and crypt formation, with IL-11 amplifying this effect. Future studies will need to dissect the specific signals and developmental mechanisms that underlie the emergence of this novel neuronal-stromal-endocrine repair axis. Furthermore, it would be important to explore whether these pathways are also operative in the context of immune injury in other tissues.

Pokatayev and colleagues identified inflammation-associated fibroblasts induced by FCN1⁺ IL1B⁺ macrophages that secrete IL-11 as a profibrotic factor in the cycle of chronic inflammation and fibrosis in IBD.⁴³ In our acute T-cell-driven GI GVHD model, *Dio2*⁺ fibroblasts co-express *IL11* and are present at sites of intense macrophage and cytotoxic T-cell infiltration. We speculate that this overlap may not be coincidental, as fibrosis is a critical pathogenic feature of chronic GVHD. The chronic fibroinflammatory cycle could originate in acute GVHD as an early, localized form of the same reparative response, which shifts from healing to scarring if it surpasses the window for effective repair. Fibrosis may be an ISC-protective response that persists excessively, but this remains to be formally tested. Teshima and colleagues demonstrated that external IL-11 reduces GVHD, although the source was unknown; Nishina and colleagues found that mice lacking IL-11 or *Il11ra1* develop more severe DSS colitis, but their spatial context remained unclear.^{44,45} Our data fill this gap: *Dio2*⁺ fibroblasts produce IL-11 specifically where crypt structures are most

507 damaged, highlighting its biological significance and showing that IL-11 enhances the ISC-trophic
508 effects of locally produced T3.

509
510 Our study has limitations. The 5,000-gene panel may under-sample low-abundance niche ligands.
511 Segmentation at tight epithelial-stromal borders introduces boundary ambiguity, and no validated
512 GVHD-specific single-cell reference atlas yet exists benchmark assignments. Furthermore,
513 whether similar processes underpin stem cell niches in other target organs of GVHD remains to
514 be explored with organ-specific spatial analyses. The day-7 time point captures an acute lesion;
515 however, the relevance of these observations and the behavior of *Dio2*^{+/}*IL11*⁺ fibroblasts under
516 steroids or other therapeutics, in chronic GVHD, or during recovery will need to be specifically
517 addressed. Nonetheless, the identification of a stromal-endocrine niche that controls ISC
518 regeneration, when taken together with data from human Crohn's disease, suggests that this is
519 likely a generalized mechanism in the context of severe immune injury.

520
521 Our findings on the emergence of a spatially distinct *Dio2*^{+/}*IL11*⁺ fibroblast niche at sites of crypt
522 failure point to a previously unappreciated and a broader principle of immunopathology: when
523 immune responses disrupt homeostatic niches, tissues can form alternative, injury-driven
524 microenvironments that provide trophic signals absent from the original niche. This is akin to the
525 formation of tertiary lymphoid structures, in which chronic inflammation drives stromal and
526 immune cells to organize into lymphoid-like structures outside secondary lymphoid organs.⁴⁶
527 Similar processes occur in macrophage biology; for example, after skeletal muscle injury,
528 "dwelling" macrophages create a temporary niche essential for satellite-cell proliferation via local
529 NAMPT, while lipid-associated macrophages in the injured liver form limited niches to clear debris
530 and promote regeneration.^{47,48} Thus, our data on the *Dio2*^{+/}*IL11*⁺ stromal-endocrine compartment
531 exemplify a general adaptive strategy: development of specialized niches when the original ISC
532 tissue niche is compromised during immunopathology.

533

534 GI GVHD and IBD have been treated by suppressing immune responses or promoting immune
535 tolerance.⁴⁹ Emerging data on tissue repair and resilience are driving the development of
536 additional strategies to promote tissue tolerance.^{11,50} Our data suggest new opportunities for
537 treating immune-mediated intestinal diseases: a non-immunosuppressive, spatially focused, and
538 mechanistically distinct approach, namely the stromal-endocrine niche the ISC needs for repair
539 in the context of severe immune-mediated damage. Our data suggest that suppression of CD3⁺
540 cytotoxicity is necessary but may not be sufficient if the *Dio2*⁺*Il11*⁺ fibroblasts cannot deliver the
541 ISC-trophic factors. Restoration of local availability of T3 and IL-11 in injured crypts may
542 complement immunosuppression.

543 In summary, we identified a *Dio2*⁺*Il11*⁺ fibroblast niche that arises only under inflammatory stress
544 and generates distinct trophic signals for ISC regeneration following disruption of the homeostatic
545 niche by pathogenic immune responses. This local endocrine repair compartment may represent
546 a novel target for tissue-directed therapy.

547

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Pavan Reddy, at pavan.reddy@bcm.edu

Materials availability

This study did not generate new unique reagents.

Data and code availability

The spatial transcriptome data have been deposited at Gene Expression Omnibus (GSE342936). This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

T.I., A.Y., and P.R. designed the study and wrote the manuscript. T.I. performed the experiments. A.Y. and T.J.L. performed the spatial transcriptome analysis. K.A.M., M.C.W., Y.S., N.P.B., Y.K., V.L.O., A.S., D.Z., Y.S., and L.M. provided technical support and intellectual guidance for this study. W.H.H. reviewed the data and edited the manuscript. P.R. conceived and supervised the study.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Figure Legends.

Figure 1. Spatial transcriptomic profiling reveals epithelial and immune cell heterogeneity in murine gastrointestinal GVHD

(A) Workflow for preparation of mouse intestinal Swiss-roll sections for 10x Genomics Xenium in situ spatial transcriptomic analysis.

(B) Representative hematoxylin and eosin (H&E)-stained images of mouse small-intestinal Swiss-roll sections (left panels) and corresponding cell boundary segmentation generated using the 10x Genomics cell segmentation kit with overlaid transcript signals (right panels). Blue, nuclei; Red, cell boundaries; Yellow, RNA; Green, protein. Scale bars, 2000 μ m.

(C) Uniform manifold approximation and projection (UMAP) visualization of all identified cell populations, colored according to parental cell clusters.

(D) Subclustering of epithelial cells and dot plots of representative marker genes.

(E) Relative abundance of epithelial cell subclusters across the Control, Syn, and Allo groups. n=2 Control, n=4 Syn, n=4 Allo mice.

(F–M) GO Biological Process enrichment analysis of cell-type-specific transcriptional changes.

GO Biological Process enrichment was assessed by over-representation analysis of significantly upregulated genes identified by pseudobulk DESeq2 analysis. Genes were selected using a Benjamini–Hochberg-adjusted P value < 0.05 and a log₂ fold change > 0.25 in the indicated direction. All Entrez-mapped genes included in the corresponding differential expression analysis were used as the background gene universe. The x axis indicates GeneRatio, defined as the proportion of input genes associated with each GO term. Dot size indicates the number of input genes associated with the term, and dot color indicates $-\log_{10}$ of the Benjamini–Hochberg-adjusted P value.

(F and G) Dot plots showing GO terms enriched among genes upregulated in Allo relative to Syn or in Syn relative to Allo in Paneth cells (F) and transit-amplifying (TA) cells (G). Terms enriched among genes upregulated in Allo are displayed on the right, whereas those enriched

825 among genes upregulated in Syn are displayed on the left. Negative x-axis positions are used
826 solely for mirrored visualization and do not indicate negative enrichment.

827 (H and K–M) GO terms enriched among genes upregulated in Allo relative to Syn in IEC4 and
828 IEC7 (H), CD4 2 (K), CD8 6 (L), and CD8 7 (M).

829 (I) Subclustering of immune cells and dot plots of representative marker genes.

830 (J) Relative abundance of immune cell subclusters across experimental groups. n=2 Control,
831 n=4 Syn, n=4 Allo mice.

832 ISC, intestinal stem cell; TA, transient amplifying cell; EEC, enteroendocrine cell; B, B cell;
833 cDC1, conventional dendritic cell 1; ILC, innate lymphoid cell; moDC, monocyte-derived
834 dendritic cell; pDC, plasmacytoid dendritic cell; Plasma, plasma cell.

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836 .

Figure 2. Spatially resolved analysis of epithelial, lamina propria, and smooth muscle niches in the murine small intestine.

(A) Representative 10x Genomics Xenium Explorer image showing the epithelial layer, lamina propria, and smooth muscle compartments of the mouse small intestine.

(B) Comparison of cell-type composition within crypt regions between the Syn and Allo groups.

(C) Dominant cell–cell interactions identified within crypt regions of the Syn group, ranked according to interaction weight.

(D) Dominant cell–cell interactions identified within crypt regions of the Allo group, ranked according to interaction weight.

(E) Differential cell–cell interaction analysis within crypt regions, showing interactions enriched in the Allo group (positive scores) and reduced in the Syn group (negative scores).

(F) Comparison of cell-type composition within the lamina propria between the Syn and Allo groups.

(G) Dominant cell–cell interactions identified within the lamina propria of the Syn group, ranked according to interaction weight.

(H) Dominant cell–cell interactions identified within the lamina propria of the Allo group, ranked according to interaction weight.

(I) Differential cell–cell interaction analysis within the lamina propria, showing interactions enriched in the Allo group (positive scores) and reduced in the Syn group (negative scores).

(J and K) Heatmaps showing pathways upregulated within the crypt niche and lamina propria niche. Columns represent experimental groups, and rows represent the indicated genes and pathways.

n=4 Syn; n = 4 Allo mice. Each graph represents one group.

Figure 3. Spatial characterization of the crypt microenvironment reveals epithelial–stromal–immune interactions in gastrointestinal GVHD

(A) Representative Xenium Explorer image showing crypt regions and the surrounding microenvironment. Crypt niches were defined as regions within a 30- μ m radius surrounding ISCs and TA cells. Scale bar, 100 μ M.

(B) Comparison of cellular composition within the crypt microenvironment between the Syn and Allo groups.

(C) Volcano plots showing differential gene expression between Allo and Syn in CD8 3, Fibroblast 2, and Macrophage 2. The x axis indicates the average log2 fold change, with positive and negative values denoting higher expression in Allo and Syn, respectively, and the y axis indicates $-\log_{10}$ of the Benjamini–Hochberg-adjusted P value. Genes with an adjusted P value < 0.05 and an absolute average log2 fold change > 1 are shown. Blue and red indicate genes expressed at higher levels in Syn and Allo, respectively. Dashed lines indicate the corresponding adjusted P -value and fold-change thresholds. Gene labels represent the union of the 15 genes with the largest absolute log2 fold changes and the 15 genes with the lowest adjusted P values in each direction. To minimize potential cross-cell-type contamination, PanglaoDB (released March 27, 2020)-annotated epithelial marker genes were excluded from all plots, with additional exclusion of non-target-cell markers from the CD8 3 and Macrophage 2 plots.

(D) Heatmap showing the expression of selected cell-death-related genes in transit-amplifying (TA) cells from Syn and Allo recipients. Normalized expression values were averaged across TA cells within each biological sample and converted to row-wise z scores for each gene across samples. Columns represent individual biological samples, and rows represent the indicated genes. Colors indicate relative expression for each gene across samples, with blue and red representing lower and higher z scores, respectively. The top annotation indicates the experimental group.

(E) Neighborhood interaction networks of the crypt microenvironment in Syn and Allo groups. Condition-specific networks were constructed from sample-level spatial neighborhood enrichment scores. ISC and TA clusters were used as seeds, and only directly connected first-order neighbors (hop ≤ 1) were retained. Edges represent positive spatial associations with a mean enrichment Z score ≥ 3 and Z ≥ 3 in at least two samples. Edge width and opacity indicate the mean enrichment Z score, and node size represents cell abundance. Cell populations represented by fewer than 100 cells were excluded. Left panel; Syn. Right panel: Allo.

(F) Representative spatial image showing CD8⁺ T cells localized in proximity to crypt epithelial cells. Left panel; Syn. Right panel: Allo. Scale bar, 100 μ M.

(G-J) Cell-cell communication was inferred separately in the Syn and Allo groups using CellChat v2 from log-normalized Xenium expression data and spatial coordinates. Interaction probabilities were calculated using the mouse CellChat database with paracrine and contact ranges of 250 and 15 μ m, respectively, and communications involving fewer than 10 cells were excluded. In the butterfly plots, ligand-producing clusters are positioned centrally, with target clusters in Syn and Allo shown on the left and right, respectively; colors denote ligand-receptor pairs. In the chord diagrams, directed links indicate signaling from source to target clusters. Line width represents interaction probability.

(G) Butterfly plot comparing selected ligand-receptor interactions, including Granzyme-A (GZMA)-associated signaling, between CD8 T cells and TA or ISCs in the crypt.

(H and I) Chord diagrams comparing SPP1- (H) and midkine (MK)-mediated (I) signaling within the crypt microenvironment. SPP1-mediated interactions were detected only in Allo, whereas MK-mediated interactions were detected only in Syn; no interactions meeting the CellChat analysis criteria were detected in the respective comparison group.

(J) Butterfly plot comparing Neuregulin (NRG1)-mediated ligand-receptor interactions between the Syn and Allo groups, highlighting the Syn-specific NRG1-ERBB2/ERBB3 interaction.

(K) Schematic summary of the major findings shown in (G-J). n = 4 Syn; n = 4 Allo mice.

Figure 4. Region-of-interest analysis identifies enrichment of *Dio2*⁺ *IL11*⁺ fibroblasts in severe gastrointestinal GVHD lesions

(A) Spatial mapping of region-of-interest (ROI) areas across the Syn group and moderate and severe lesion regions from the Allo group.

(B) Comparison of overall cell-type composition across experimental groups. Data are derived from n=4 Syn samples, n=6 Allo samples, and n=3 severe lesions.

(C) Representative spatial image showing localization of *Dio2* transcripts relative to fibroblasts within regions of disrupted epithelial architecture. Scale bar, 100 μ m.

(D) Dot plots showing *Dio2* expression across enteric nerve (EN) and fibroblast subclusters in spatially defined Syn and Allo ROIs. Dot size indicates the percentage of expressing cells, and color represents mean expression. Values were calculated per ROI and averaged by group and intestinal location.

(E) Volcano plot showing differential gene expression between *Dio2*-positive (red dots) and *Dio2*-negative (blue dots) fibroblasts within Allo severe ROIs. Differentially expressed genes (DEGs) were identified by comparing *Dio2*-positive (n = 973; red) and *Dio2*-negative (n = 6,052; blue) fibroblasts within the Allo Severe group (Total n = 7,025). To avoid arbitrary cutoffs, the threshold for *Dio2* positivity (log-normalized expression > 1.03) was statistically determined by identifying the local minimum in the kernel density estimation of *Dio2* expression across the target cell population. The x-axis shows the log₂ fold change, and the y-axis represents the -log₁₀ adjusted *P* value. Significant DEGs (adjusted *P* < 0.05 and |log₂ fold change| $\geq$ 1) are colored to indicate upregulation in *Dio2*⁺ (Positive) or *Dio2*⁻ (Negative) cells, with the top 30 most significant genes labeled. Non-significant genes are shown in grey.

(F) Spatial distribution of sympathetic neuron-associated gene expression across all EN clusters within each ROI region. Left panel, *Dbh* expression (norepinephrine biosynthetic gene). Right panel, *Slc18a2* expression (norepinephrine transporter gene). Dot size indicates the percentage of expressing cells, and color represents mean expression. Values were calculated per ROI and

938 averaged by group and intestinal location.

939 (G) Spatial neighborhood enrichment networks across intestinal conditions. Networks for the
940 Syn_Ileum (a), Allo_Ileum (b), and Allo_Severe (c) groups. Nodes represent cell clusters, with
941 sizes proportional to total cell counts. Edges indicate significant spatial colocalization (median Z-
942 score ≥ 2.0), evaluated via k-nearest neighbor ($k=8$) spatial graphs and permutation testing
943 ($n=100$). Edge thickness reflects the median enrichment Z-score across all ROIs. For clarity,
944 only the top 8 strongest interactions per cluster are displayed, and isolated nodes lacking
945 significant connections are omitted.

946 (H) Heatmap showing the aggregated spatial communication probabilities of identified signaling
947 pathways across three experimental groups: Syn, Allo (non-Severe region), and Allo_Severe.
948 Cell-cell communication analysis was performed using CellChat (v2) on spatial transcriptomics
949 data, incorporating spatial distances and cell proximity. For each pathway, raw communication
950 probabilities from individual samples were aggregated by group and subsequently normalized
951 as Z-scores across the groups. The color gradient represents the relative signaling strength,
952 ranging from blue (downregulated) to white (mean) and red (upregulated). Unsupervised
953 hierarchical clustering (left dendrogram) groups pathways exhibiting similar activation dynamics
954 corresponding to the progression of disease severity.

955 Syn, $n = 2$ mice; Allo (moderate), $n = 6$ mice; Allo (severe), $n = 3$ mice.

Figure 5. Spatial emergence of *Dio2*⁺ *IL11*⁺ fibroblast and the impact of thyroid hormone and IL11 in gastrointestinal GVHD.

(A) T3 quantification in the terminal ileum of Syn and Allo mice at day 7 after HSCT. Statistical significance was determined by t-test. **p* < 0.05.

(B) RNAscope analysis of the small intestine at day 7 after HSCT. Left panel: Representative image of Syn sample. Right panel: Representative Image of Allo sample. *Il11* (yellow), *Dio2* (cyan), *Pdgfra* (green), and DAPI (blue) signals were analyzed using ImageJ. Binary masks were generated using channel-specific thresholds that were kept constant across experimental conditions, and the *Il11*⁺*Dio2*⁺*Pdgfra*⁺ overlapping area was identified by sequential AND operations. The triple-positive area was normalized to the DAPI-positive area. Each dot represents one region of interest (ROI).

(C) Quantification was performed using images acquired from multiple distinct locations within the same Swiss roll.

(D) *Dio2* and *Il11* gene expression in primary small intestinal fibroblasts following 10 μ M forskolin treatment for 8 hours. *n*=3 control, *n*=3 forskolin-treated. **p* < 0.05, ***p* < 0.01

(E) Time-course analysis of *Ppargc1a* gene expression following treatment with 400nM T3 in organoids cultured for 5 days from Allo-derived crypts. *n*=3 baseline, *n*=3 4hour, *n*=3 8hour, *n*=24 hour. ***p* < 0.01, ****p* < 0.001, ns;not significant.

(F) Representative images of organoids derived from Syn crypts and Allo crypts treated with T3 and/or NH-3. Scale bar, 500 μ m.

(G) Comparison of organoid size (μ m²) between Syn-derived organoids and Allo-derived organoids treated with 400 nM-1 μ M T3 or 1-10 μ M NH-3 after 5 days of culture. **p* < 0.05, ***p* < 0.01, *****p* < 0.0001.

(H-I) Comparison of organoid size after 5 days culture in crypts under basal conditions (H) or following treatment with 100 pg/ml IFN γ (I), with or without 1ng/ml human IL11 and/or 400 nM T3. *****p* < 0.0001.

982 (J) Representative images of control intestinal organoids, organoids co-cultured with intestinal
 983 fibroblasts, and organoids co-cultured with 10 μ M forskolin-treated intestinal fibroblasts. Scale
 984 bar, 500 μ m.

985 (K) Comparison of organoid size after 5 days of co-culture with primary interstitial fibroblasts or
 986 fibroblasts pretreated with 10 μ M forskolin for 8 hours. **** $p < 0.0001$.

987 (L) Representative RNAscope images of the colon from untreated mice and mice with 3% DSS-
 988 induced colitis. Left panels: H&E staining and fluorescent RNA *in situ* hybridization images of
 989 the colon from untreated mice. Right panels: corresponding images of the colon from mice with
 990 3% DSS-induced colitis. Scale bar, 50-750 μ m.

991 (M) Quantification of *Dio2*⁺ *IL11*⁺ fibroblasts in the colons of the control mice and mice after 7
 992 days of 3% DSS administration. Lesional areas were analyzed in DSS-treated mice, whereas
 993 regions of interest were randomly selected in control mice. n=3 control, n=3 3%DSS. Ns; not
 994 significant.

995 (N-O) *DIO2* and *Il11* expression in Stromal 2 cells. Sample-level pseudobulk expression of *DIO2*
 996 (N) and *Il11* (O) in Stromal 2 cells from healthy control (HC) and Crohn's disease tissues. Each
 997 dot represents one sample; boxes show the median and interquartile range. Differential
 998 expression was assessed using DESeq2, with Benjamini–Hochberg-adjusted P values shown.
 999 HC, n=7; Crohn's disease, n=12.

1000 (P) Representative spatial distribution of cells within an ulcerated lesion of the ileum. Highlighted
 1001 are transit-amplifying cells (TAs, blue) and undifferentiated epithelium (green) alongside Stromal
 1002 2 (orange). Other cell lineages are shown in gray.

1003 (Q) Spatial two-step neighborhood network centered Stromal 2 in Crohn's disease (radius $\leq$ 50
 1004 μ m). Node size corresponds to cell count, and color indicates cell lineage. Edge thickness
 1005 reflects the significance of spatial co-occurrence ($\log_{10}$ mean Z-score $\geq$ 2).

1006 Data are represented as mean $\pm$ SEM. Scale bars, 50-400 μ m. Statistical significance was
 1007 determined by ordinary one-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

1008

METHODS**1009 Key resources table**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Recombinant Human IL-11 (carrier-free) 10 ug	Biolegend	585902
NH-3	TargetMol	T40548
IntestiCult mouse organoid growth medium	Stem cell technologies	06005
Matrigel growth factor reduced basement membrane	Corning	356230
Hematoxylin Solution, Gill No.3	Sigma-Aldrich	GHS316-500ML
Alcoholic Eosin Y 515	Leica Biosystems	3801615
Dako bluing buffer	Agilent Technologies	CS-702
Collagenase from <i>Clostridium histolyticum</i>	Sigma-Aldrich	C7657
Dispase® II	Sigma-Aldrich	D4693
Forskolin	Fisher scientific	50-295-2141
Dextran sulfate sodium salt, MW	Thermo scientific	J63606.22
Triiodo-L-thyronine	Sigma-Aldrich	T6397
Critical commercial assays		
Triiodothyronine (T3) competitive ELISA kit	Fisher scientific	EIAT3C
RNAscope Multiplex Fluorescent Reagent Kit	Advanced Cell Diagnostics	
Xenium in situ Prime 5K Mouse Pan Tissue	10X Genomics	1000672
Xenium Prime 5K Add-on Custom up to 50 Gene Panel (12 rxns) - CDP# BJJ8BU	10X Genomics	1000672
Xenium Thermocycler Adaptor Kit	10X Genomics	1000623
Xenium Slides & Sample Prep Reagents (2 slides, 2 rxns)	10X Genomics	1000460
Xenium Cell Segmentation Add-on Kit (2 rxns)	10X Genomics	1000662
Xenium Decoding Consumables (1 run, 2 slides)	10X Genomics	1000487
Xenium Decoding Reagents (1 run, 2 slides)	10X Genomics	1000461
Reagent or resource		
RNAscope™ Probe - Mm-Il11-C2 - Mus musculus interleukin 11 (Il11) transcript variant 2 mRNA	Bio-technie	552461-C2
RNAscope™ Probe - Mm-Dio2-C4 - musculus deiodinase iodothyronine type II (Dio2) mRNA	Bio-technie	479331-C4
RNAscope™ Probe - Mm-Pdgfra - Mus musculus platelet derived growth factor receptor alpha polypeptide (Pdgfra) transcript variant 1 mRNA	Bio-technie	480661
ClariTSA™ Fluorophore 520	Bio-technie	323271
ClariTSA™ Fluorophore 650	Bio-technie	323273
Opal 570 Reagent Pack	Akoya Biosciences	FP1488001KT
Opal 780 Reagent Pack	Akoya Biosciences	FP1501001KT
RNeasy kit	QIAGEN	74104
ITaq™ Universal SYBR Green One-Step Kit	BIO-RAD	1725151
Deposited data		

Spatial transcriptome data of human Crohn disease	Colleen et el. Nature 2026.	DOI:10.17632/mxy6p6wfmy.2
Raw and analyzed data-spatial transcriptome	This paper	GEO: GSE342936
Experimental models: Organisms/strains		
Mouse: C57BL/6	Charles River	Cat#027
Mouse: BALB/c	Charles River	Cat#028
Oligonucleotides		
Primers for RT-qPCR analysis, see Table S5	Sigma-Aldrich	N/A
Software and algorithms		
GraphPad Prism10	GraphPad	https://www.graphpad.com
R	Tha R Foundation	http://www.r-project.org
Xenium Explorer	10X Genomics	https://www.10xgenomics.com/support/jp/software/xenium-explorer/latest
ImageJ	NIH image	Version 1.54g
Other		
MouseCD90.2microbeads	Miltenyi Biotec	130-121-278
Pan T Cell Isolation Kit II, mouse	Miltenyi Biotec	130-095-130

1010

Bone marrow transplantation model and DSS-induced colitis model.

Eight to twelve weeks C57BL6 (#027, B6, H-2Kb) and BALB/c (#028, H-2Kd) were purchased from Charles River Laboratories. Mice were maintained in specific-pathogen-free (SPF) conditions. Institutional animal care and use committee (IACUC) protocol number: AN-8909. An allogeneic mouse model of GVHD was generated as previously described.⁵¹ Briefly, splenic T cells from BALB/c or C57BL/6 donors were enriched by utilizing Pan T Cell Isolation kits, and bone marrow was collected from the same donors. CD90.2 MicroBeads were used for T cell depletion. On day 0, 3×10^6 T cells and 5×10^6 bone marrow cells were injected intravenously into C57BL/6 recipient mice. Recipient mice received 10 Gy of irradiation on day -1. The mice were randomly assigned to syngeneic or allogeneic groups in each experiment. DSS-induced colitis was established as previously described.²² Briefly, mice were provided with 3% (w/v) dextran sulfate sodium (DSS) in drinking water ad libitum for 7 consecutive days. Control mice received regular drinking water throughout the experiment.

Intestinal organoid generation

Intestinal crypt-derived organoids culture was adapted from previous reports.¹⁰ Briefly, the small intestine was harvested from mice, and luminal contents were removed by washing with ice-cold PBS. The intestine was opened longitudinally and cut into approximately 2-mm fragments. Tissue fragments were incubated in 10 mM EDTA in PBS at 4 degrees for 40 minutes and subsequently transferred to ice-cold PBS. Crypts were mechanically dissociated by gently pipetting and filtered through a 70 μ m cell strainer. The procedures were repeated 3-5 times, and the number of isolated crypts was counted. Crypts were embedded in Matrigel and cultured in crypt culture medium. Crypts were seeded at a density of 2-5 crypts per μ l in 50 μ l Matrigel droplets plated in 24 well plates. Plates were incubated at 37 degrees for 30 minutes to allow Matrigel solidification, after which crypt culture medium was added. The culture medium

was replaced every two days. For passage, Matrigel was disrupted with ice-cold PBS on day 7; crypts were collected and counted, and cultures were re-seeded at the same density.

Isolation and culture of primary intestinal fibroblasts

Isolation and culture of primary mouse intestinal fibroblasts were performed as previously described with minor modifications.⁵² Briefly, mice were euthanized, and the ileum was harvested, opened longitudinally, and washed thoroughly with CMF buffer (Ca²⁺/Mg²⁺-free Hanks' balanced salt solution supplemented with 25 mM sodium bicarbonate and 2% fetal bovine serum) to remove luminal contents. The tissue was cut into ~5-mm fragments and incubated in 1× HBSS containing 5 mM EDTA, 1 mM DTT, and 1% penicillin-streptomycin at 37°C with shaking (200 rpm) for 20 min to remove the epithelial layer. Tissue fragments were then washed in the CMF buffer and vortexed vigorously for 15 seconds three times. The remaining tissue was digested in DMEM/F12 containing 300 U/mL collagenase type XI and 0.08 U/mL dispase at 37°C with shaking (200 rpm) for 40 min to isolate stromal cells. Following enzymatic digestion, the suspension was vigorously triturated, passed through a 70-µm cell strainer, and centrifuged at 280 × g for 10 min at room temperature. Cell pellets were resuspended in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin and cultured at 37°C in a humidified incubator with 5% CO₂. Culture medium was replaced every 2 days.

Xenium *in situ* spatial imaging analysis

FFPE tissues were analyzed using the 10x Genomics Xenium Analyzer according to the Xenium in situ Gene Expression protocols (CG000580, CG000760, CG000246, and CG000613). Briefly, 5 µm FFPE tissue sections were mounted onto Xenium slides, followed by deparaffinization and permeabilization to allow access to mRNA. A probe set targeting 5000 pre-deigned genes and 56 custom genes (Supplementary Table 2) was hybridized overnight for

16 hours. After hybridization, the probe ends were ligated to the targeted RNAs, forming circular DNA probes with high specificity. Rolling Circle Amplification was then performed to amplify each RNA-binding event, thereby enhancing the signal-to-noise ratio. To minimize tissue autofluorescence, background fluorescence was chemically quenched. Sections were subsequently stained with DAPI, and the slides were loaded onto the Xenium instrument for imaging and transcript decoding (Xenium software version 2.0.0). Secondary analysis, including cell segmentation and transcript assignment, was performed directly on the instrument. Data output quality, initial morphology images, subcellular transcript localization, segmentation, and clustering were evaluated and visualized using Xenium Explorer.

Thyroid hormone extraction and quantification

Because T3 is a lipophilic hormone, samples were prepared for ELISA analysis according to previously published protocols with minor modifications to the manufacturer's protocol. For plasma samples, plasma was mixed with six volumes of ethyl acetate, vortexed for 2 min, and incubated at room temperature for 5 min. Samples were then frozen on dry ice, and the upper organic phase was collected. The solvent was evaporated using a SpeedVac concentrator for 1 h, and the dried extracts were used for ELISA analysis.

For tissue samples, approximately 100 mg of terminal ileum tissue was collected and homogenized in 1 mL of ethyl acetate using a tissue homogenizer. Homogenates were centrifuged at 5,000 rpm for 15 min at 4°C, and the supernatants were collected. The solvent was evaporated using a SpeedVac concentrator for 1 h. The resulting pellet was resuspended in 100 µl of ethanol and diluted with 400 µl of assay buffer. Samples were vortexed thoroughly and incubated for 5 min at room temperature. The extracts were subsequently diluted 1:10 in assay buffer before analysis.

T3 concentrations were quantified using a commercial ELISA kit according to the manufacturer's instructions.

1088

1089 **RNAscope Multiplex Fluorescent *In Situ* Hybridization**

1090 Paraffin embedded mouse gut tissue sections were stained using the RNAscope Multiplex
1091 Fluorescent Reagent kit v2 (ACDbio Bio-technique), following the vendor's procedure (UM
1092 323100/Rev D). Tissues were pretreated with protease and treated with H₂O₂ to remove
1093 endogenous peroxidase activity. ACD gene probes were hybridized to the tissue sections
1094 targeting the following mRNA molecules: *Dio2*, *Pdgfra* and *Il11*. The bound molecules were
1095 amplified using the ACDbio RNAscope technology. The final step is to bind Opal or Vivid dyes
1096 to the signaling molecules using tyramide signal amplification (TSA) to covalently bind the
1097 fluorophores to the tissue. *Dio2* was detected with Vivid 520, *Pdgfra* with Opal 570 and *Il11* with
1098 Vivid 570. These images were acquired using a Leica Stellaris confocal microscope.

1099

1100 **RNA isolation and RT-PCR**

1101 Total RNA from mouse primary fibroblasts or dissociated intestinal organoids was isolated using
1102 the RNeasy Kit. One-step RT-qPCR was performed using the iTaq™ Universal SYBR® Green
1103 One-Step Kit (Bio-Rad) with gene-specific primers according to the manufacturer's instructions.
1104 Gene expression levels were normalized to β-actin expression within each sample, and relative
1105 gene expression was calculated using the ΔΔCt method.

1106

1107 **Triiodothyronine and NH-3 treatment of organoid**

1108 Freshly isolated crypts were embedded in Matrigel, and every 2 days the existing medium was
1109 replaced with fresh standard organoid culture mediums containing the indicated concentrations
1110 of T3 or NH-3. The number of organoid buds was counted, and representative images were
1111 acquired on day 5 of culture.

1112

1113 **Recombinant mouse cytokine treatment of organoids**

1114 Freshly isolated crypts were embedded in Matrigel, and every 2 days the existing medium was
1115 replaced with fresh standard organoid culture medium containing the indicated concentrations of
1116 IFNg. The number of organoid buds was counted, and representative images were acquired on
1117 day 5 of culture

1118

1119 **Coculture of intestinal organoids with primary small intestinal fibroblasts**

1120 Coculture of small intestinal organoids with primary small intestinal fibroblasts was performed as
1121 previously described. Primary small intestinal fibroblasts were first isolated and seeded at $4 \times$
1122 10^5 cells per well on 48-well plates. Cells were cultured for 16–24 h at 37°C in a CO₂ incubator
1123 to allow fibroblast attachment. The culture medium was then removed, and 50 freshly isolated
1124 small intestinal crypts suspended in 50 µl of Matrigel were plated on each well. After incubation
1125 at 37°C for 30 min to allow Matrigel polymerization, fresh standard organoid culture medium
1126 was added. The medium was replaced every 2 days, and organoid size was quantified on day
1127 5. Representative images were acquired at the same time point.

1128

1129 **Organoid measurement and image**

1130 Organoid measurement was performed as previously report.^{53,54} The number of organoids and
1131 crypts were manually counted, and crypt formation was scored as the number of crypts divided
1132 by the number of organoids each well. The organoids were imaged by an Invitrogen EVOS
1133 M500 inverted Microscope or CellVoyager CV8000 High-Content Screening System
1134 (Yokogawa). Unless otherwise specified in the figure legends or method details, organoid
1135 assays 3 wells per group with at least 2 different mice were analyzed.

1136

1137 **Bioinformatics analysis**

1138 **Spatial transcriptomics data acquisition and preprocessing**

1139 10x Genomics Xenium spatial transcriptomics data were generated and processed using the
1140 standard 10x Genomics Xenium pipeline
1141 ([https://www.10xgenomics.com/support/software/xenium-onboard-analysis/latest/algorithms-](https://www.10xgenomics.com/support/software/xenium-onboard-analysis/latest/algorithms-overview/xa-algorithms)
1142 [overview/xa-algorithms](https://www.10xgenomics.com/support/software/xenium-onboard-analysis/latest/algorithms-overview/xa-algorithms)). Cell segmentation and initial k-means clustering were performed
1143 within the 10x pipeline output.

1144 Seurat v5.4.0 objects were created for each individual sample (four biological replicates each for
1145 the naive and syngeneic [Syn] conditions, and four for the allogeneic [Allo] condition, with two
1146 additional Allo replicates included specifically for downstream ROI and severe region analyses).
1147 Quality control was conducted for each sample individually to remove phantom cells and
1148 doublets. The scuttle package was used to create the cut offs for both instances with the
1149 function is Outlier. Following this quality control, samples were merged into a single Seurat
1150 object using the merge function with merge.dr set to TRUE.

1151

1152 **Cell clustering and annotation**

1153 Unsupervised clustering was performed using the FindNeighbors and FindClusters functions
1154 from Seurat on the merged dataset. Clusters were annotated based on established cell-type–
1155 specific marker genes such as *Myh11*, *Epcam*, and *Pecam1* to assign primary cell identity
1156 labels. Each primary cell population was then subset and subjected to secondary sub-clustering
1157 to resolve finer cellular heterogeneity. A resolution of 0.5 was used for each of the subsetted
1158 primary clusters. Clusters such as the immune cells needed further clustering with a higher
1159 resolution, so the clustertree package (Ver 0.5.1) was used to further isolate the resolution to
1160 1.1. Findallmarkers was run for each of the subclustering to create a fine-tuned labeling. In cases
1161 where subclusters were more consistent with an alternative primary lineage, they were
1162 reassigned to the appropriate primary cell type.

1163

1164 **Spatial niche and anatomical analysis**

1165 Spatial niche analysis was performed using Seurat's BuildNicheAssay function. Each sample
1166 was run independently for their field of view (FOV) to account for image-specific spatial
1167 organization. The default setting for the niches.k of 4 and default setting of neighbors.k of 20
1168 was applied to identify major anatomical regions within each sample. To further resolve the
1169 epithelial spatial heterogeneity into the crypt and villus domains, the k-means clustering
1170 provided by the 10x analysis was used to isolate cells that could show the separation. For each
1171 sample, a different k-means clustering was used after reviewing the Xenium files with Xenium
1172 Explorer v4.1.1.

1173

1174 **Cell–cell communication analysis**

1175 Cell–cell interaction analyses were conducted using CellChat (Ver 2.2.0.9001) within each
1176 defined anatomical niche and for each individual sample. All the CellChat functions were run
1177 with default parameters except for filterCommunication which was changed to have a min.cell of
1178 5. Following niche-specific interaction inference, datasets were merged to enable comparative
1179 analyses between Allo and Syn conditions. Results were visualized with netVisual_circle and
1180 netVisual_heatmap.

1181

1182 **IMAP spatial projection analysis**

1183 IMAP analysis was performed to visualize spatial relationships between cells relative to
1184 epithelial and smooth muscle compartments. Smooth Muscle cells that were used for distance
1185 calculations included the sub labeled clusters SMC 2, SMC 3, SMC 4, and SMC 6. To ensure

that the distance to the closest smooth cell was on the same row of the Swiss roll a center point was used so that the smooth muscle cell that was used must be closer to that center point than the cell being analyzed. IMAP calculations were conducted separately for each FOV to prevent spatial overlap artifacts. Following per-FOV IMAP computation, datasets were merged to generate final composite IMAP visualizations.

Region-of-interest analysis (ROI)

Regions of interest were defined in Xenium Explorer for anatomically matched regional analysis. For each sample, jejunal and ileal ROIs were selected to contain comparable numbers of cells across samples. In allogeneic samples, an additional inflammatory ROI was selected from the area with the most prominent local inflammatory changes within the same tissue section. Cells within each ROI were extracted from the corresponding sample-level Xenium Seurat object using spatial coordinates and were used for downstream analyses of local cellular composition, spatial proximity, and cell–cell communication.

Crypt-proximal niche

Crypt-proximal niches were defined by spatial proximity to stem cell (ISC) and transit-amplifying (TA) populations, which were identified using fine-grained cell annotations in Sub_Labels. For each sample, Euclidean distances from all cells to the nearest ISC or TA cell were calculated using spatial coordinates. Cells within a 30 μm radius were classified as crypt-proximal niche cells. This threshold was selected based on visual inspection in Xenium Explorer to capture the immediate microenvironment while avoiding the non-biological inclusion of cells from adjacent layers in the Swiss-roll tissue configuration. Niche extraction was performed independently for each sample prior to downstream pooled analyses.

1210

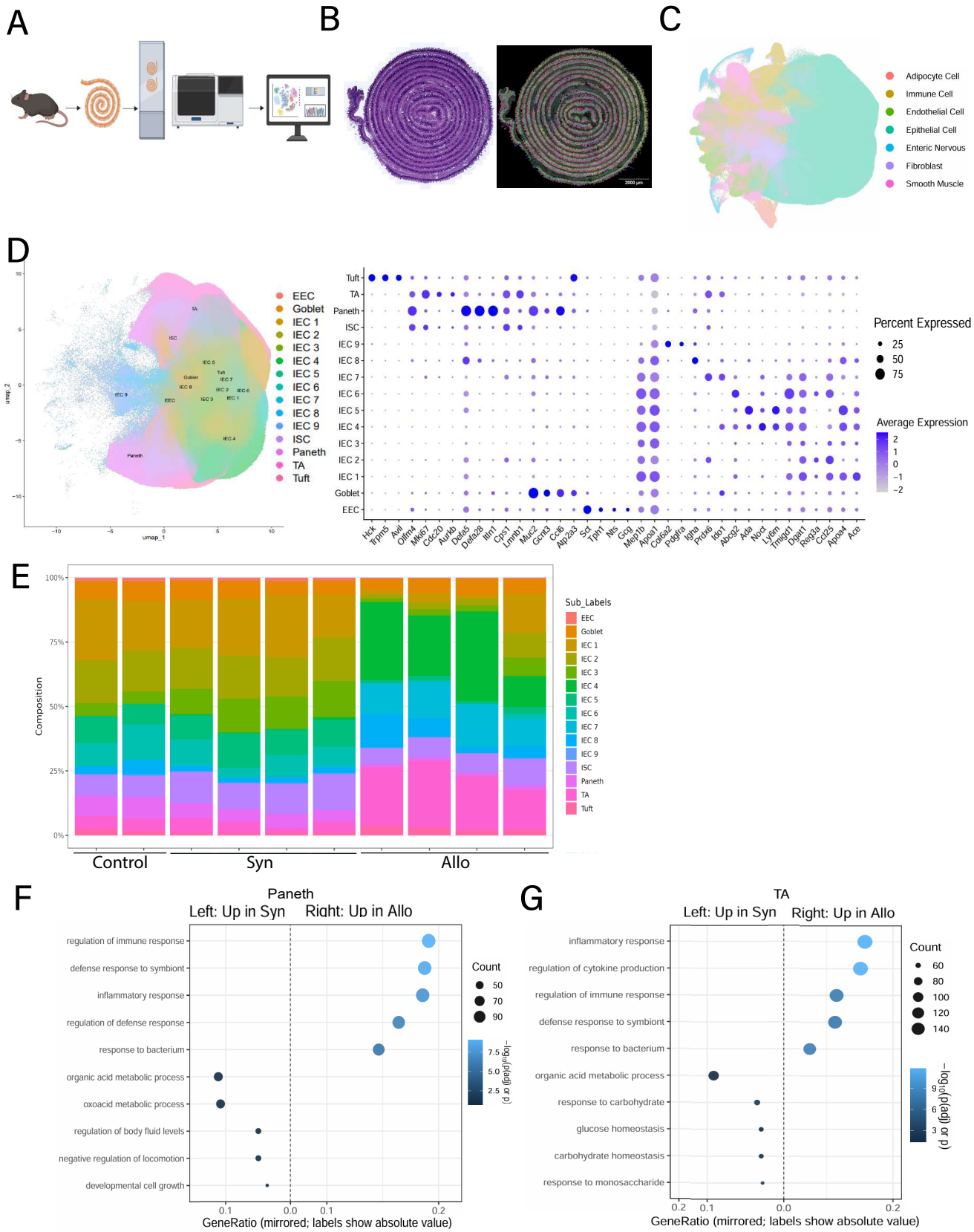
1211 **Cell–cell communication analysis**

1212 Cell–cell communication analysis was performed using CellChat v2.2.0.9001 to infer spatially
1213 informed ligand–receptor interactions for both crypt-proximal niche cells and ROI-derived cells.
1214 For each analysis, the Xenium assay was normalized, and fine-grained cell identities stored in
1215 Sub_Labels were used as CellChat identity classes. The mouse CellChat database was used
1216 for ligand–receptor inference. Spatial communication probabilities were estimated using
1217 CellChat’s spatial mode by incorporating cell coordinates into the analysis. Interactions involving
1218 cell groups with fewer than 10 cells were excluded. Pathway-level communication probabilities
1219 and aggregated interaction networks were then computed for downstream analysis. CellChat
1220 outputs were summarized at ligand–receptor and pathway levels. Pooled CellChat analyses
1221 were interpreted as spatially informed summaries of condition-associated signaling patterns,
1222 while statistical comparisons were performed using biological samples or sample-aggregated
1223 ROI measurements as the unit of analysis where applicable.

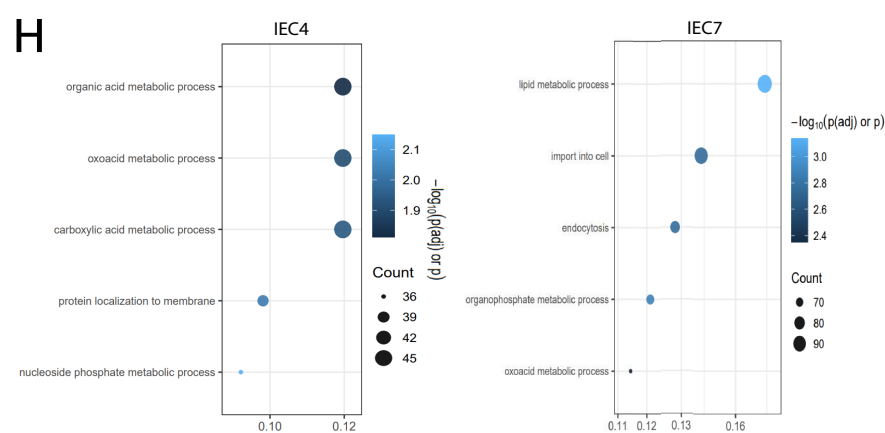
1224 [https://www.10xgenomics.com/support/software/xenium-onboard-analysis/latest/algorithms-
1225 overview/xoa-algorithms](https://www.10xgenomics.com/support/software/xenium-onboard-analysis/latest/algorithms-
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1226

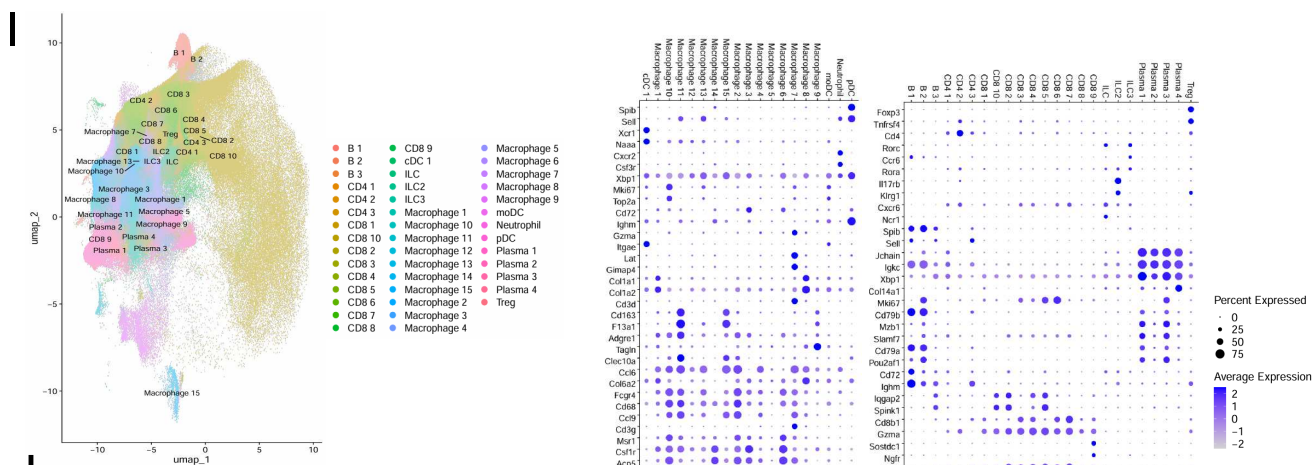
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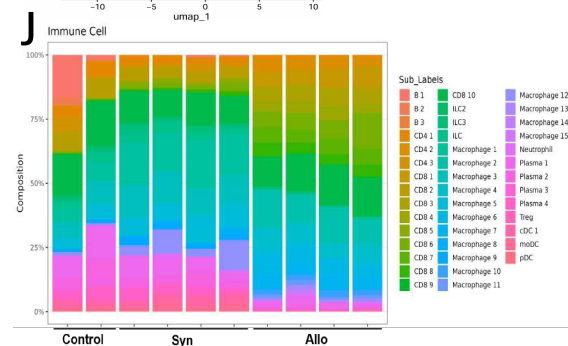
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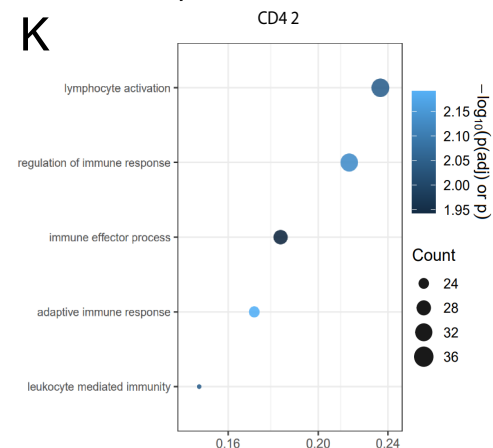
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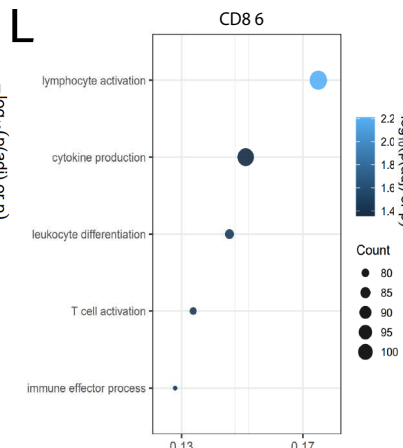
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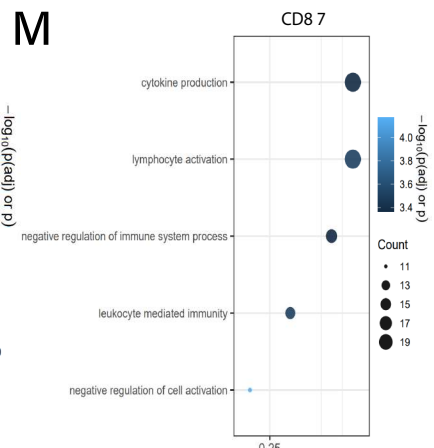
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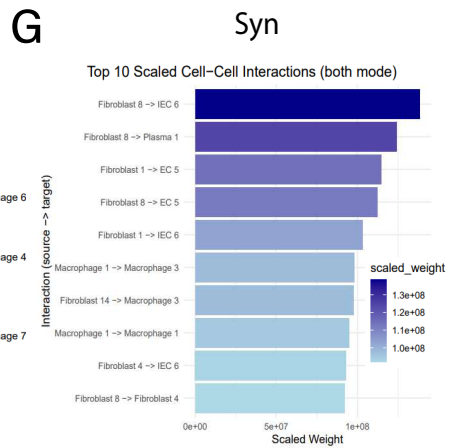
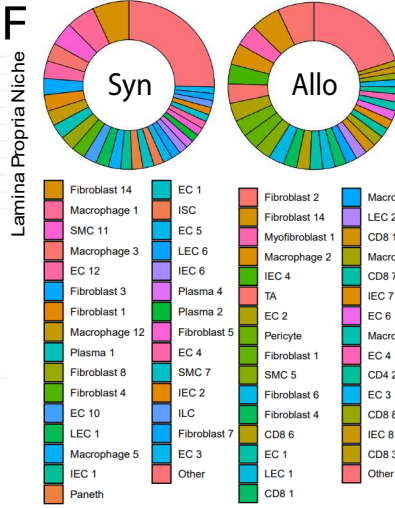
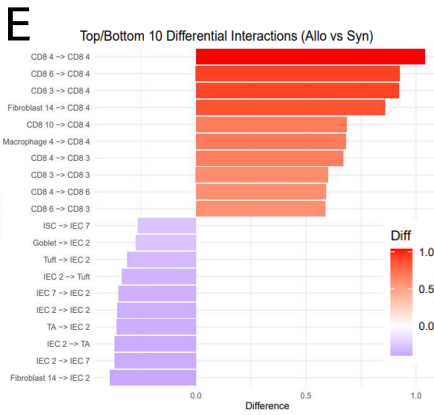
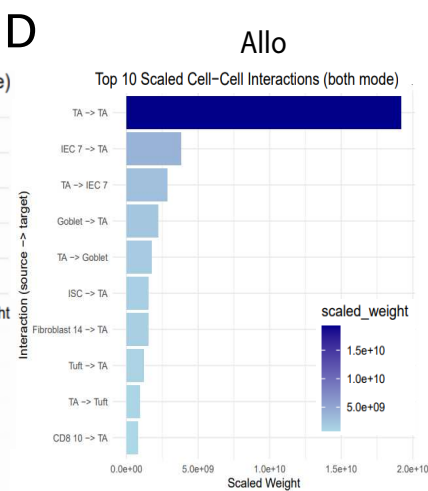
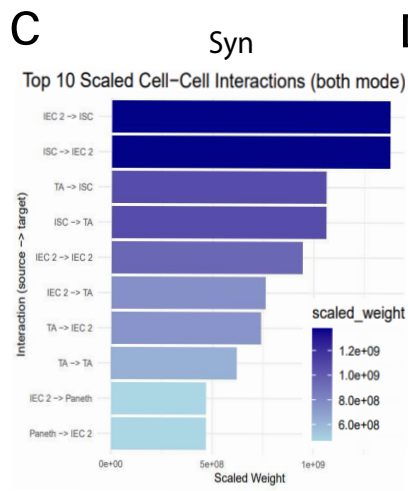
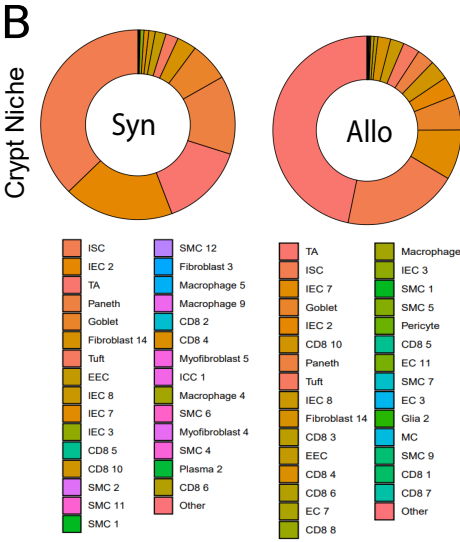
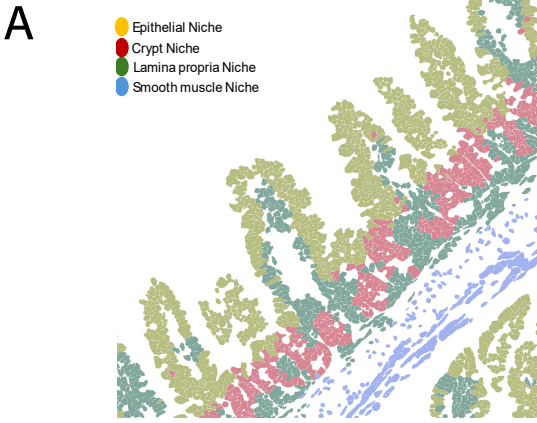


L



M

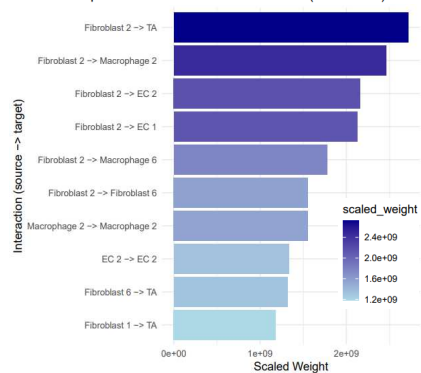




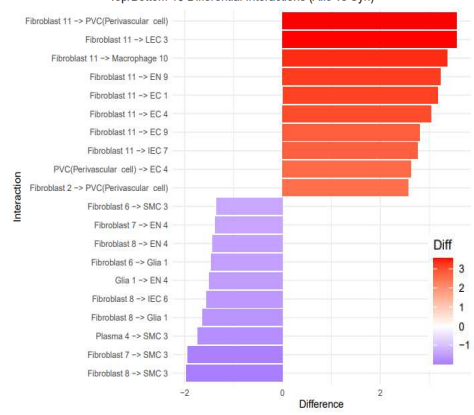
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Allo

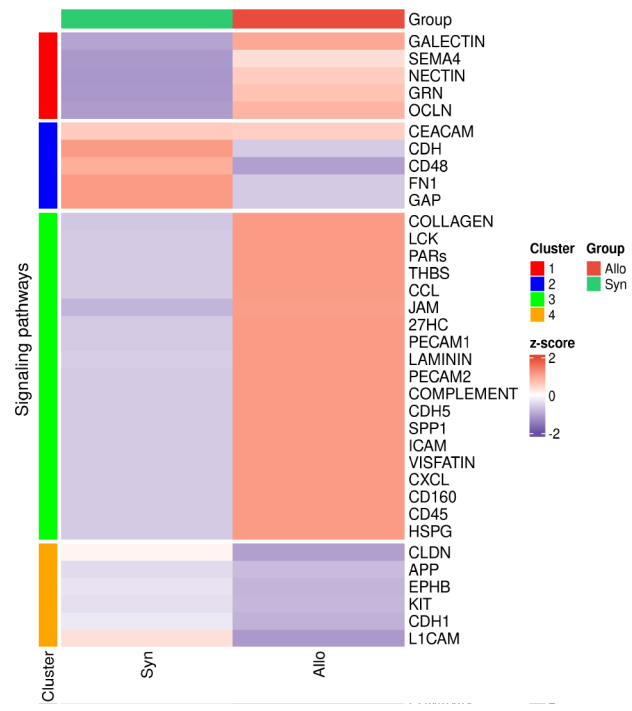
Top 10 Scaled Cell-Cell Interactions (both mode)



Top/Bottom 10 Differential Interactions (Allo vs Syn)

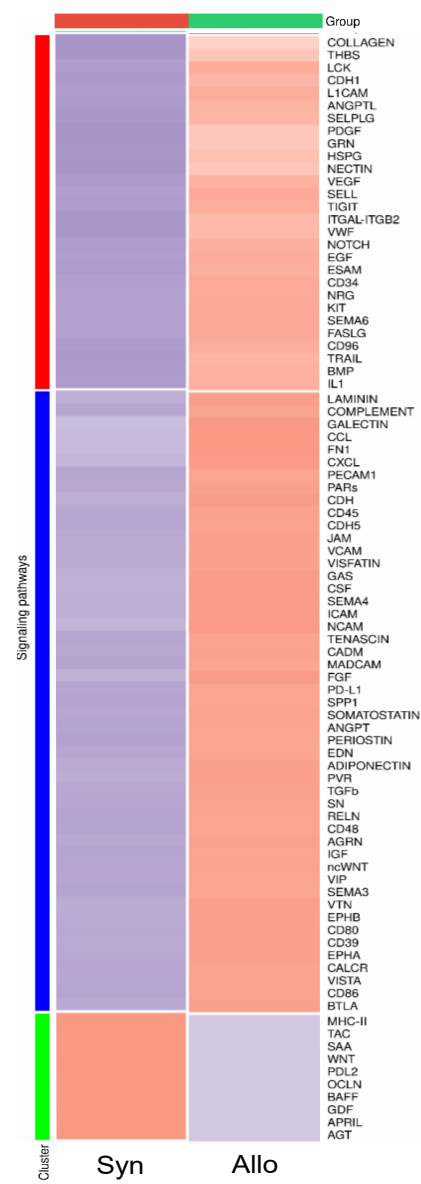


Crypt Niche

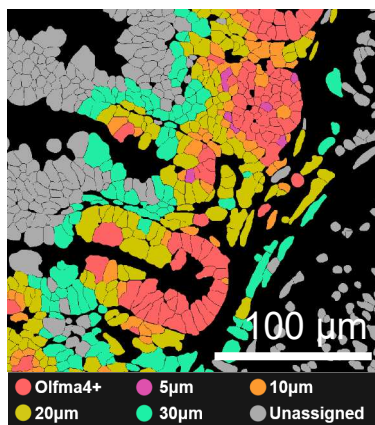


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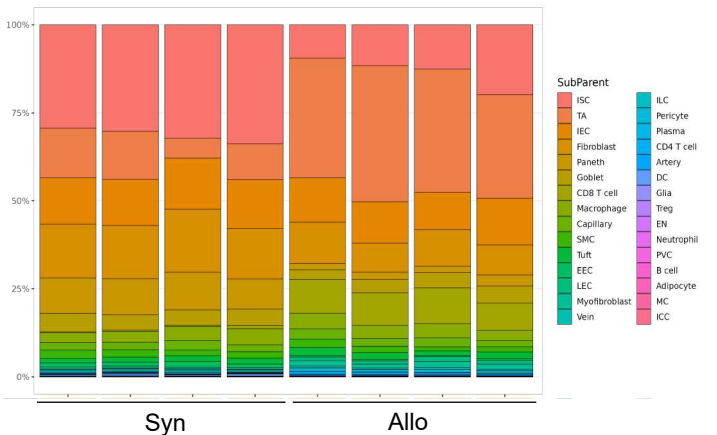
Lamina propria niche



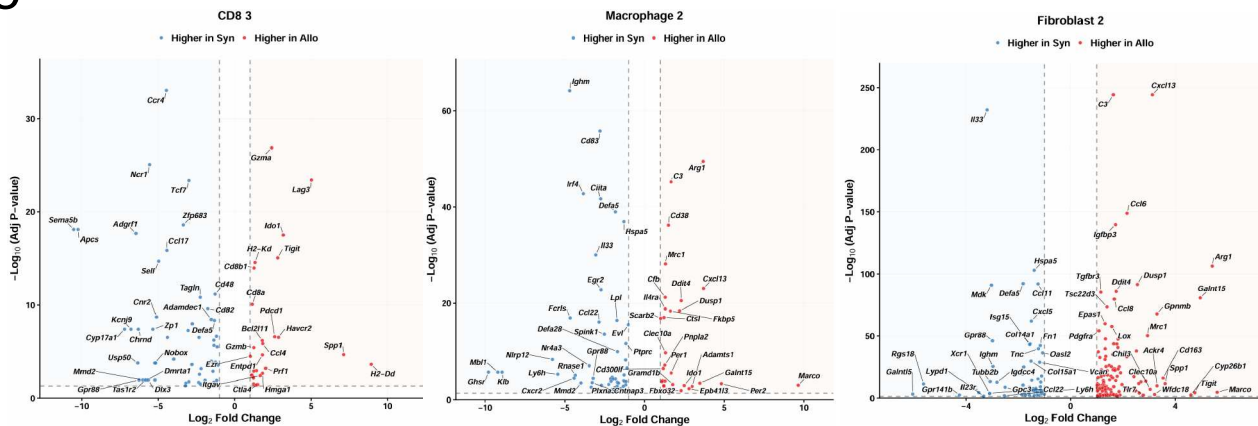
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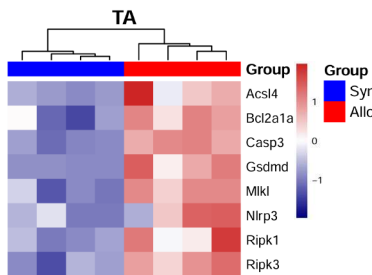
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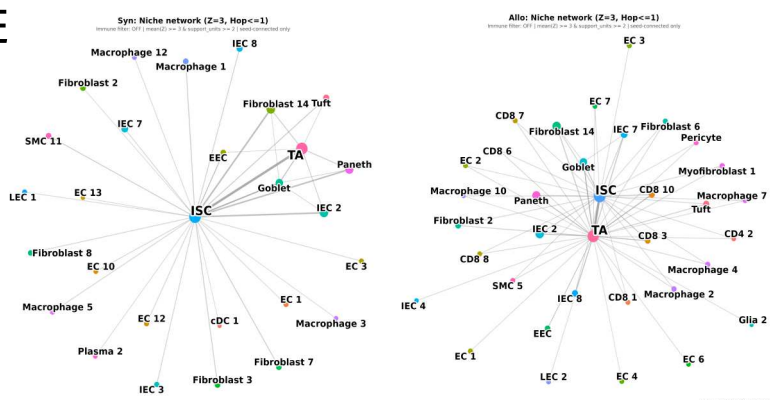
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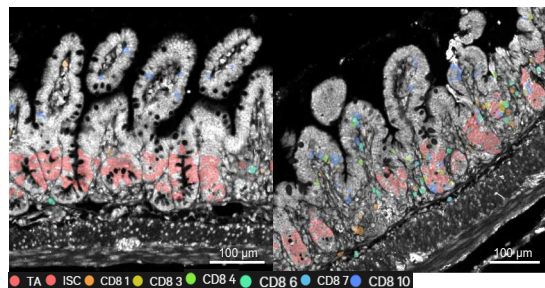
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E

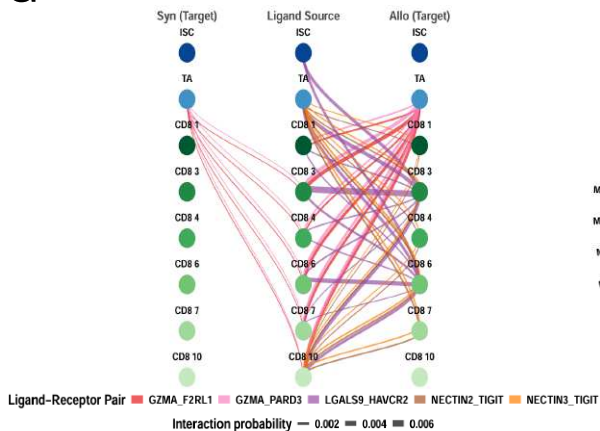


F



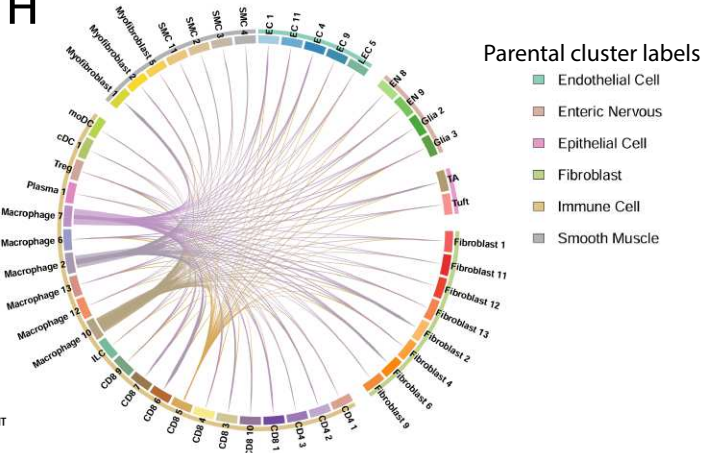
G

LR Signal Comparison: Syn vs Allo (Xenium Spatial)



H

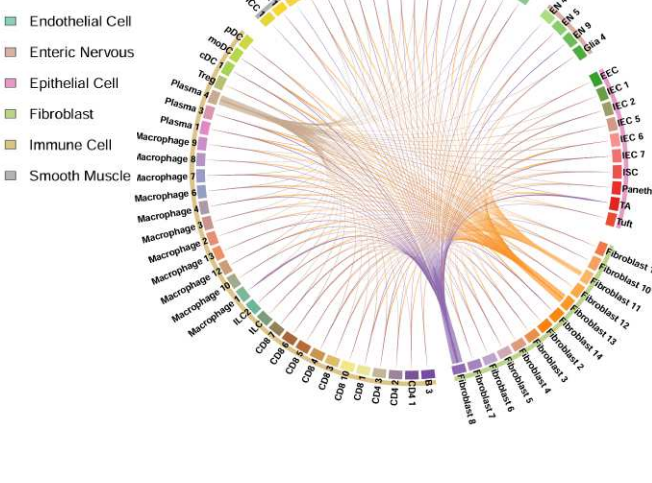
Spp1-Allo



I

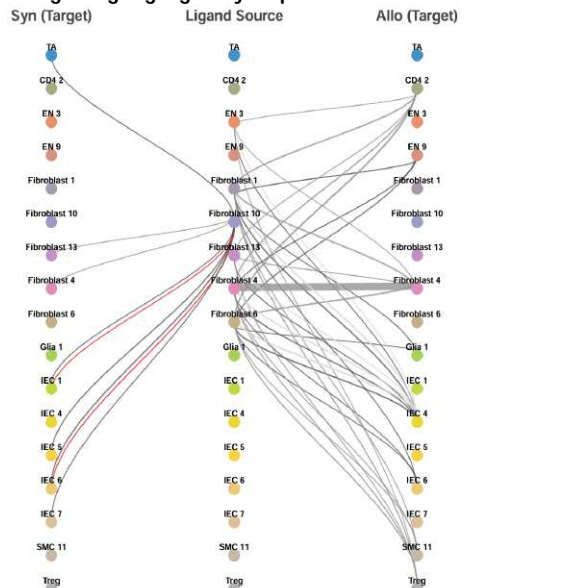
Midokine-Syn

Parental cluster labels

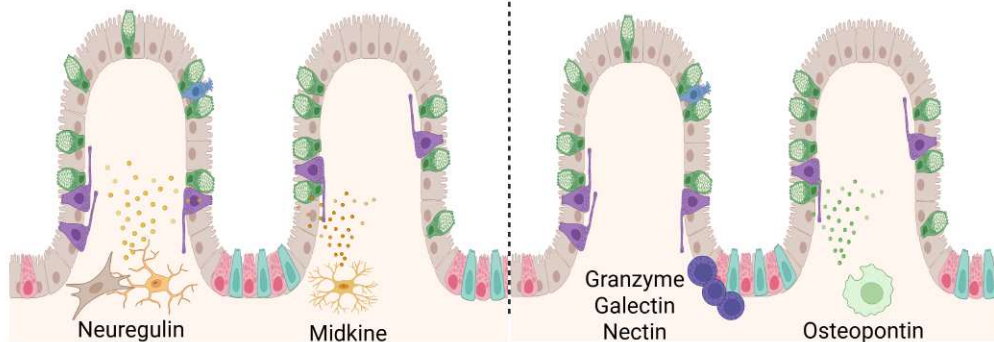


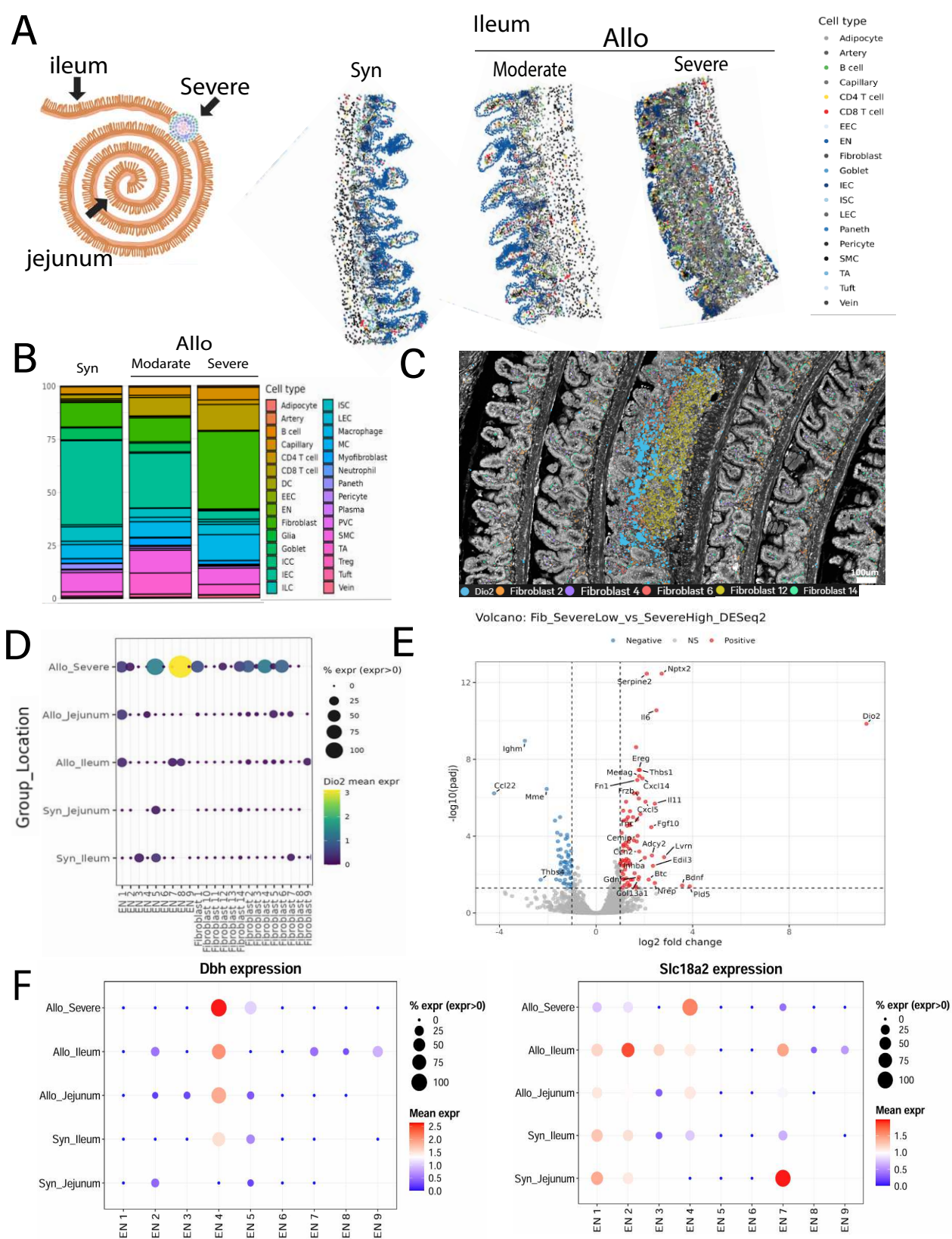
J

NRG Signaling Highlight: Syn-specific ERBB2/ERBB3



K

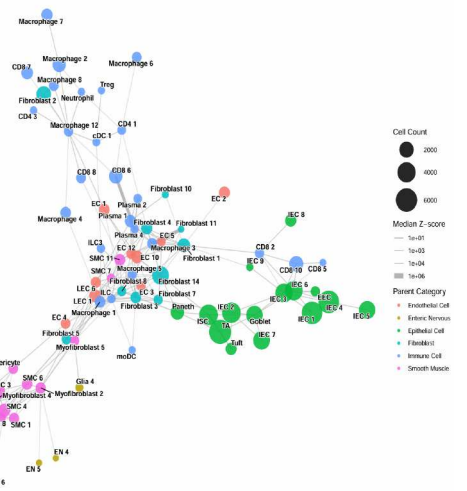




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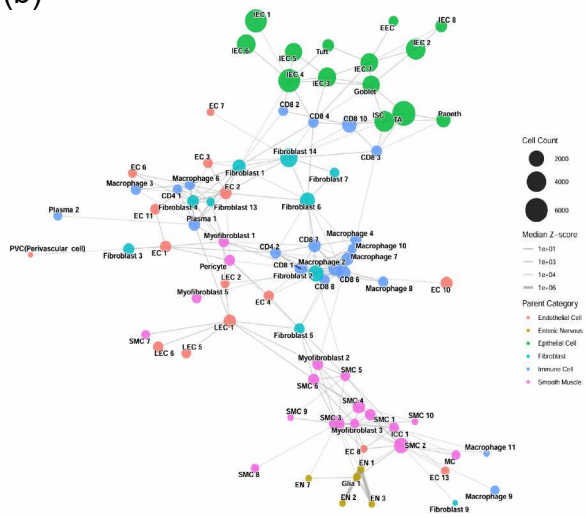
(a)

Network Map: Syn_ileum
Hybrid Strategy | Median Z = -2 | Top 8 edges/node



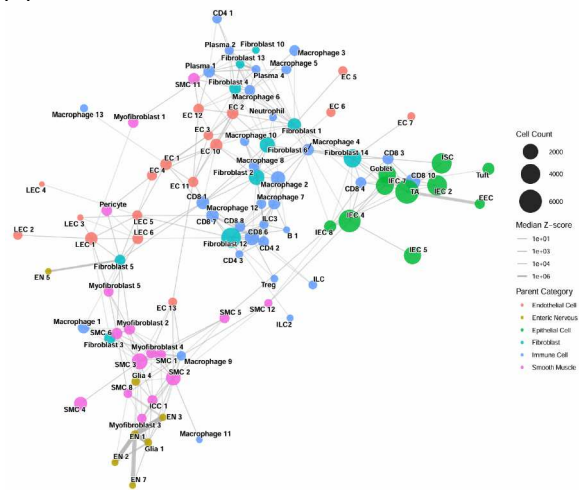
(b)

Network Map: Allo_ileum
Hybrid Strategy | Median Z = -2 | Top 8 edges/node

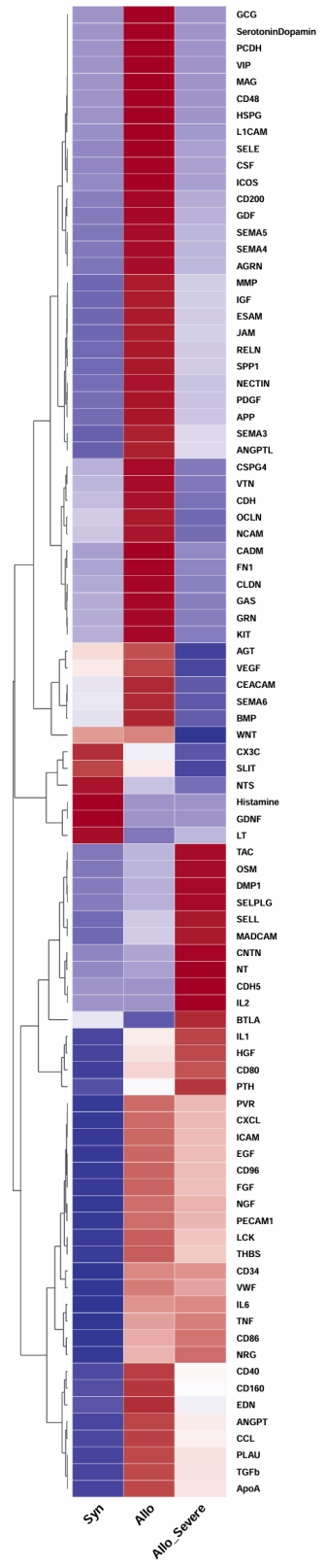


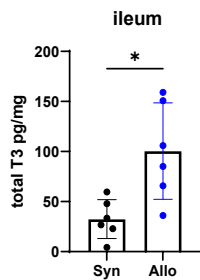
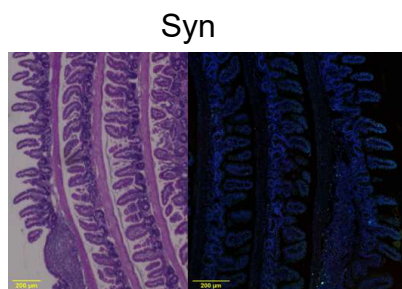
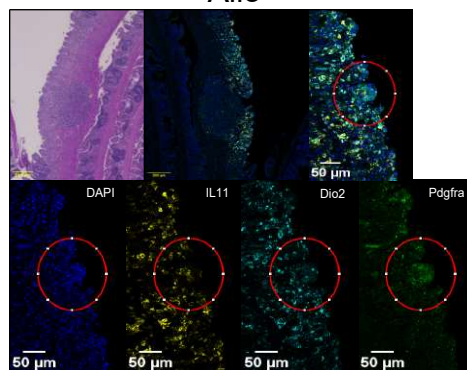
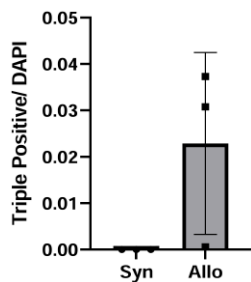
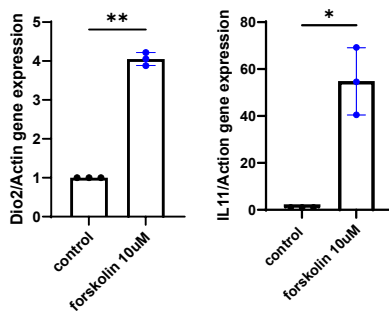
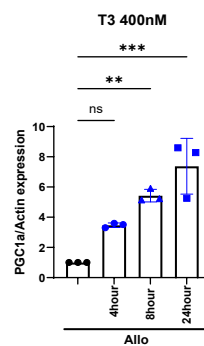
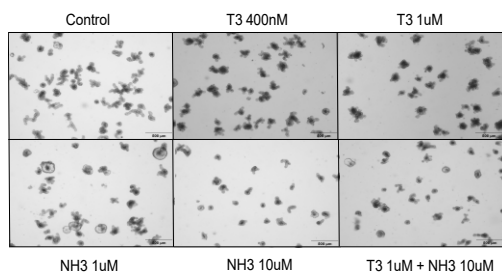
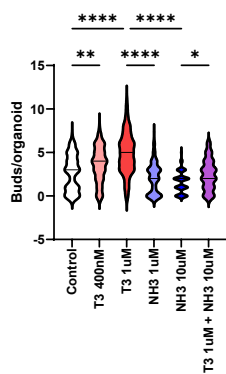
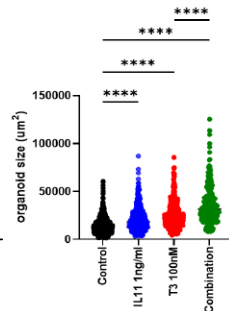
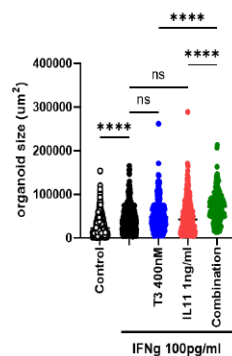
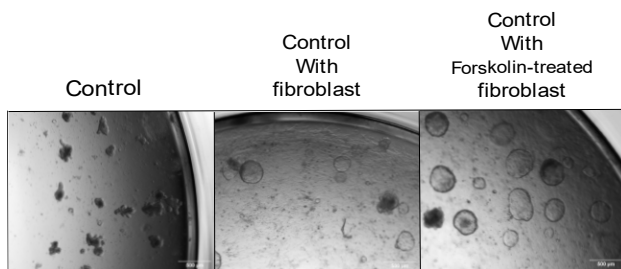
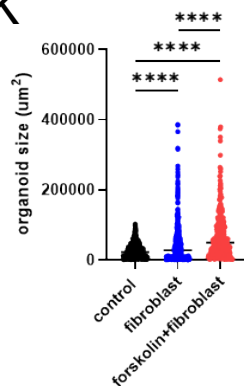
(c)

Network Map: Allo_severe
Hybrid Strategy | Median Z = -2 | Top 8 edges/node



H



A**B****Allo****C****D****E****F****G****H****I****J****K**

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ExtendedDataFig5.pdf](#)
- [ExtendedDataFig92.pdf](#)
- [ExtendedDataFig6.pdf](#)
- [Extendeddatadocument.pdf](#)
- [ExtendedDataFig7.pdf](#)
- [ExtendedDataFig91.pdf](#)
- [SupplementalTable.xlsx](#)
- [ExtendedDataFig1.pdf](#)
- [ExtendedDataFig3.pdf](#)
- [ExtendedDataFig4.pdf](#)
- [ExtendedDataFig8.pdf](#)
- [ExtendedDataFig2.pdf](#)