

1    **Extended Data**

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3    ***Dio2<sup>+</sup> Il11<sup>+</sup>* fibroblasts constitute a local stromal-endocrine niche that promotes**  
4    **intestinal stem cell regeneration during immune-mediated injury**

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10 Figure S1. Representative marker genes defining the parental niches.  
11 Representative marker genes for each parental cluster were identified by differential expression  
12 analysis. The dot plot shows scaled expression levels and the proportion of cells expressing  
13 each gene across individual clusters.  
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Figure S2. Subclustering analysis of stromal and non-hematopoietic cell compartments.

(A) UMAP visualization of fibroblast subclusters with representative marker gene dot plots.

(B) Relative composition of fibroblast subclusters across Control, Syn, and Allo groups.

(C) UMAP visualization of vascular endothelial subclusters with representative marker gene dot plots.

(D) Relative composition of endothelial subclusters across experimental groups.

(E) UMAP visualization of enteric neuronal subclusters with representative marker gene dot plots.

(F) Distribution of enteric neuronal subclusters across experimental groups.

(G) UMAP visualization of smooth muscle subclusters with representative marker gene dot plots.

(H) Relative composition of smooth muscle subclusters across groups.

(I) UMAP visualization of adipocyte subclusters with representative marker gene dot plots.

(J) Relative composition of adipocyte subclusters across experimental groups.

Fibro, fibroblast; LEC, lymphatic endothelial cell; EC, endothelial cell; PVC, perivascular cell; EN, enteric nerve; SMC, smooth muscle; MyoFibro, myofibroblast; MC, mesothelial cell; ICC, interstitial Cells of Cajal, Adipo, adipocyte.

Figure S3. Cellular composition and dominant cell–cell interactions within the villus and smooth muscle niches.

(A) Comparison of cell-type composition within villus regions across Syn and Allo groups.

(B) Dominant cell–cell interactions within the villus niche in the Syn group, ranked by interaction strength.

(C) Dominant cell–cell interactions within the villus niche in the Allo group, ranked by interaction strength.

(D) Differential cell–cell interaction analysis within the villus niche, showing interactions enriched in the Allo group (positive scores) and reduced in the Syn group (negative scores).

(E–H) Same analyses as in (A–D) performed for the smooth muscle niche.

(I) Heatmaps showing pathways upregulated within the smooth muscle niches. Columns represent experimental groups, and rows represent the indicated genes and pathways.

(J) Ligand-receptor interactions involving insulin-like growth factor (IGF) within the smooth muscle niche.

Figure S4. IMAP analysis of Tregs and spatial characterization of the crypt microenvironment.

(A) Distance-dependent distribution of total CD4, CD8 cells, and Tregs relative to ISC and TA cells. Spatial distribution and proximity of T cell subsets to intestinal stem cells (ISC) and transit-amplifying (TA) cells. (a–c) Cumulative frequency distributions of distance from CD4<sup>+</sup> T cells (a), CD8<sup>+</sup> T cells (b), and regulatory T cells (Tregs) (c) to nearest ISCs and TA cells within a 30  $\mu$ m radius in Syngeneic (Syn, red) and Allogeneic (Allo, green) groups. Bin width = 1  $\mu$ m. Statistical significance was assessed using a two-sample Kolmogorov–Smirnov test.

(B) Schematic overview of the IMAP analysis. Distances from individual cells to epithelial labels were plotted on the x-axis, whereas distances to smooth muscle labels were plotted on the y-axis, thereby projecting cellular positions into a two-dimensional spatial framework. V: villus region, C: crypt region, M: smooth muscle region.

(C) Distribution of cells located within the region defined by  $x < 50 \mu\text{m}$  and  $y < 50 \mu\text{m}$ , with highlighted cells shown in green.

(D) IMAP plots of CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and Tregs in Syn and Allo groups. The x-axis represents the distance to the nearest epithelial cell, and the y-axis represents the distance inward to the nearest smooth muscle.

(E) Proportion of cells in the Syn and Allo groups located within the region defined by  $x < 50 \mu\text{m}$  and  $y < 50 \mu\text{m}$  in IMAP space.  $n=4$  Syn,  $n=4$  Allo mice. \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; NS, not significant.

68 Figure S5. Differences in cellular composition within the crypt microenvironment based on  
69 subcluster classification.  
70 (A) Proportions of all subclustered cell types within the crypt microenvironment in Syn and Allo  
71 groups. n=4 Syn, n=4 Allo lesions.  
72 (B–E) Relative proportions of subclustered cell populations within the crypt microenvironment in  
73 Syn and Allo groups, including immune cells (B), fibroblasts (C), intestinal epithelial cells (D),  
74 and endothelial cells (E). n=4 Syn, n=4 Allo lesions.  
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Figure S6. Comprehensive comparison of epithelial cell death–associated gene expression. (A–E) Heatmaps showing the expression of cell death–associated genes in intestinal stem cell (ISC), Paneth, goblet, tuft, and enteroendocrine cell (EEC) cells across experimental groups. Normalized expression values were averaged across each group 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.

Figure S7. Global spatial interaction networks of the crypt microenvironment in Syn (A) and Allo (B) groups. Condition-specific networks were constructed from sample-level spatial neighborhood enrichment scores to map unbiased pairwise cellular interactions. Edges indicate robust positive spatial associations, defined by an enrichment Z score  $\geq 2$  in at least two independent samples per condition. Edge width and opacity are proportional to the mean of the significant Z scores, while node size corresponds to overall cell abundance. Node color denotes the parental cell lineage.

96 Figure S8. Cell-type composition of immune, epithelial, and fibroblast subclusters within ROI  
97 regions.  
98 (A) Composition of immune cell subclusters in the ileum.  
99 (B) Composition of intestinal epithelial cell subclusters in the ileum.  
100 (C) Composition of fibroblast subclusters in the ileum.

101 Figure S9. ROI-based spatial analysis of the jejunum in GVHD  
102 (A) Spatial plots of jejunum across Syn and moderate Allo groups.  
103 (B) Comparison of cell-type composition across experimental groups.  
104 (C-E) Comparison of immune, epithelial, and fibroblast subclusters between Syn and Allo  
105 jejunum. n=4 Syn, n=6 Allo lesions  
106 (F) Aggregated signaling pathway comparison in Jejunal ROI between Syn and Allo. Heatmap  
107 displaying the relative communication probabilities of signaling pathways inferred by CellChat.  
108 Probabilities from individual samples were summed within each group and normalized as Z-  
109 scores across rows. Red indicates higher relative pathway activity, while blue indicates lower  
110 activity. The left dendrogram represents hierarchical clustering of the pathways.  
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