

Supplementary Material for

Integrated cell type-specific cis-eQTL analyses of blood and gut identifies ~25 thousand regulatory modules, matching eQTL for 140 IBD risk loci and entrectinib as possible repurposing candidate.

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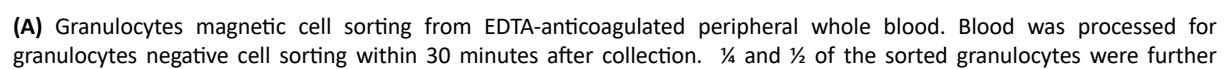
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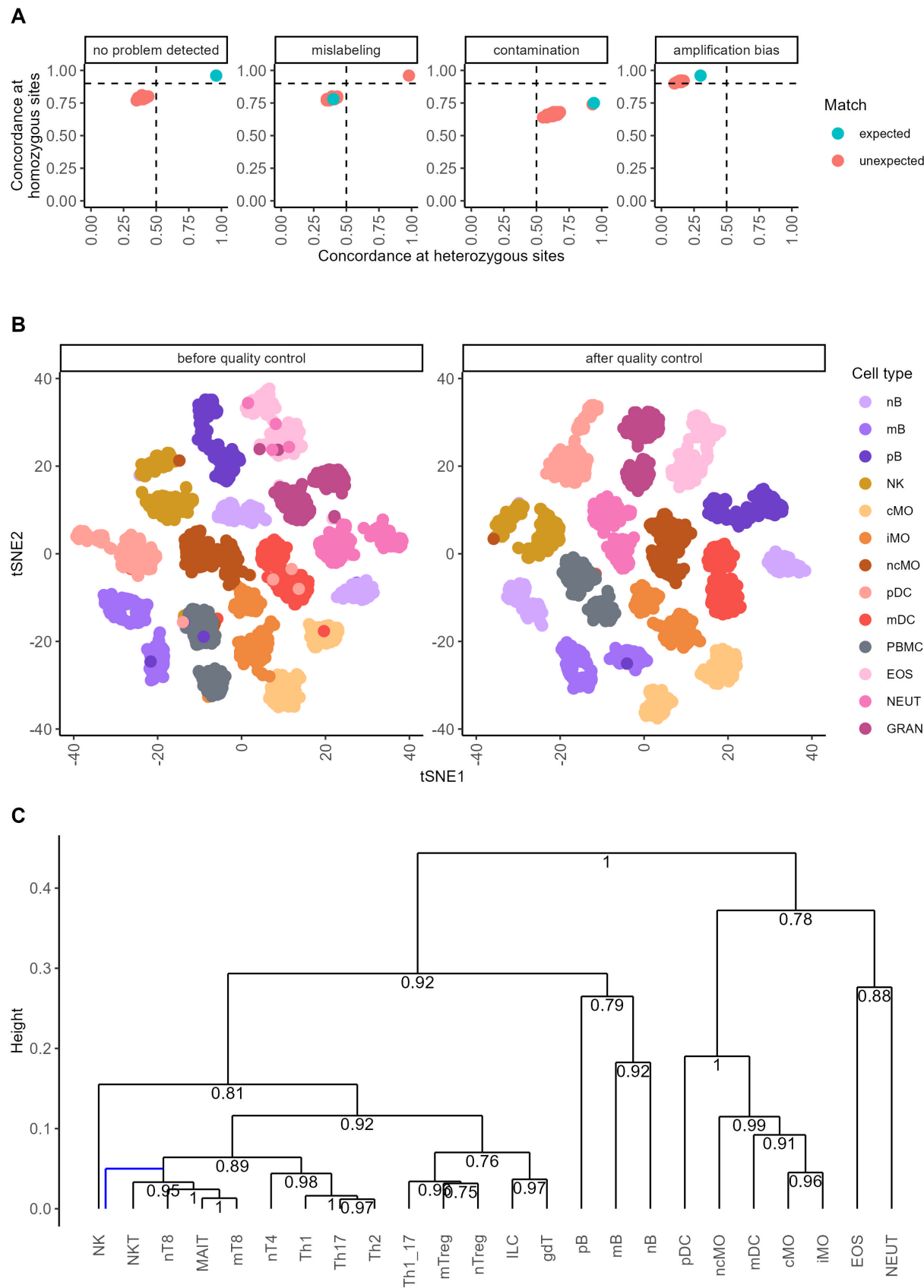
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A.



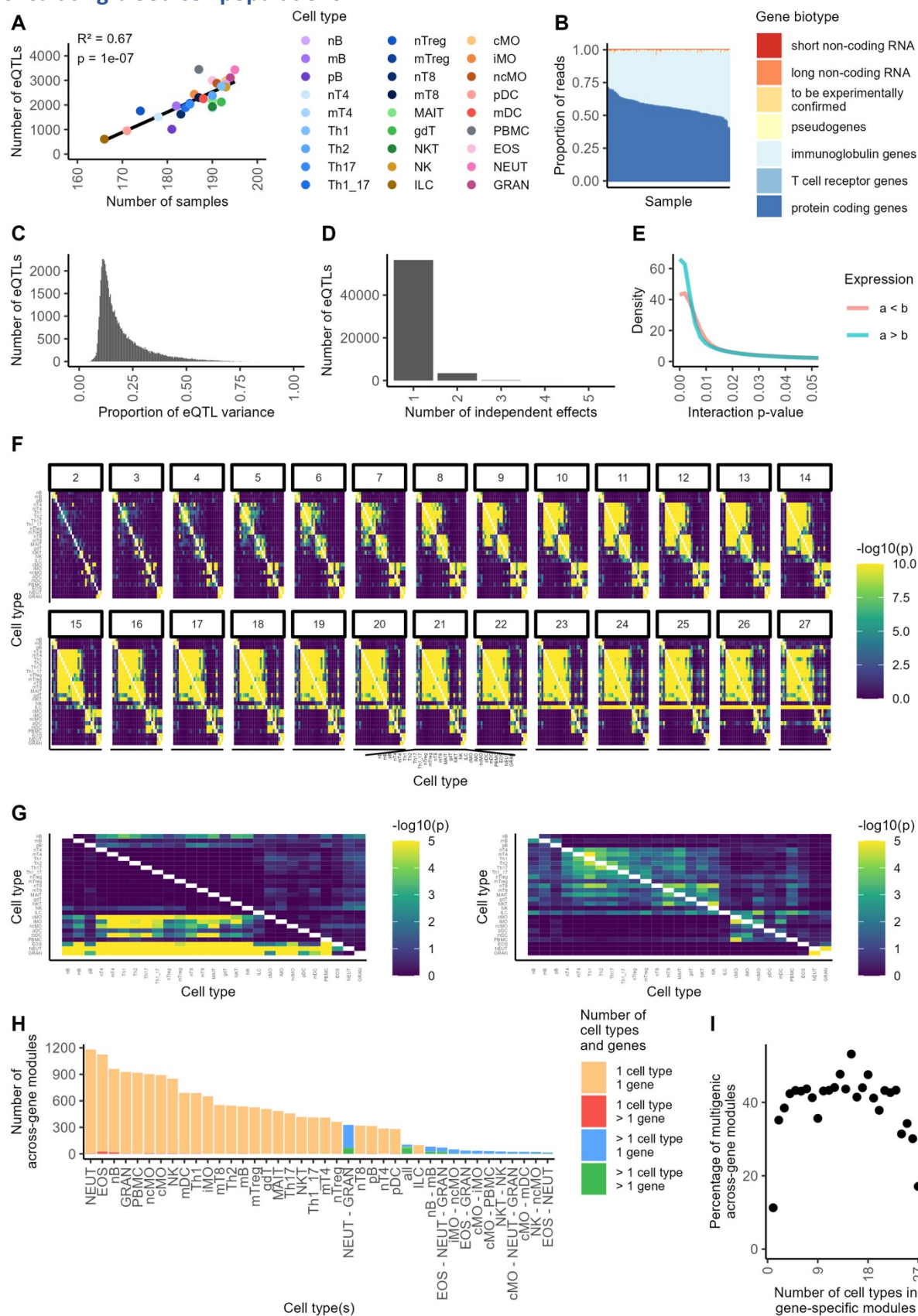
processed separately and respectively for Neutrophils and Eosinophils selection. The remaining ¼ was immediately frozen until use. For QC of the cell sorting, an aliquot before and after each sorted fraction were stained using anti-CD15 and anti-Siglec-8 antibodies, together with viability dye. Left panel shows the percentage of each sorted population before and after sorting. **(B)** Flow cytometry cell sorting and QC of PBMC derived cell populations. Representative FACS plots illustrating gating strategies and QC after cell sorting for B cells (upper right panel), MAITs, gd T and ILC cells (middle left panel), monocytes and dendritic cells (middle right panel), NK and NKT cells (lower left panel), and conventional CD8 and CD4 T cells (lower right panel). For a detailed description of subset names, see Suppl. Data 2. For information on the flow-cytometry antibodies used in this study, see Suppl. Data 29. Red quadrants indicate the target sorted cell types. Created in BioRender. Rahmouni, S. (2026) <https://BioRender.com/rr75p65>, <https://BioRender.com/z6ywjdx>.

Supplementary Figure 2: Quality control of the bulk RNA-Seq data on 27 circulating immune cell populations.



(A) Examples of issues detected by confronting genome and transcriptome genotypes. From left to right: (i) Example of an RNA sample that matches its presumed donor (green dot); the red dots correspond to the confrontation of that RNA sample with the DNA of all other individuals. (ii) Example of an RNA sample that matches the DNA of an individual that is not the presumed donor. (iii) Example of an RNA sample that is likely contaminated (mixture between two samples), as it is “too heterozygous” and hence shows a drop in concordance at homozygous genotypes for both the presumed donor (green dot) and the contaminating individual (co-positioned red dot). (iv) Example of a likely PCR issue; the RNA appears “too homozygous” and hence shows a drop in concordance at heterozygous genotypes. **(B)** Example of issues detected when confronting presumed cell type vs inferred transcriptome-based cell type: tSNA map of 2,444 samples from 13 cell types labeled by presumed cell type of origin before (left) and after (right) quality control. Based on the expression level of 500 most variable genes. **(C)** Hierarchical tree for 24 of the 27 cell types (PBMC, granulocytes and memory CD4 were not included because being mixtures of cell types) obtained from $1 - |\text{Spearman's correlation}|$ between the mean (across all samples) expression levels of the 4,000 most variable (ANOVA F value of cell type effect) coding genes using “average” method. The numbers on the nodes correspond to the proportion of trees recapitulating the same split when varying the number of variable genes considered from 250 to 8,000 (32 analyses) and using the same method. The tree shown is the best supported tree in the method. Nearly identical (most supported) trees were obtained when applying the “ward.d” and “ward.d2” methods. The blue line marks the only differences that was observed with the “ward.d2” method.

Supplementary Figure 3: eQTL mapping and construction of regulatory modules in 27 circulating blood cell populations.



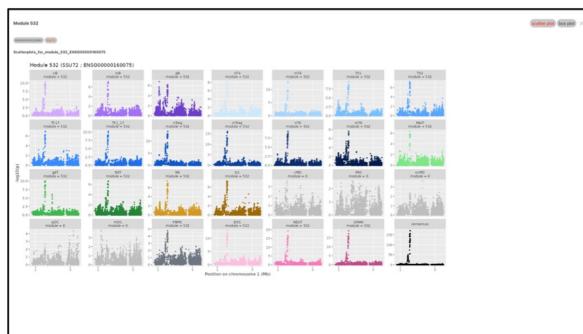
(A) Number of detected eQTL as a function of the number of usable samples for the corresponding (color labeled) cell type. **(B)** Demonstration of the inter-individual variation in the abundance of immunoglobulin reads (light blue) in plasmocytes of 181 individuals. **(C)** Distribution of the proportion of variation in gene expression level accounted for by the (primary) detected *cis*-eQTL. **(D)** Results of the conditional analysis for the detection of independent secondary *cis*-eQTL effects. **(E)** Density of interaction *p*-values for genes whose expression is higher in cell type A than in cell type B (red, $n = 442,829$) and genes whose expression is lower in cell type A than in cell type B (green, $n = 290,142$). The mass of very low *p*-values was slightly higher for genes whose expression was higher in cell type A than B ($n = 442,829$), when compared to genes whose expression was lower in cell type A than B ($n = 290,142$). This could be due in part to the fact that eQTL effects were present yet underestimated in cell type B because the gene's expression was lower and noisier in cell type B (and the interaction *p*-value therefore also lower). However, estimates of π_1 (corresponding f.i. to $1 - 2 \times \text{proportion of test with } p\text{-value} > 0.5$) were nearly identical: 0.95 for genes with higher expression in cell type A vs cell type B, and 0.93 for genes with higher expression cell type B vs cell type A. **(F)** $\text{Log}(1/p\text{-value})$ of the excess number of shared modules over expectations (given number of detected eQTLs in the respective cell types) for all possible pairs of cell types. Computations are performed by including modules with two active cell types (upper left facet), three or less active cell types, four or less active cell types, ..., 27 or less active cell types (lower right facet). **(G)** (Left) $\text{Log}(1/p)$ -values of the excess of observed module switches between pairs of cell types over random expectations computed using a binomial test. (Right) $\text{Log}(1/p)$ -values of the excess of observed module sharing between pairs of cell types over random expectations computed using a binomial test. Analyses restricted to genes that are assigned to more than one module. **(H)** Across-gene regulatory modules grouped by the combination of cell types in which they are active and ordered by the frequency of occurrence of the corresponding pattern. Modules encompassing one gene and active in one cell type only are labeled in yellow ($n = 15,926$). Modules encompassing more than one gene yet active in only one cell type are labeled in red ($n = 84$). Modules encompassing one gene yet active in more than one cell type are labeled in blue ($n = 3,433$). Modules encompassing more than one gene and active in more than one cell type are labeled in green ($n = 1,451$). Only the 40 more common activity patterns (out of 2,959 in total) are shown. These include the 27 patterns corresponding to modules active in only one cell type. **(I)** Estimation of the proportion of gene-specific modules that merge with another gene-specific module to create a multigenic module when considering gene-specific modules active in 1, 2, 3, ... 27 cell types (x-axis). Interestingly, gene-specific modules active in one cell type rarely (11.3%) fuse with others to form multigenic modules. Likewise, gene-specific modules that are active in all 27 cell types rarely (17.1%) become multigenic modules. A contrario, gene-specific modules that are active in 2 to 26 cell type more often (average 41.4%; range: 30.1% - 53.2%) fuse with others to form multigenic modules. To make sure that the probability of merging between gene-specific modules was not affected by the number of EAPs in the modules, we exclusively used one EAP per gene-specific module, namely the consensus EAP for modules with more than one EAP.

Supplementary Figure 4: Screenshots from the CEDAR website (<https://tools.giga.uliege.be/cedar>) for the 27 circulating immune cell populations.

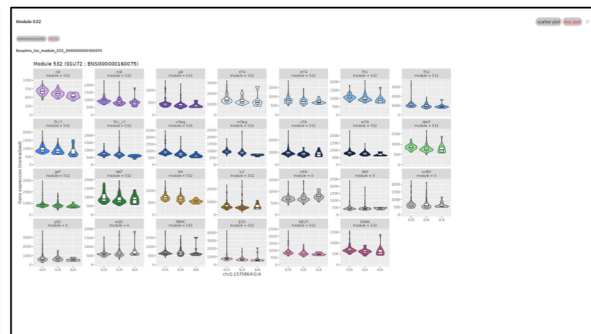
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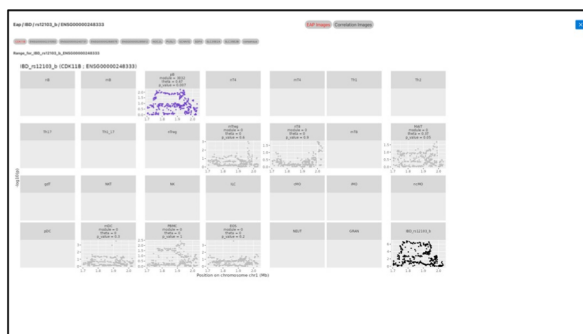
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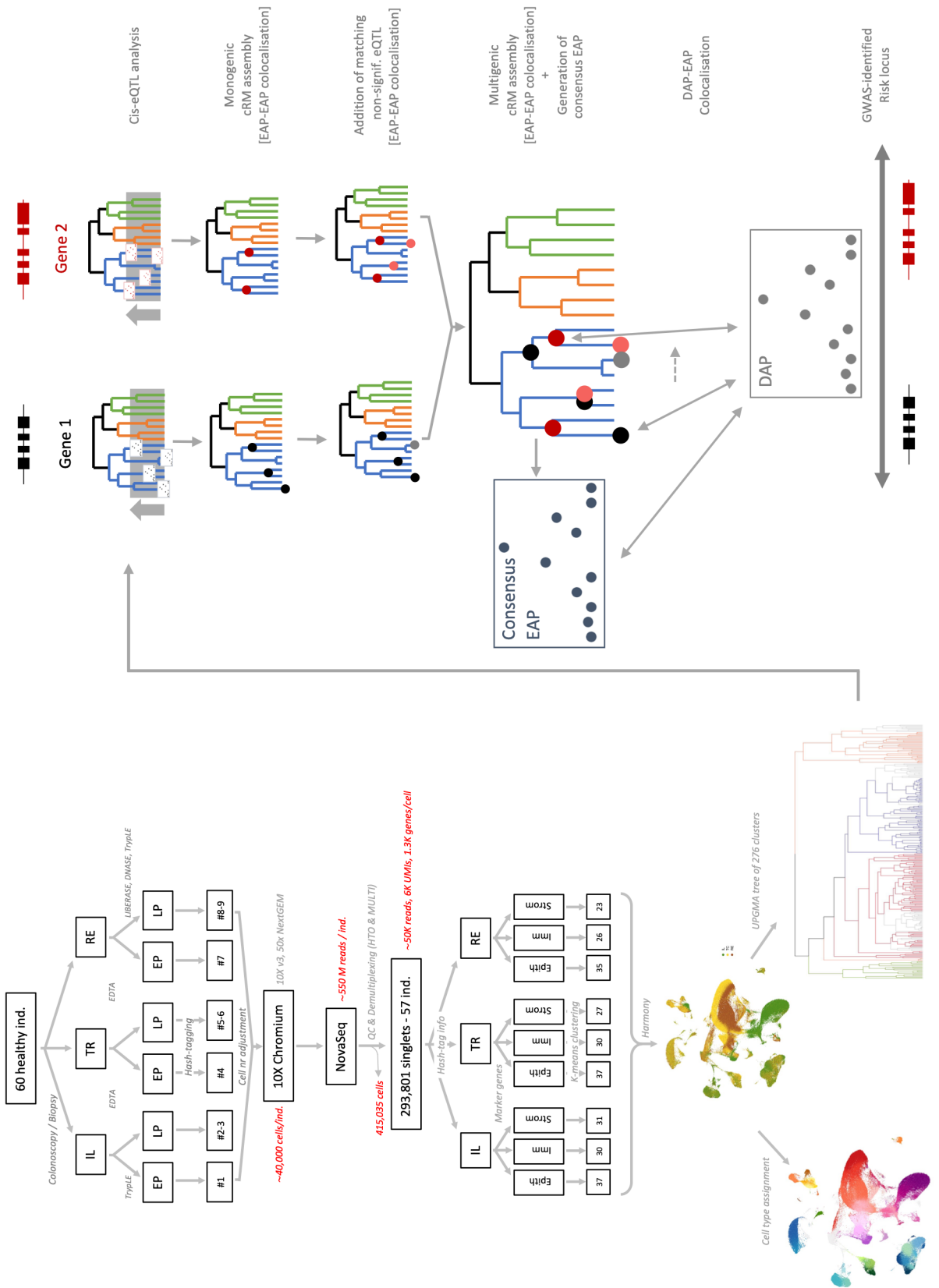
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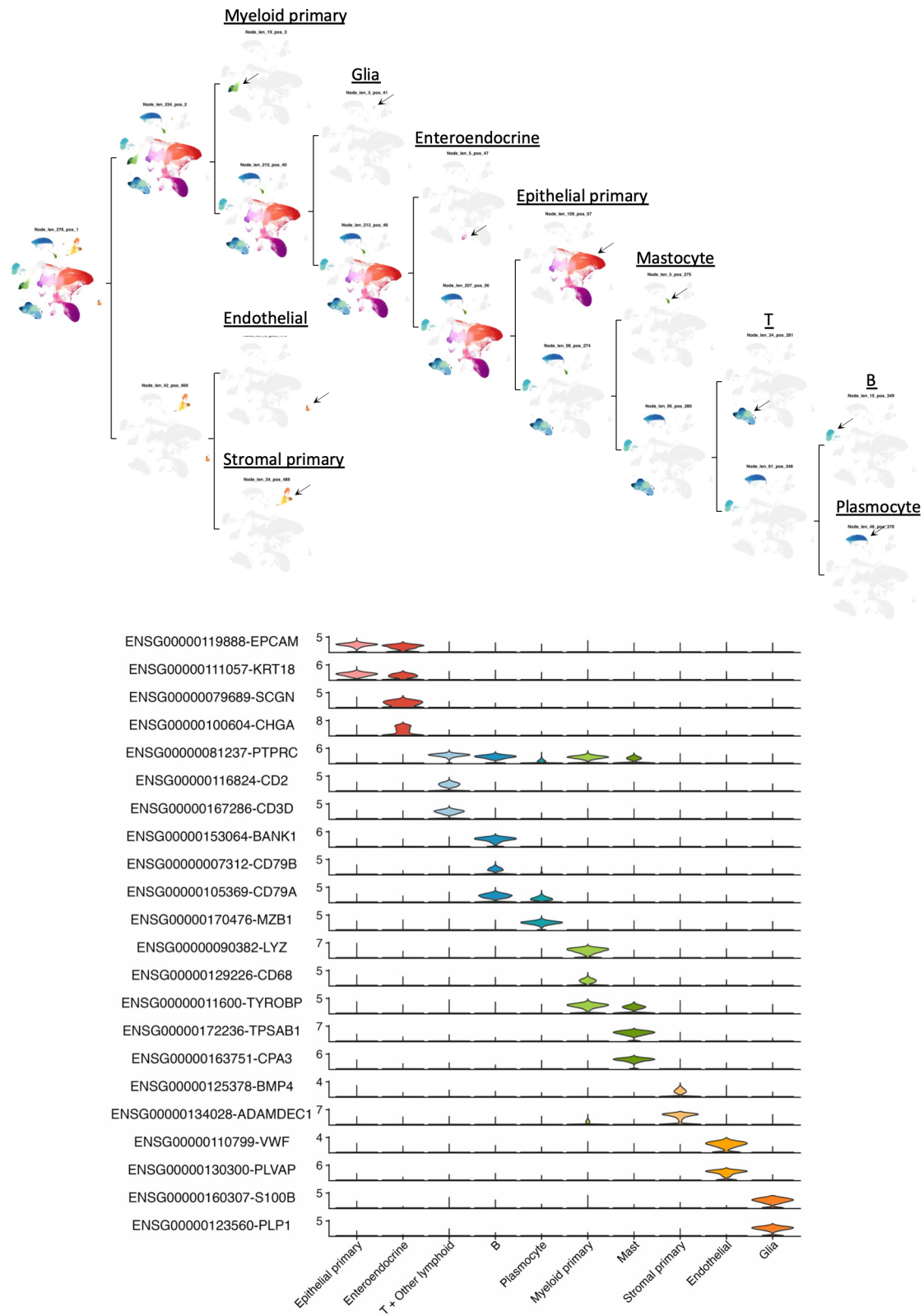
(A) Top panel: genome browser showing the position of genes and risk loci for inflammatory bowel disease (IBD). Bottom panel: activity of the eQTL and *cis*-acting regulatory modules (RM). Significant eQTL are marked by large arrows (↑). Arrows for eQTL that are part of the same module have the same color. eQTL in a module with effect sign opposite to the most significant eQTL in the module ("representative") are marked by a downward pointing arrow (↓). Non-significant eQTL that are part of a module by virtue of matching EAP ($|\theta| \geq 0.6$) are marked by small arrows (Λ or V). eQTL that are alone in their own module are in grey. Modules can be highlighted by clicking on them. (B) By clicking on a module, one has access to the EAP graphs showing the activity of the corresponding eQTL in all 27 cell types by gene (one page per gene). EAPs that are part of the selected module are colored, others are shown in grey. If the gene was not expressed in a given cell type (and

the eQTL could not be tested) the EAP graph is “empty”. The last facet (lower right) corresponds to the “consensus” EAP of the module. **(C)** By clicking on a module, one also has access to violin plots at the lead SNP of the consensus EAP showing the distribution of DESeq2 normalized gene expression values for individuals sorted by SNP genotype. **(D)** By clicking on the bars corresponding to the disease risk loci, one has access to the EAP in all 27 cell types of all genes (one page per gene) that have a matching DAP-EAP ($|\theta| \geq 0.6$, $p \leq 0.05$) with the corresponding DAP (lower right facet). **(E)** By clicking on the bars corresponding to the disease risk loci, one also has access to the theta-plots (reference 15 in main text) in all 27 cell types of all genes (one page per gene) that have a matching DAP-EAP ($|\theta| \geq 0.6$, $p \leq 0.05$).

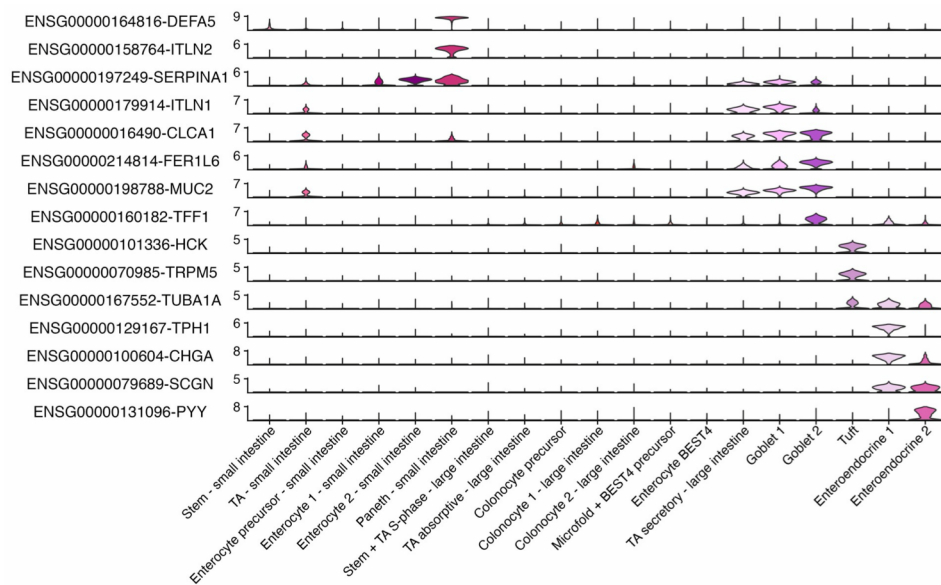
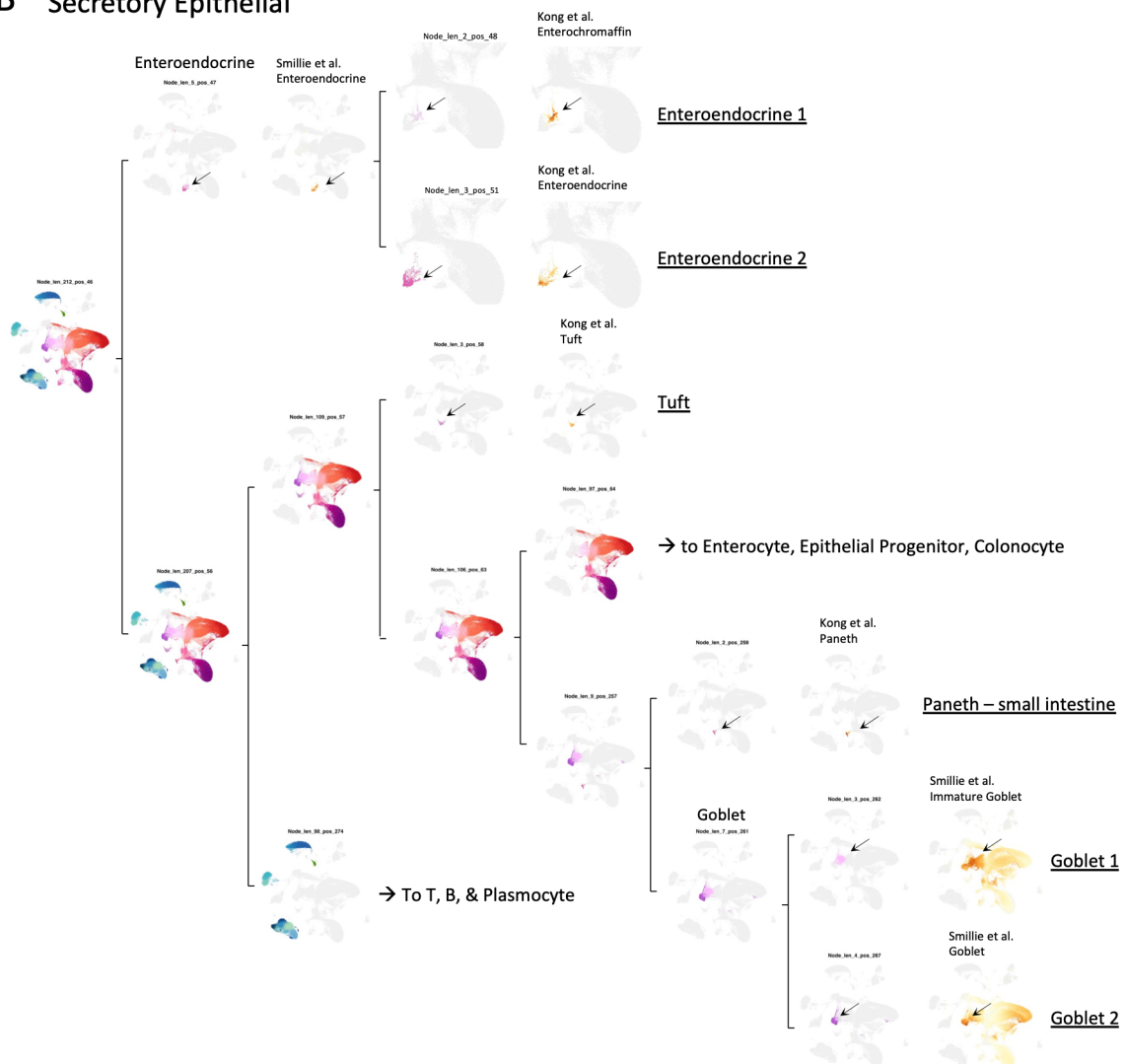
Supplementary Figure 5: Graphical abstract of workflow of *cis*-eQTL analyses in scRNA-Seq data from intestinal biopsies.



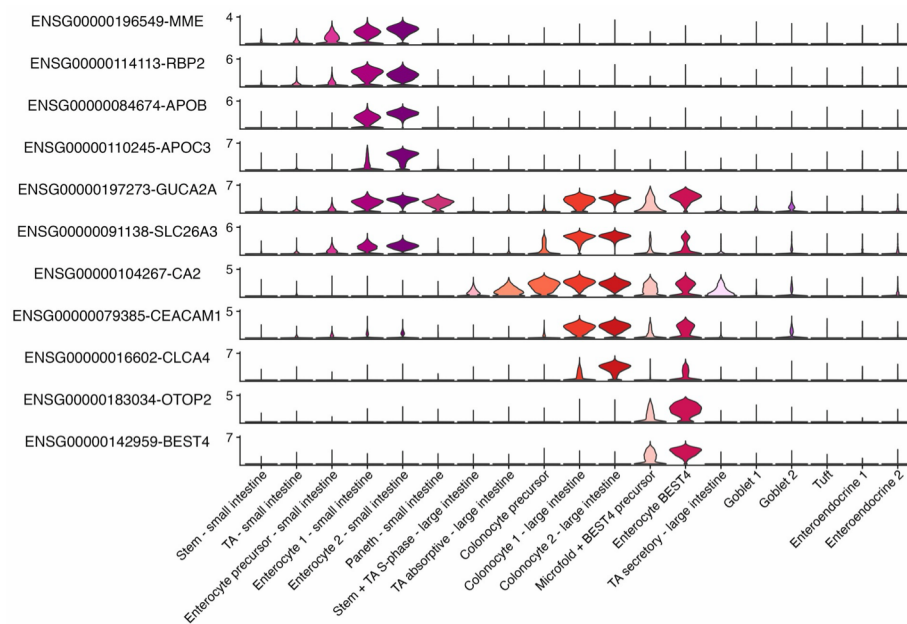
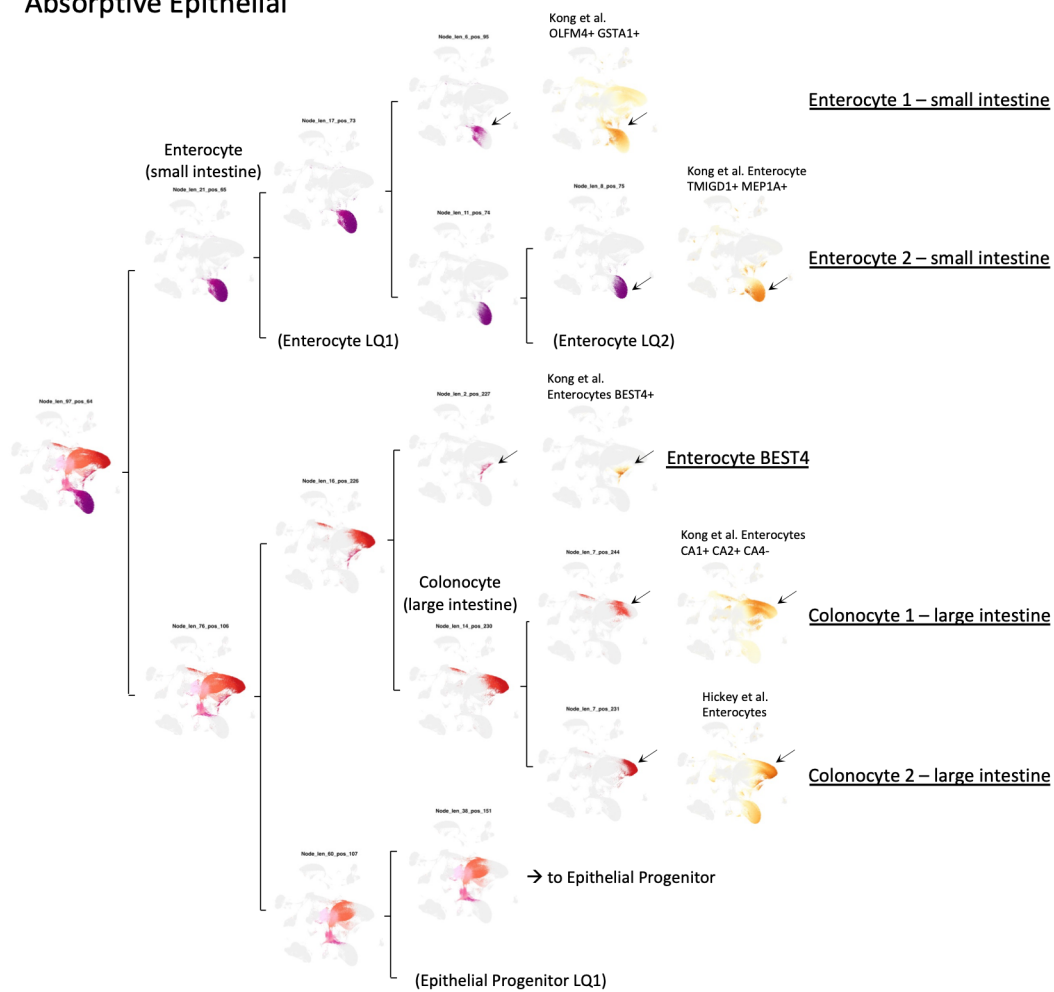
A 10 grand clusters



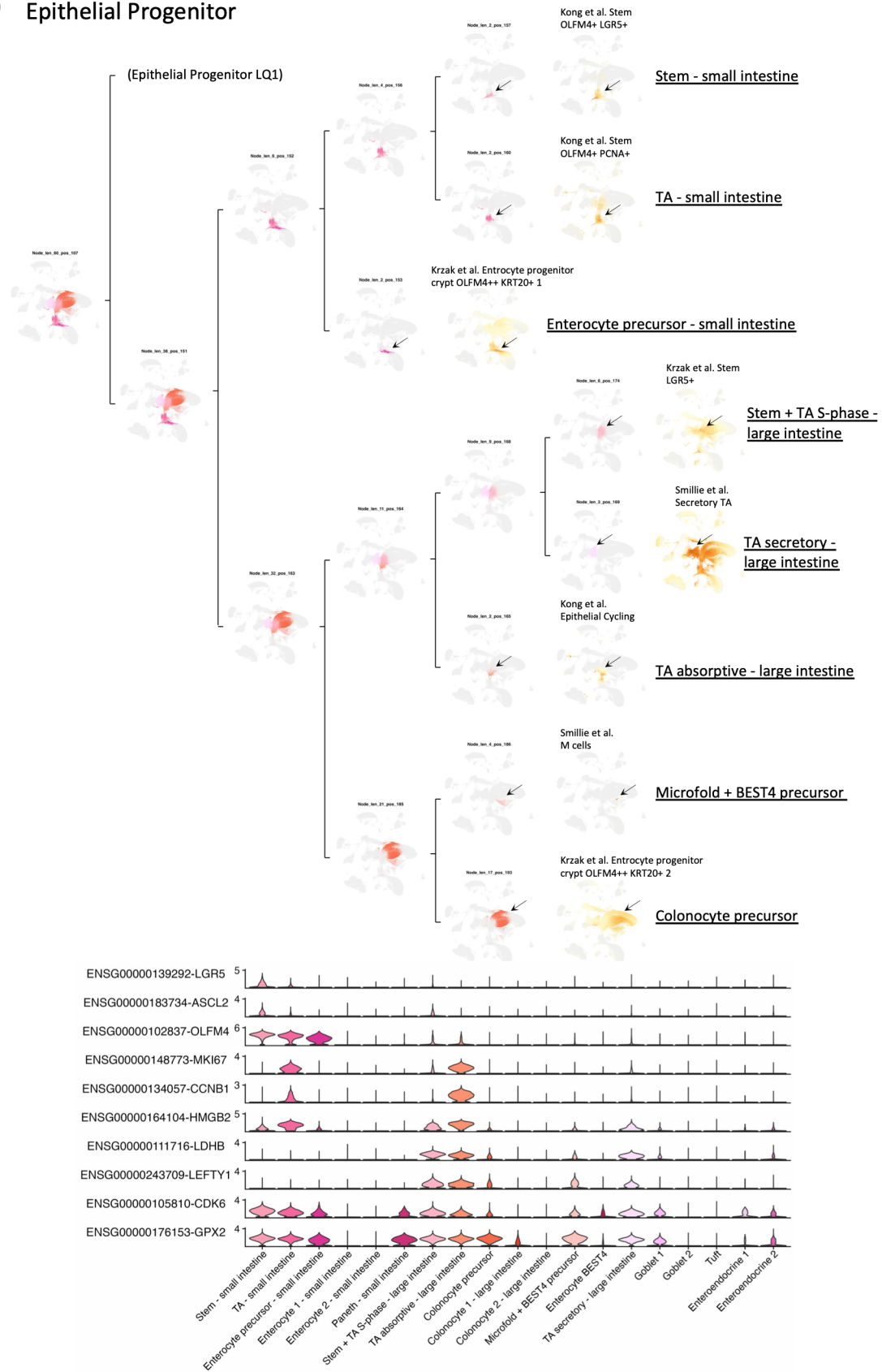
B Secretory Epithelial



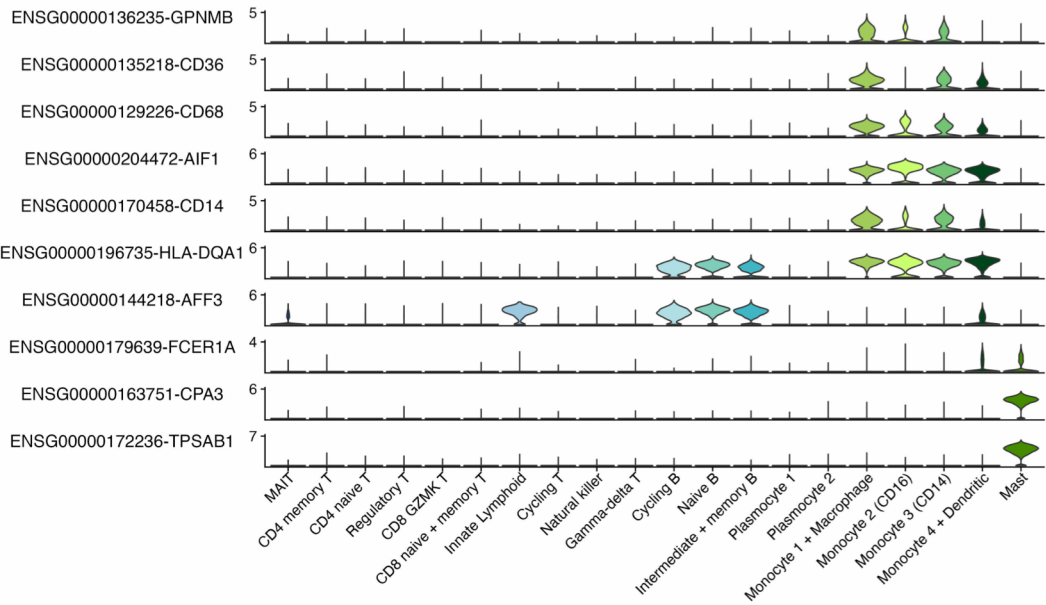
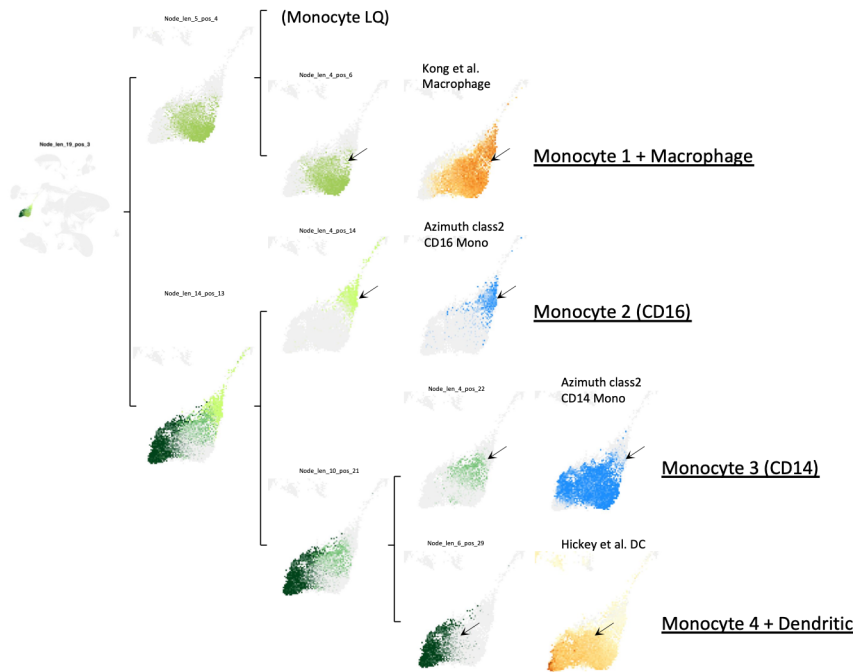
C Absorptive Epithelial



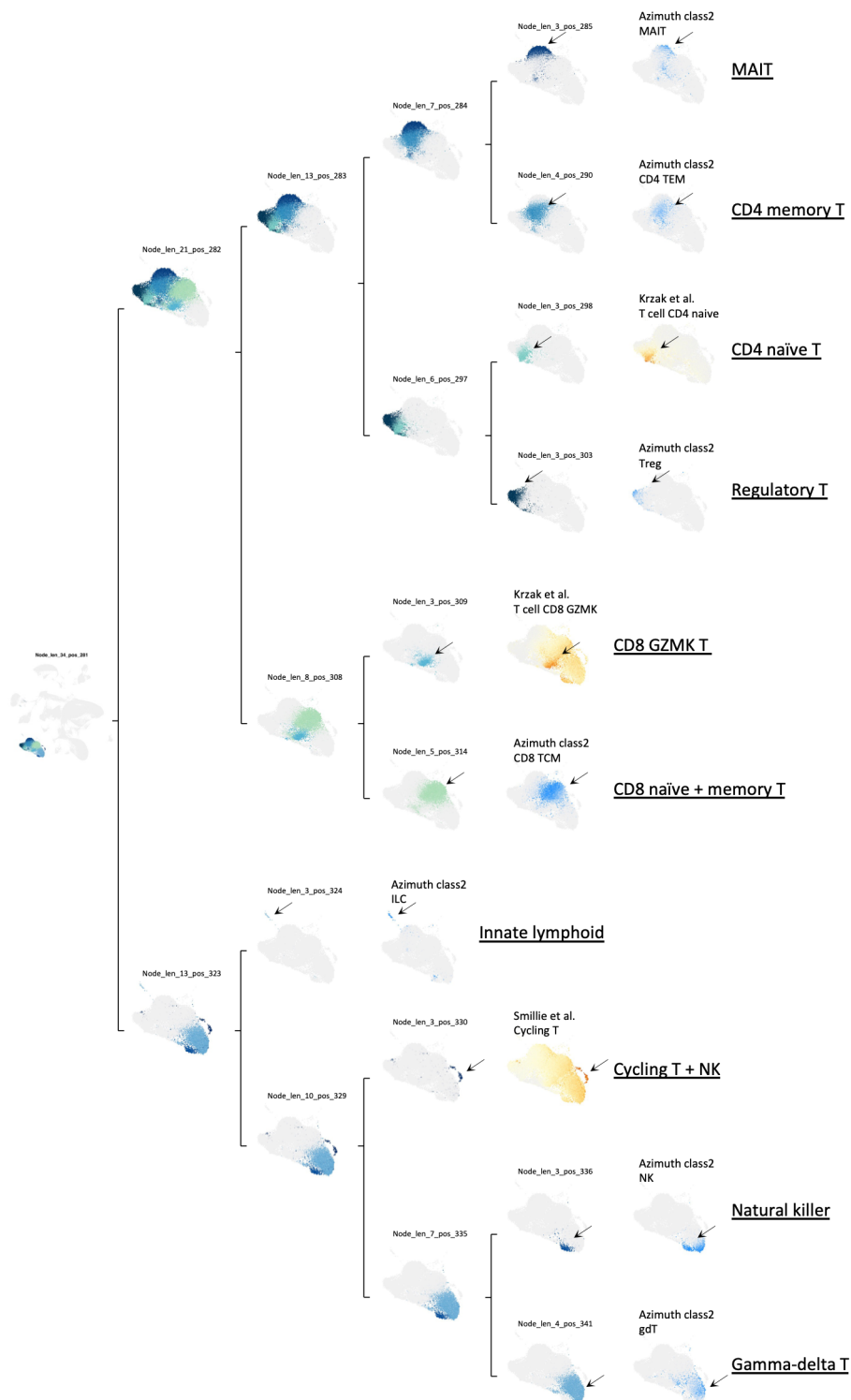
D Epithelial Progenitor

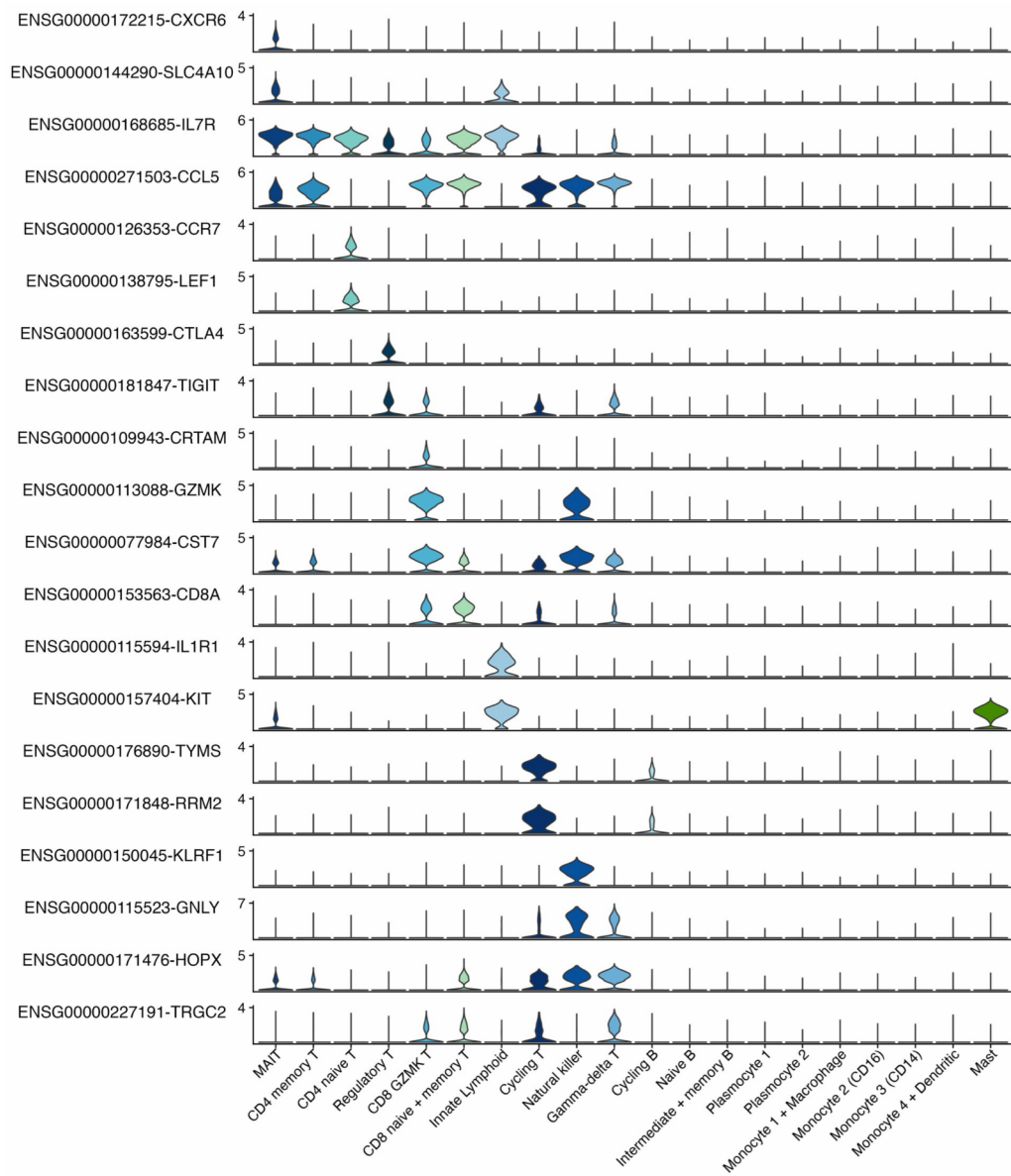


E Myeloid primary

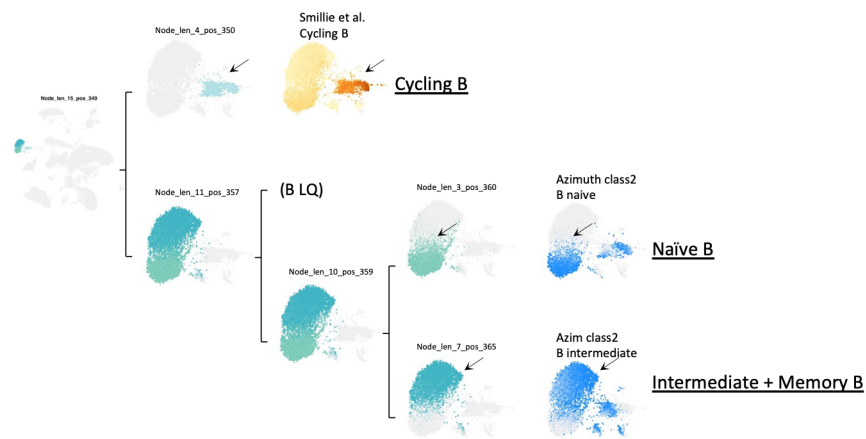


F T + Other lymphoid

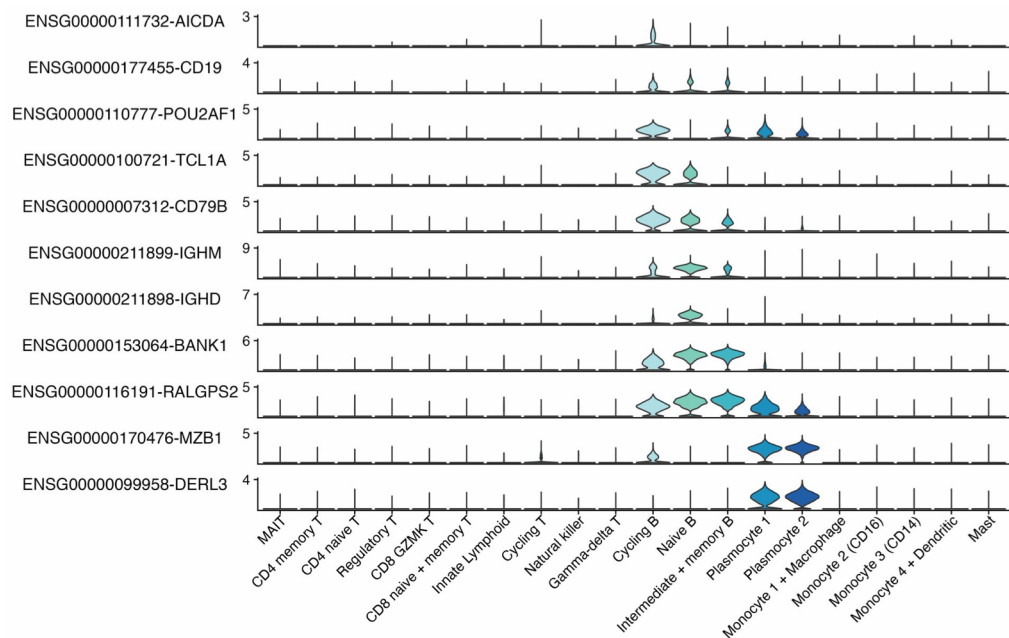
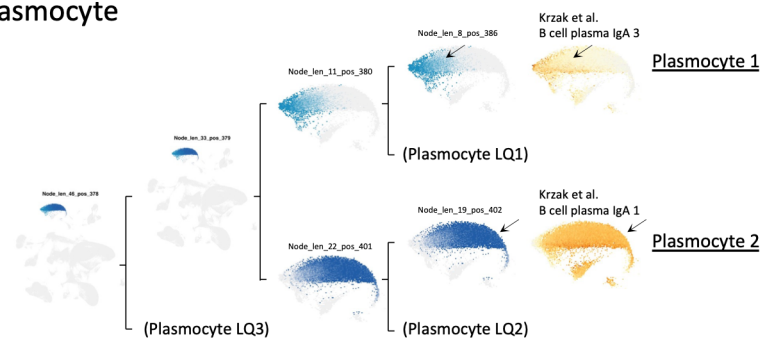




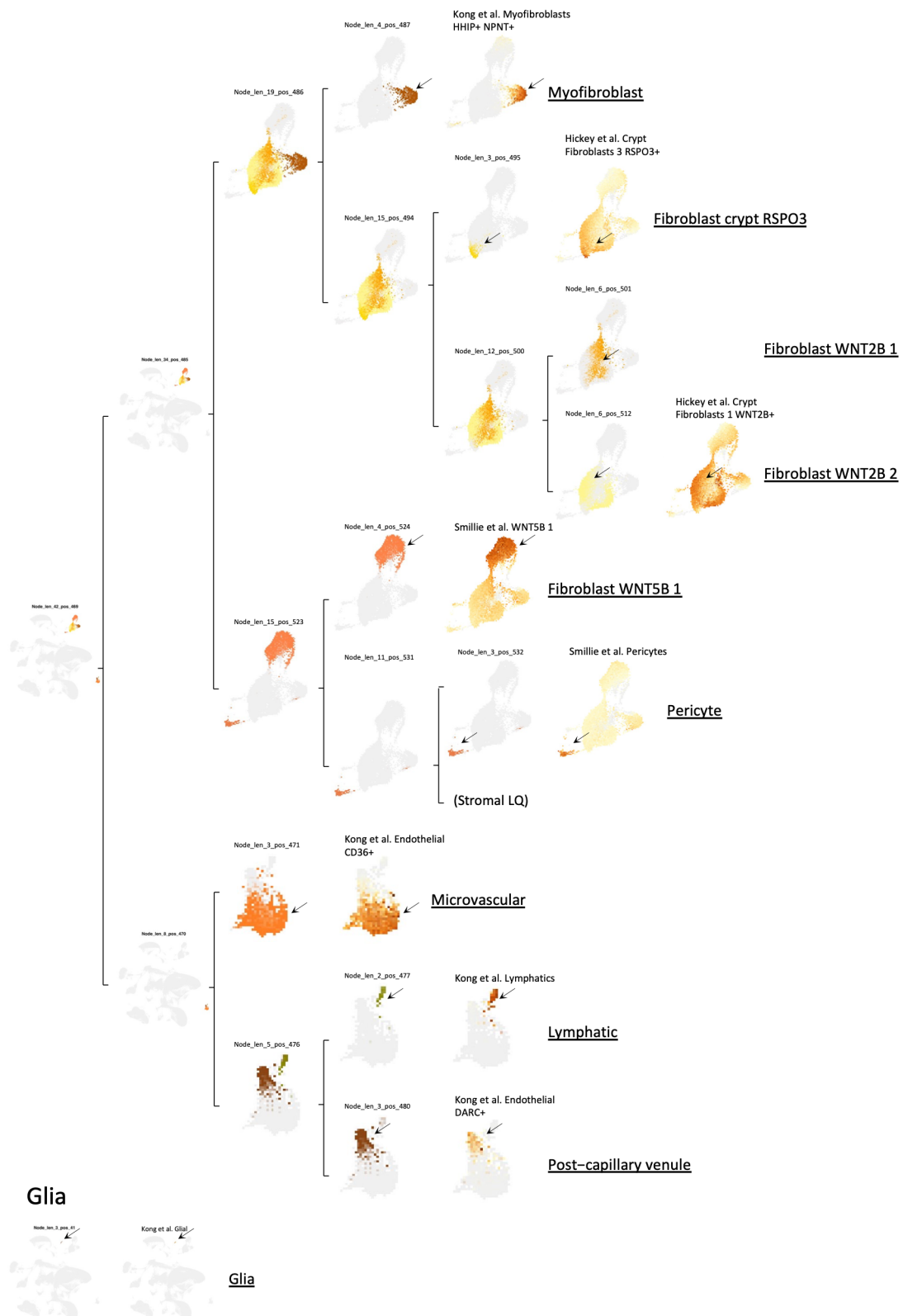
G B cell

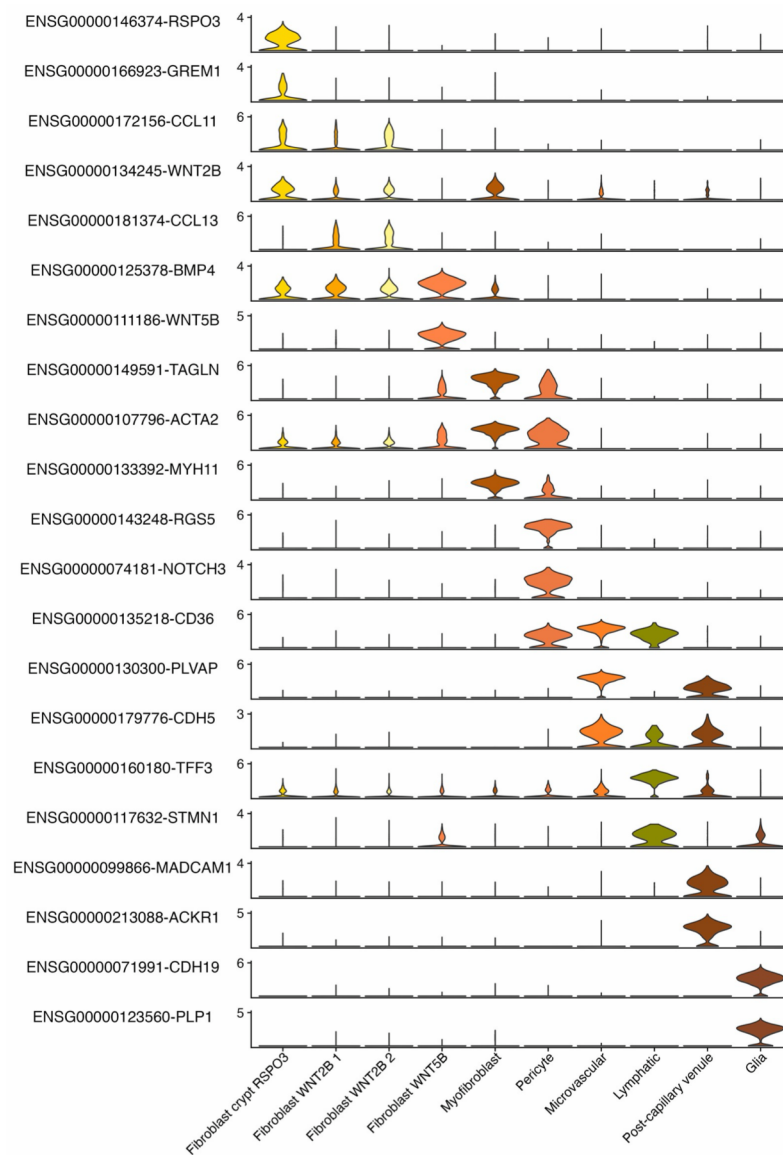


Plasmacyte

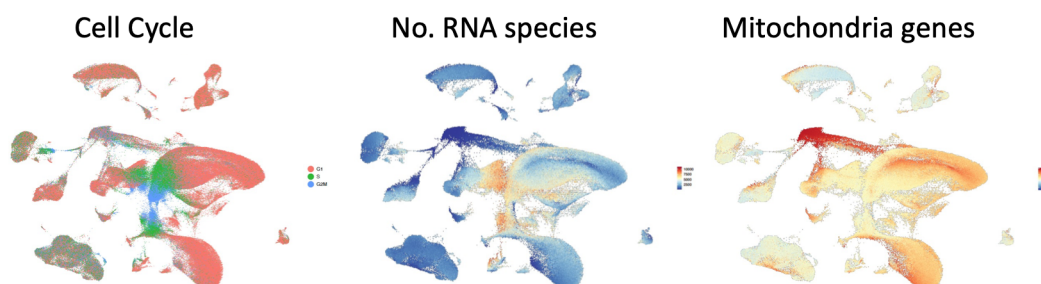


H Stromal primary

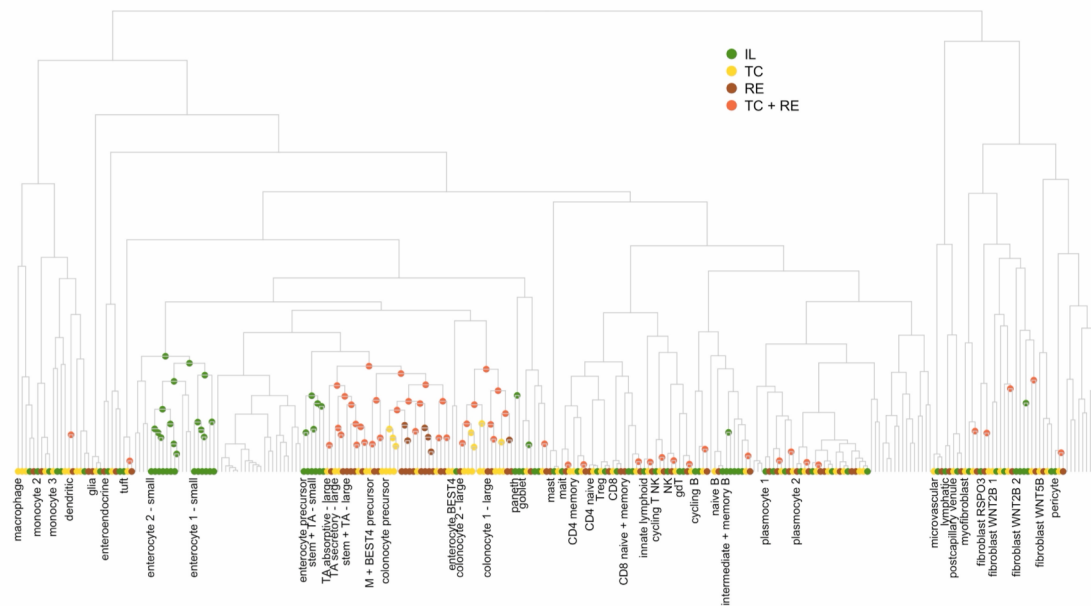




I



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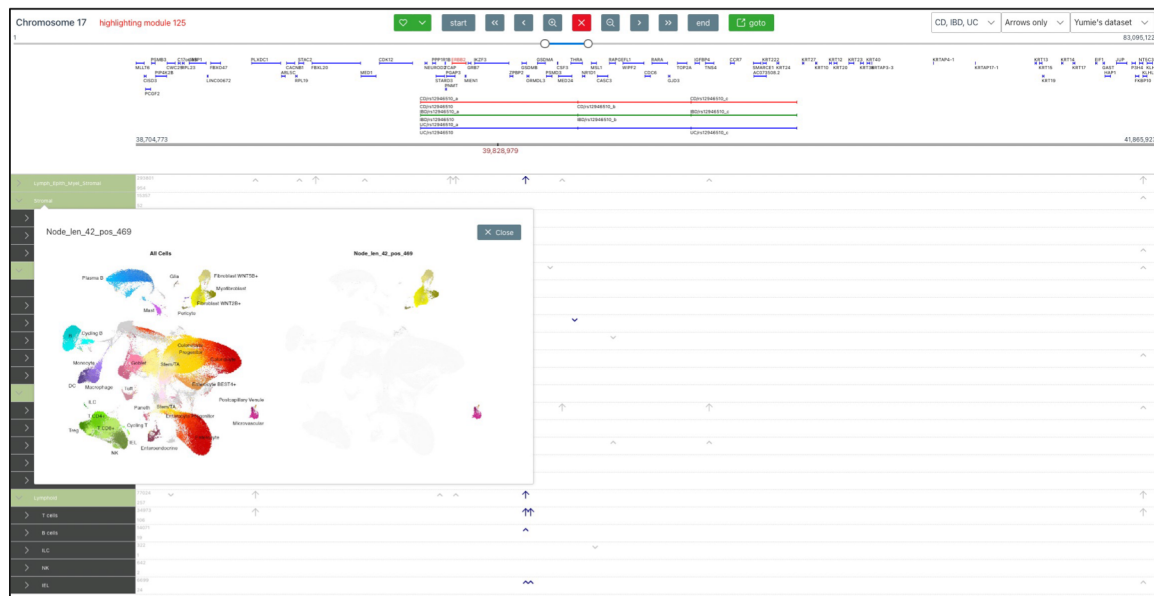
(A-H) Illustration of the cell content of the nodes and leaves of the hierarchical tree of cell clusters. The different panels sequentially walk the reader through the tree from the root towards the leaf. In terms of cellular content, each node in the tree corresponds to the agglomeration of the cells of all dependent leaves. For each node, we show the global UMAP yet color only the cells that are part of the node. The colors used correspond to the colors of Fig. 2D, with epithelial cells in reddish tones, lymphoid cells in bluish tones, myeloid cells in greenish tones, and stromal cells in orange tones. For part of the nodes, corresponding to what were, in the end, nodes identified as the upper levels of one of 49 specific cell types, we add the same UMAP yet this time labeled in pseudo-color measuring the intensity of expression of a set of signature genes. The source of the corresponding gene signature is provided, as well as the name of the corresponding cell type (underlined). If the source is one of the following references [references 22, 27, 28, 29 in main text], we use yellowish tones. If the source is the Azimuth human PBMC reference mapping program [reference 24 in main text], we use bluish tones. The reason why we herein mention 49 cell types as opposed to 43 in f.i. Fig. 2A, is because we herein (SFig. 6) separately highlight ileal, colonic and rectal cell clusters for some epithelial cell type, while these are considered as one in Fig. 2A. Each lower panel shows expressions of a subset of marker genes (rows) characteristic for the indicated cell types (columns) in references [reference 22, 23, 24, 25, 26, 27, 28 in main text]. Cells annotated as low quality (LQ) were ignored.

(I) Seurat cell cycle phase scores, the number of RNA detected and module scores for mitochondrial genes are shown on the UMAP.

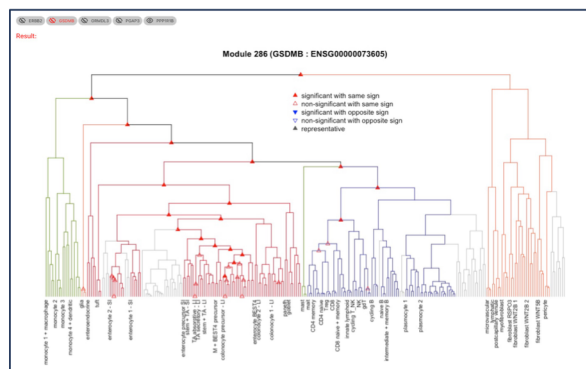
(J) Nodes, in the hierarchical tree, for which all dependent leaves derive from the same anatomical location (terminal ileum, transverse colon, rectum) are color-labeled accordingly. It appears that one has to go quite “high” (i.e., towards the leaves) in the tree, before colors appear. This means that for most cell types, the cells have very similar transcriptome across anatomical locations. This is especially true for myeloid and lymphoid cell types. Separation by anatomical location occurs a bit earlier in the tree for epithelial and, to a lesser extent, for stromal cells.

Supplementary Figure 7: Screenshots from the CEDAR website (<https://tools.giga.uliege.be/cedar>) for the scRNA Seq analyses of the intestinal biopsies.

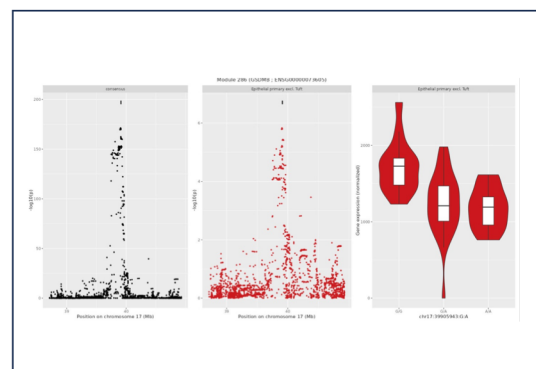
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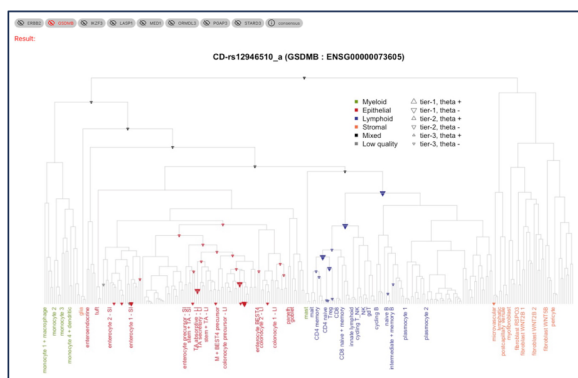
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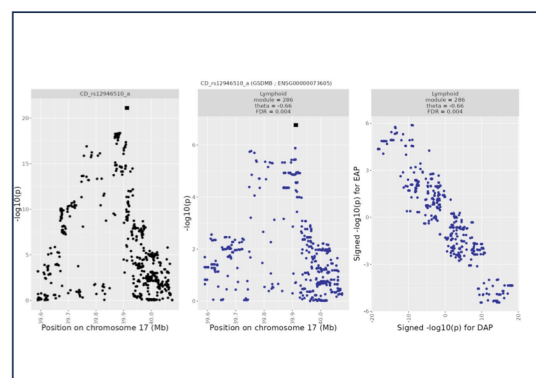
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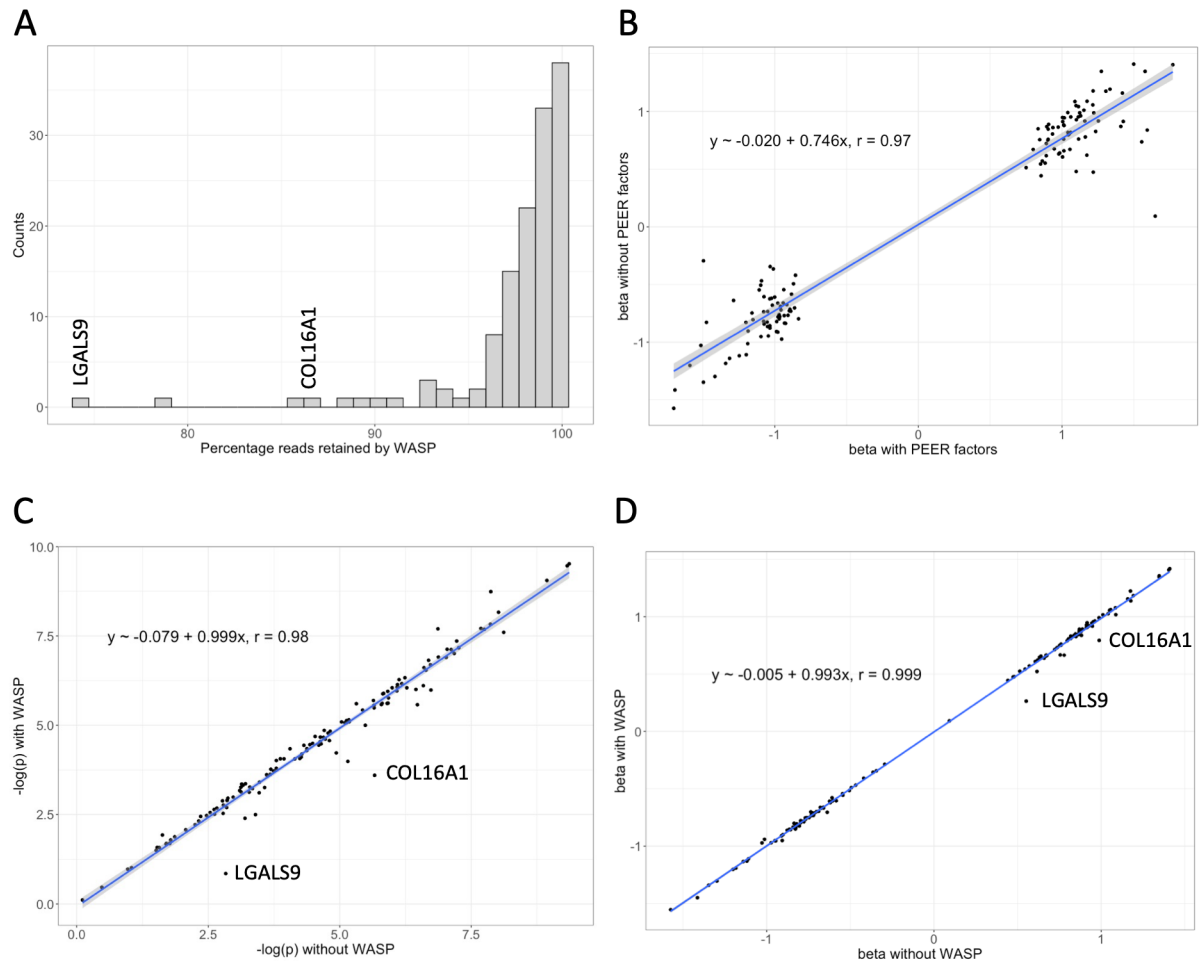
E



(A) The X axis of the main page corresponds to the genome coordinates showing, in the top panel, the position of the genes as well as the boundaries of color-coded disease risk loci (IBD, CD and UC in the example), and in the lower panel the expression levels (marbles, not shown) and eQTL activity (arrows, as in SFig. 4 for blood) for the different genes. The Y axis corresponds to the cell type hierarchy guided by the tree resulting from

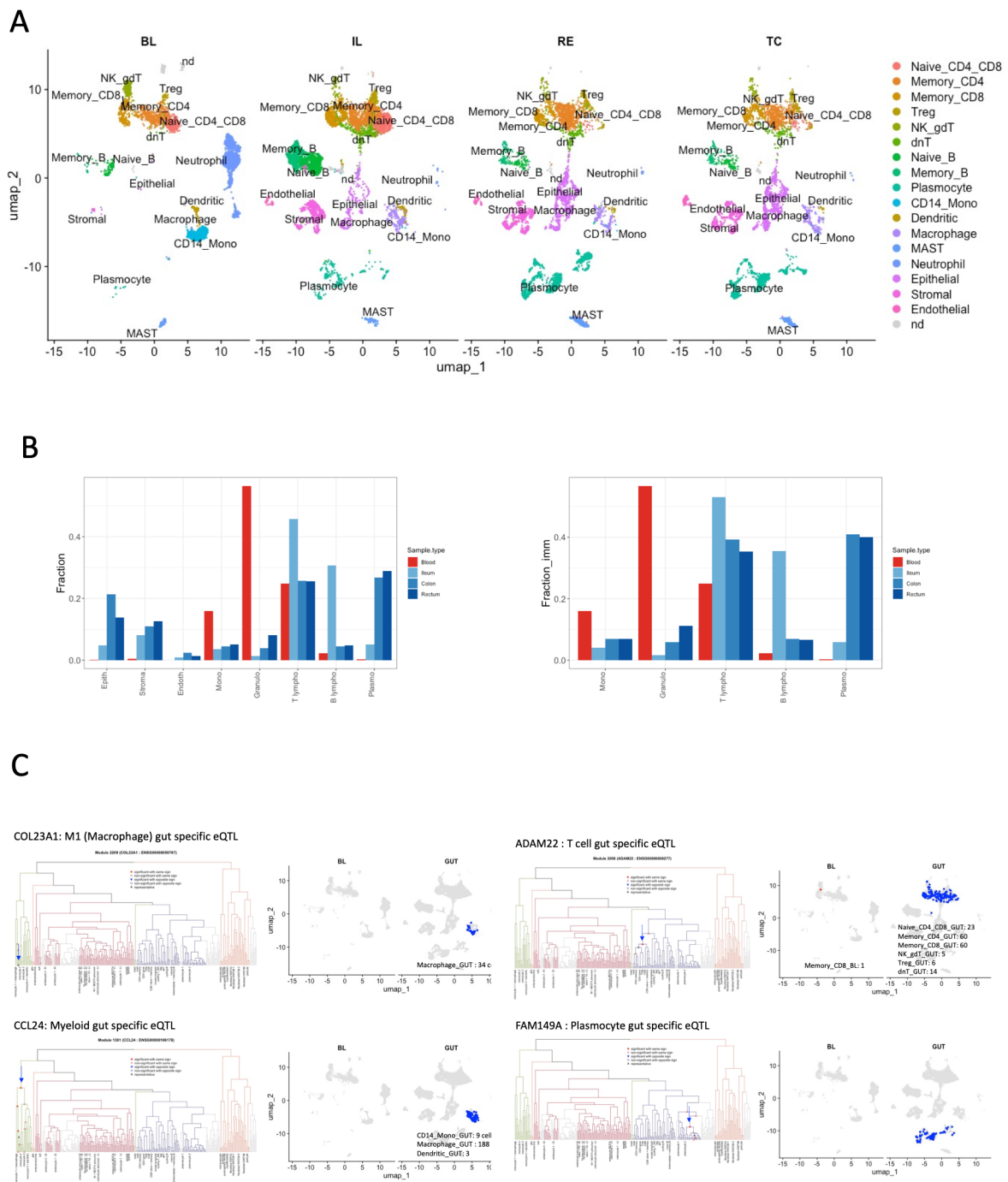
hierarchical clustering of the cell clusters and their annotations to specific intestinal cell types. Clicking on a level in the cell type hierarchy shows the corresponding cell selection on the 2D UMAP. **(B)** Clicking on an arrow (corresponding to an eQTL effect) calls a set of pages for each gene in the corresponding module, each with the hierarchical tree with symbols showing the activity of the eQTL (full circles: significant eQTL, empty circles: non-significant eQTL yet matching the consensus EAP of the module). **(C)** Clicking on the symbols calls a next page that shows the consensus EAP of the module, the EAP for the selected gene for the selected position in the tree and the corresponding violin plot for the top variants of the (consensus) EAP. **(D)** Clicking on a disease locus calls a set of images corresponding to trees for all genes that show a matching EAP-DAP for the selected risk locus. **(E)** Clicking on the corresponding symbols in the image, calls gene-specific pages showing the DAP for the corresponding risk locus, the EAP for the corresponding eQTL and the theta plot.

Supplementary Figure 8: Evaluating allele-specific mapping efficiency as a cause of spurious compartment-specific eQTL.



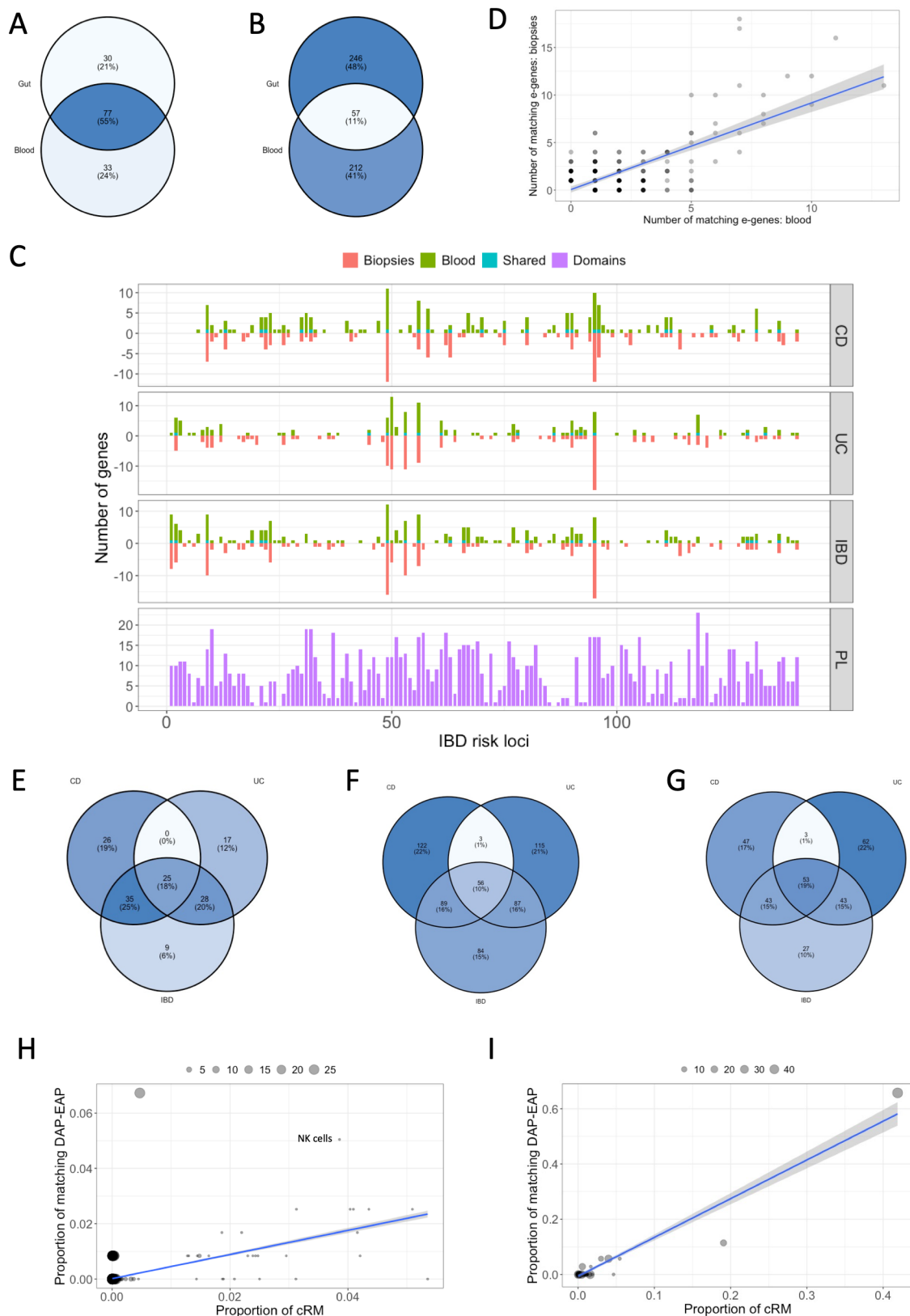
The eQTL-detection pipeline used for blood included a preprocessing step with WASP [reference 31 in main text], to mitigate variant effects on mapping efficiency of RNA reads. WASP was not part of the pipeline used for the analysis of the intestinal scRNA-Seq data, and this could have generated false-positive gut-specific eQTL. Thus, we applied WASP to the candidate gut-specific eQTL. For 14 of these, WASP had no effect at all on the number of mapped reads, and hence could not have had an effect on the eQTL results. For the remaining, WASP had an effect on the number of utilized reads, but it was modest: on average, WASP excluded 2.3% of the reads, ranging from 0% to 25.64% (*LGALS9*) (Panel A). We nevertheless tested whether the eQTL effect was affected by WASP preprocessing. Thus, we performed eQTL analysis before and after WASP preprocessing. We did not correct expression levels for PEER factors in these analyses, as the correlation between the eQTL effects (i.e., the beta's) with and without the use of PEER factors was 0.97 (panel B). The correlation between the $\log(1/p)$ values before and after WASP was 0.98 and the slope of the regression line was 0.99 (Panel C), while the correlation between the corresponding beta-values was 0.99 and the slope of the regression line was 1 (Panel D). Only for two genes, did the β -value drop significantly after WASP: *LGALS9* in gut-resident plasmacytes (Bonferroni-corrected $p = 5.1 \times 10^{-12}$), and *COL16A1* in B cells (Bonferroni-corrected $p = 7.4 \times 10^{-5}$). These were dropped from the list of compartment-specific eQTL (Suppl. Data 18). Therefore, we conclude that allele-specific mapping efficiency did not cause a significant number of false-positive gut-specific eQTL.

Supplementary Figure 9: Integrated blood, ileal, colonic and rectal GEM-X Flex scRNASeq



(A) UMAP of integrated blood, ileal, colonic and rectal GEM-X Flex scRNASeq data for 23,261 cells. Cell types were annotated using Seurat [reference 24 in main text] **(B)** Proportion of the different cell types (*left panel*: all cell types; *right panel*: immune cell types only) for blood (red), ileum (light blue), transverse colon (blue), rectum (dark blue). **(C)** GEM-X Flex expression profiles for genes that are subject to compartment-specific eQTL: (i) myeloid lineage: *COL23A1* and *CCL24* are two genes that are expressed in macrophages (*COL23A1*, *CCL24*) and dendritic cells (*CCL24*), cells in which they are subject to a gut-specific eQTL. (ii) T lymphocytes: *ADAM22* is expressed in multiple types of T lymphocytes in the gut but not in blood. (iii) B lymphocytes: *FAM149A* is expressed in gut-resident plasmocytes but not in circulating plasmocytes.

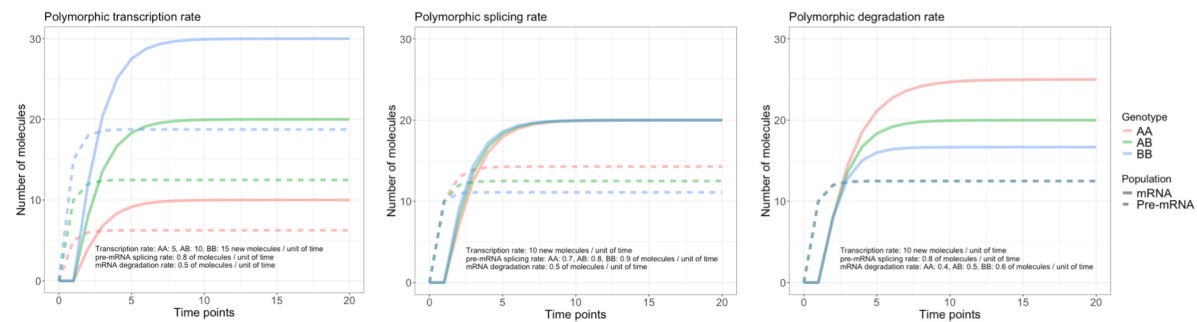
Supplementary Figure 10:



(A) Number of IBD risk loci with matching DAP-EAP in intestinal biopsies only (gut, n=30), in circulating immune populations only (blood, n=33), and both in gut and blood (n=77). **(B)** Number of e-genes with matching DAP-EAP in intestinal biopsies only (gut, n=246), in circulating immune populations only (blood, n=212), and both in gut and blood (n=57). **(C)** Number of e-genes with matching DAP-EAP in 206 IBD risk loci (ordered by

chromosomal position along the X-axis) in blood (green bars) and biopsies (orange bars, numbers x -1). The blue bars correspond to the number of e-genes shared between blood and biopsies for a given risk locus. Numbers are given separately for CD, UC and IBD risk loci (see Suppl. Data 21). Intestinal biopsies only (gut, n=246), in circulating immune populations only (blood, n=212), and both in gut and blood (n=57). The panel with the violet bars at the bottom, labeled “PL” (-eiotropy), reports the sum of the number of “trait domains” that are influenced by the pleiotropic genomic loci, defined by Watanabe *et al.*, 2019, that overlap with the corresponding IBD risk locus. **(D)** The number of matching e-genes (i.e., with matching DAP-EAP) in blood (X-axis) and biopsies (Y-axis) is highly correlated across the IBD risk loci, despite the fact that the corresponding e-genes mostly differ between blood and biopsies (see blue bars in panel C). **(E)** Numbers of risk loci with matching e-genes for CD, UC and IBD, with corresponding overlaps (i.e., risk loci with matching e-genes for CD and UC but not IBD (n=0), CD and IBD but not UC (n=35), UC and IBD but not CD (n=28), CD, UC and IBD (n=25)). **(F)** Numbers of matching e-genes for CD, UC and IBD, with corresponding overlaps (i.e., e-genes matching the DAP for CD and UC but not IBD (n=3), CD and IBD but not UC (n=89), UC and IBD but not CD (n=87), CD, UC and IBD (n=56)). **(G)** As for (F), yet restricting the analysis to 81 loci that are risk loci for both CD and UC. **(H)** The proportion of matching DAP-EAP involving “blood” cis-acting regulatory modules (= cRM) with a given “activity vector” (i.e., the combination of the 27 cell types in which the module is active) is correlated with the proportion of regulatory modules (= RM) with that “activity vector”. Yet, some types of modules stand out. Regulatory modules that are active in all cell types account for only 0.0047 of all across-gene modules, yet account for 0.0672 of DAP-EAP matches (> 14-fold excess), reminiscent of Momozawa *et al.* [reference 15 in main text]. Each dot corresponds to one of 3,572 observed activity vectors. The size of the dot corresponds to the number of “1”s in the vector (i.e., the number of cell-types out of 27 in which the module is active). The graph was obtained considering across-gene modules and the three diseases (CD, UC and IBD) combined. **(I)** The proportion of matching DAP-EAP involving “biopsy” regulatory modules encompassing a number of cell types (i.e., out of the 43 distinguished cell types; see also Fig. 2A) is correlated with the overall proportion of cognate regulatory modules (RM = cis-acting regulatory module). Yet regulatory modules assigned to the root of the hierarchical tree (Fig. 2F), and hence encompassing mostly modules that are active in many cell types, account for the highest proportion of DAP-EAP matches. Each dot corresponds to one of 64 observed cell-type groups. The size of the dot corresponds to the number of cell types (out of 43) included in the group. The graph was obtained considering gene-specific modules and the three diseases (CD, UC and IBD) combined.

Supplementary Figure 11: Pre-mRNA and mature mRNA-specific eQTL due to variants affecting transcription, splicing and degradation rate, respectively



The steady state levels of pre-mRNAs and mature mRNAs in cells reflect an equilibrium between transcription and splicing rate, for pre-mRNAs, and between pre-mRNA levels, splicing and degradation rate, for mature mRNAs. Genetic variants that affect the **transcription rate** will generate different steady state levels between individuals of different genotypes (say AA, AB and BB) for both pre-mRNAs and mature mRNAs (left panel). Genetic variants that affect **splicing rate** will generate different steady state levels between individuals of different genotypes for pre-mRNAs but not for mature mRNAs (middle panel). Genetic variants that affect mRNA stability (and hence their **degradation rate**) will generate different steady state levels between individuals of different genotypes for mature mRNAs but not for pre-mRNAs (right panel).

Accordingly, the detection of eQTL caused by variants affecting splicing or degradation rate may be affected by the RNA extraction and sequencing methods. To detect eQTL due to variants affecting splicing rate requires that the extracted RNA include sufficient pre-mRNA molecules and that the sequencing pipeline considers reads corresponding to non-spliced transcripts. To detect eQTL due to variants affecting degradation rate requires that the extracted RNA include sufficient mature mRNA molecules while the sequencing pipeline may only consider spliced transcripts.

The RNA extraction, and cDNA synthesis methods used for the blood and gut samples were different. This may have affected the ratio of interrogated pre-mRNA versus mature mRNA molecules. And this may have differentially impacted the power to detect eQTL resulting from variants affecting splicing or degradation rate, hence possibly causing false-positive compartment-specific eQTL. However, as the genome space devoted to the control of transcription rate is substantially larger than the genome space devoted to the control of alternative splicing, splicing or degradation rate, we believe that such mechanisms could only account for a minority of observed gut-specific eQTL.

Supplementary Material 1: Comments on candidate drugs for repurposing to treat IBD with genetic support from this study.

CFTR. Well-studied chloride channel essential for ion transport and fluid secretion in tissues, including the gastrointestinal tract, and playing an important role in mucin maturation in the intestine¹. Nullizyosity for *CFTR* causes cystic fibrosis (CF), while hemizyosity may increase resistance to infectious diseases². The prevalence of IBD was shown to be 7 times higher in CF patients compared to controls³. *A contrario*, *CFTR* hemizyosity was recently shown to protect against CD⁴. Also, glibenclamide, a (*CFTR*) inhibitor, was reported to protect rats against 2,4-dinitrobenzene sulfonic acid (DNBS)-induced gastrointestinal inflammation⁵. In agreement with this, we herein shown that increased *CFTR* expression in intestinal epithelial and stromal cells is associated with increased risk for UC. Note, however, that there is some evidence (albeit non-significant) from our data that decreased expression of *CFTR* in specific enterocyte populations may increase CD risk, deserving further analysis in a larger dataset. Also, plasma levels of *CFTR* were found to be increased in IBD patients. This suggests that downregulating *CFTR* may improve IBD. Of note, *CFTR* has been shown to act as a pathogen recognition molecule that can induce NF- κ B activation⁶, and serves as epithelial receptor for *S. Typhi* transluminal migration⁷. Also, *CFTR* KO mice secreted more intestinal mucus^{8,9}, which plays a protective role in IBD. While most drug development targeting *CFTR* aims at restoring function in the context of CF, there exist at least one presumed *CFTR* inhibitor, namely crofelemer, approved for HIV-associated diarrhea¹⁰. Crofelemer is a natural, (not so) small molecule derived from the *Croton lechleri* tree. It is taken orally to treat secretory diarrhea (as opposed to diarrhea resulting from an underlying inflammatory process) in various contexts. It has proven effective in HIV-seropositive patients on stable antiretroviral therapy¹¹. Being primarily used for non-inflammatory diarrheal conditions, crofelemer may alleviate diarrheal symptoms IBD patients but it is unclear whether it also affects the underlying inflammatory process. Of note, inhibition of *CFTR*-mediated intestinal chloride secretion could be a potential therapy for bile acid diarrhea, a condition frequently present in IBD¹². It seems important to determine whether the apparent effect of *CFTR* expression on IBD risk depends on *CFTR*'s chloride channel activity (affected by crofelemer) or of distinct functions of this gene (such as pathogen recognition).

CYP3A5. Codes for a member of the cytochrome P450 monooxygenases metabolizing various endo- and xenobiotics. In particular CYP3A enzymes participate in bile acid biotransformation, which – if perturbed – could contribute to the disruption of gut homeostasis in colitis patients including by affecting microbiota composition¹³⁻¹⁵. We observe that an increase of CYP3A5 expression in naïve CD8 increases CD risk (tier 1). However, in enterocytes of the small intestine, the opposite seems to occur (negative theta), albeit with FDR above 0.10 but potentially more relevant. There was no detectable effect of disease status on CYP3A5 expression/abundance in blood, biopsies and plasma. There exist at least two small molecule inhibitors of CYP3A5: cobicistat and ritonavir, used to boost the effectiveness of other drugs (particularly antiretroviral). Ritonavir may have independent anti-inflammatory action, as well as affect the microbiome but this appears unrelated to the observed DAP-EAP match. Genotype at CYP3A5 variants has been proposed as a guide to tacrolimus dosage in the treatment of ulcerative colitis^{16,17}, yet this bears no relationship with DAP-EAP matching.

IL18R1 and IL18RAP. IL18R1 and IL18RAP code for the IL18 receptor and its accessory subunit (enhancing IL18 binding), respectively. With IL1, IL18 is activated upon PAMP-recognition by the inflammasomes. Our data indicates that downregulation (predominantly) of both genes (IL18R1 and IL18RAP), primarily (but not exclusively) in circulating immune cells (including ILC, MAIT, and Th17), increases the risk to develop CD (predominantly), hence that “activating” both might have a beneficial effect. In agreement with these findings, IL18R KO mice are more susceptible to DSS-induced colitis¹⁸, and pre-treatment of mice with IL18 protected against DSS-induced colitis¹⁹. However, IL18 administration at later stages of DSS-induced colitis exacerbates symptoms¹⁹, while administration of IL18 neutralizing antibodies reduces the severity of DSS-induced colitis [f.i. ²⁰]. In humans, abundance of IL18 is generally increased in IBD patients, both in blood and mucosa²¹, while mendelian randomization indicates that increase in IL18 concentrations increases IBD risk²². We observe an increase of IL18R1 mRNA abundance in naïve B cells of active CD patients, while Eldjarn *et al.*²³ report an increased abundance of IL18R1 in IBD patients. Thus, the IL18 axis appears to have an anti-inflammatory effect prior to disease declaration, yet a pro-inflammatory effect after. Iboctadekin is reported as an agonist of IL18R1 and IL18RAP, and is in fact recombinant human IL18. Iboctadekin has been tested in the context of infectious diseases and oncology, but with mediocre results, and its development was discontinued. Given the apparent dual function of the IL18 axis in inflammation, the use of iboctadekin in the treatment of IBD appears difficult to support. Nevertheless, an iboctadekin-based treatment that might be considered in the context of IBD, is to prevent relapse, particularly after disease remission has been achieved following surgery, rather than its use during the active phase of the condition.

INPP5D. Encoding inositol Polyphosphate-5-Phosphatase D (=SHIP1). SHIP acts as a multifunctional protein controlled by multiple regulatory inputs, and influences downstream signaling via both phosphatase-dependent and -independent means. SHIP1 has functions in B cells, T cells, NK cells, dendritic cells, mast cells, and macrophages²⁴. Downregulation is generally considered to be pro-inflammatory. SHIP deficiency causes Crohn's disease-like ileitis in the mouse²⁵, while deficiency in Inflammatory Bowel Disease is associated with severe Crohn's disease and peripheral T cell reduction²⁶. Accordingly, we observe that *INPP5D* downregulation in circulating T lymphocytes, MAIT and NK cells, as well as resident monocytes increases CD risk. Also, *INPP5D* expression is decreased in circulating monocytes from IBD patients. The small molecule rosiptor, the most developed and characterized allosteric activator of INPP5D, has been unsuccessfully tested as anti-inflammatory drug to treat interstitial cystitis and bladder pain syndrome and its development has been discontinued [<https://www.pharmaceutical-technology.com/news/aquinox-halts-development-drug-bladder-pain-syndrome/>]. Binding of rosiptor to SHIP1 was shown to be weak when compared to other agonists which may in part explain poor clinical results^{27,28}. We conclude that rosiptor is probably not an optimal candidate for repurposing.

LAMA2. Codes for an α subunit of laminins, an essential component of the basement membrane with important roles in tissue organization. LAMA2 deficiency causes a muscular dystrophy accompanied by an exacerbated innate immunity response, autophagy and cell death²⁹. Intriguingly, somatic LAMA2 mutations were recurrently observed in colonic mucosa of DSS-treated mice³⁰. LAMA2 may play a role in intestinal barrier homeostasis. Along those lines, mutations in laminins have been associated with skin blistering³¹. We observe that variants that increase *LAMA2* expression, including in precursor and mature enterocytes,

consistently increase CD risk. However, *LAMA2* expression was found to be downregulated in fibroblasts of IBD inflamed terminal ileum. Ocriplasmin is an injectable (intravitreally) protease inhibitor acting on laminins (and fibronectin) that is used to treat vitreomacular adhesion^{32,33}. Whether it is the down- or up-regulation, or any, that is desirable in this case is uncertain. There is a need for further exploration of the link between *LAMA2* and IBD, which may also involve its role in autophagy³³.

NEK7. NIMA Related Kinase 7, plays a central role in assembly of the NLRP3 inflammasome³⁴. Missense *NLRP3* mutation cause dominant congenital multisystem inflammatory diseases (Cinca (MIM 607115) and Muckle-Wells syndromes (MIM 191900)). Downregulating NEK7 by intraperitoneal injection of lentiviruses expression anti-NEK7 shRNAs attenuates DSS-induced colitis³⁵. We observe that variants that increase NEK7 expression in circulating naïve B cells increase CD risk. Of note, however, these same variants appear to decrease NEK7 expression in gut-resident lymphoid and myeloid immune cells, albeit with FDR > 0.10, hence inverting the association with CD (decreased expression increases risk). Disease affects NEK7 expression but the sign of the affect depends on cell type: expression is increased in enterocytes from non-inflamed colon of patients when compared to controls, while expression is increased in some and decreased in other circulating immune cells of patients. Entrectinib (ENB) is a potent tyrosine multikinase small-molecule inhibitor that targets the NTRK, ROS1 and ALK oncogenes, approved by the FDA for the treatment of various tumors³⁶. It was recently shown to bind to NEK7 R121, and interferes with its interaction with NLRP3. In mouse models, ENB effectively reduced symptoms of NLRP3 inflammasome-related diseases (other than colitis), indicating its potential as a therapeutic candidate³⁷. ENB has a high safety profile and is well tolerated by almost all patients without cumulative toxicity³⁸. Inhibition of the inflammasome has shown beneficial effects in various inflammatory pathologies. ENB appears may be a repurposing candidate for IBD.

PRKAA1. PRKAA1 codes for the α_1 isoform of the catalytical subunit of the AMP-activated ser/thr protein kinase (AMPK) that plays a central role in cellular energy homeostasis by activating ATP-producing catabolic processes under conditions of high AMP/ATP ratios. Several AMPK-activating drugs are known, including AICAr (aka acadesine) which is converted intracellularly into the AMP-mimic ZMP, that binds to the regulatory γ AMPK subunit(s). AMPK stimulation inhibits the proliferation of several cell types, justifying the use of AMPK activators (including AICAr) to treat leukemias. Moreover, AICAr increases extra- and intracellular concentrations of adenosine, a potent vasodilator, underpinning its use to improve post-ischemic heart recovery³⁹. AMPK is increasingly recognized as a central player in immune homeostasis as well, affecting both adaptive and innate immunity⁴⁰ (Suppl. Data 26). In general, activation of AMPK has anti-inflammatory effects. AMPK also plays a role in the regulation of autophagy⁴¹. AMPK also contributes to intestinal homeostasis. Indeed, knocking PRKAA1 out in colonic epithelium of mice delays epithelial repair after DSS-induced colitis⁴², and this could involve effects on epithelial cell migration and tight junction integrity^{43,44}. Concomitantly, our colocalization data support the fact that a reduction in *PRKAA1* expression in gut-resident plasmocytes and possibly in colonocyte precursors increases the risk to develop UC. We didn't see a concomitant decrease of *PRKAA1* expression in IBD patients, but rather a modest increase in cycling immune cells in inflamed colon. However, others have reported a reduction of Thr172 phosphorylated (and hence active) α AMPK in IBD of patients⁴⁵. Interestingly, AMPK activation with AICAr or metformin has been shown to reduce

the severity of SDS- and TNBS-induced colitis in the mouse ^{42,46,47} and other models of inflammatory illnesses⁴⁰.

Taken together, these data support AMPK activation as a potential therapeutic target for IBD, as has been indicated previously^{40,48}. However, AICAr has shown poor oral bioavailability, the preferred route of administration being intravenous injection³⁹. Hence, other AMPK activating drugs, including metformin, may be more suitable repurposing candidates.

Of note, the *PRKAA1* gene is located in the vicinity of the *PTGER4* gene, which was previously incriminated as potential causative gene driving the corresponding IBD risk locus⁴⁹. An additional DAP-matching EAP for the *TTC33* gene is also found in this work for gut-resident T lymphocytes.

PTGIR. Encodes the G protein coupled receptor of prostacyclin (PGI₂). Prostacyclin is mainly known as a potent vasodilator (and inhibitor of platelet activation). Concomitantly, small molecule agonists of PTGIR (often prostacyclin mimics) are primarily used in the treatment of pulmonary arterial hypertension. However, prostaglandins, including prostacyclin, are known to affect inflammation, having either pro-inflammatory (f.i. rheumatoid arthritis) or anti-inflammatory (f.i. allergic inflammation) effects depending on context^{50,51}. PTGIR has well-documented anti-inflammatory properties by inhibiting the production of pro-inflammatory cytokines and the immune response⁵². Paradoxically, stimulating PTGIR signaling in cultured human monocytes augments calprotectin expression, a marker of inflammation⁵³. PTGIR may play a role in maintaining the intestinal epithelial barrier⁵⁴, and in regulating intestinal microcirculation which is impaired in IBD [f.i. ⁵⁵], and tissue repair. Of note, increased expression of the *PTGER4* receptor for PGE₂ (known to affect the immune system) has been associated with an increased risk for CD ⁴⁹, yet *PTGER4* knock-out mice are more susceptible to DDS-induced colitis, while *PTGER4* agonists are protective⁵⁶. We observe that reduced expression of PTGIR in multiple circulating T lymphocyte populations as well as resident monocytes is consistently associated with increased risk to develop UC. We don't see a clear effect of disease status on PTGIR expression/levels whether in circulating leukocytes, biopsies or plasma. We note that the use of cyclooxygenase inhibitors reportedly exacerbates symptoms in some IBD patients. As for IL18R1/IL18RAP, PTGIR activator-based treatments that could be considered in the context of IBD, are to prevent relapse, particularly after disease remission has been achieved following surgery, rather than their use during the active phase of the condition.

TUBB4B. The *TUBB4B* gene codes for one of the 10 human β -tubulin paralogues. α - and β -tubulins form heterodimers which assemble in protofilaments and then microtubules, one of the main components of the cytoskeleton. Microtubules play an essential role in mitotic and meiotic chromosomal segregation, intracellular transport, assembly and function of cilia and flagella (sperm), cell polarization, junction and migration. Not surprisingly, microtubules fulfil important functions in cells of the adaptive and innate immune systems (f.i. role in immune synapse, and leukocyte migration) as well as of the intestinal mucosa ⁵⁷⁻⁵⁹. There exist several drugs perturbing the function of microtubules, including the microtubule destabilizing colchicine and microtubule stabilizing taxol and derivatives. With regards to IBD, levels of acetylated α -tubulin (supposedly a marker of stable microtubule structure) were shown to be reduced in colonic samples of IBD patients and mice with DSS-induced colitis, while taxol was shown to mitigate colitis symptoms in DSS-treated mice⁶⁰. Also of interest, colchicine (but not taxol) hampers the activation of the pyrin inflammasome, yet this effect is abrogated by pyrin

mutations (i.e., in the *MEFV* gene) causing the common Familial Mediterranean Fever. The beneficial effect of colchicine in the treatment of FMF is attributed to its effect on leukocyte motility and phagocytosis^{61,62}. On a cautionary note, the use of taxol derivatives in chemotherapy has been associated with cases of drug-induced inflammatory colitis and typhlitis⁶³. Also, the use of colchicine in the mouse, was shown to cause serious intestinal injuries, inhibit inflammatory pathways, and increase intestinal permeability⁶⁴.

More specifically, *TUBB4B* codes for an isoform of β -tubulin harboring a conserved C-terminal motif suggesting that it participates in the formation of cilia. In the mouse, *TUBB4B* localizes to cilia of multi-ciliated cells including in respiratory, oviductal and ependymal epithelial cells, and these structures are affected in *TUBB4B* KO mice⁶⁵. In humans, mutations in *TUBB4B* cause dominant Leber congenital amaurosis⁶⁶, primary ciliary dyskinesia (PCD)⁶⁷, and are associated with left-handedness⁶⁸. With regards to IBD, *TUBB4B* was identified as part of a gene expression signature defining UC in remission⁶⁹, while the tubulin inhibitor mebendazole, emerged amongst the top 10 candidates from a CMap-based analysis of genes differentially expressed in UC⁷⁰.

From our data *TUBB4B* appears fairly highly expressed in many cell types including intestinal epithelial cells [<https://tools.giga.uliege.be/cedar/publihpq/>]. Variants that increase *TUBB4B* levels in BEST4 enterocytes and WNT2B fibroblasts (and to a lesser extent in colonocyte precursors and resident T cells), increase the risk to develop IBD. *TUBB4B* was significantly overexpressed in circulating plasmocytes and underexpressed in circulating granulocytes of CD patients (Suppl. Data 22). Of note, the corresponding IBD risk locus overlaps the *CARD9* and *INPP5E* genes, while the *TUBB4B* gene is located at ~750 Kb.

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