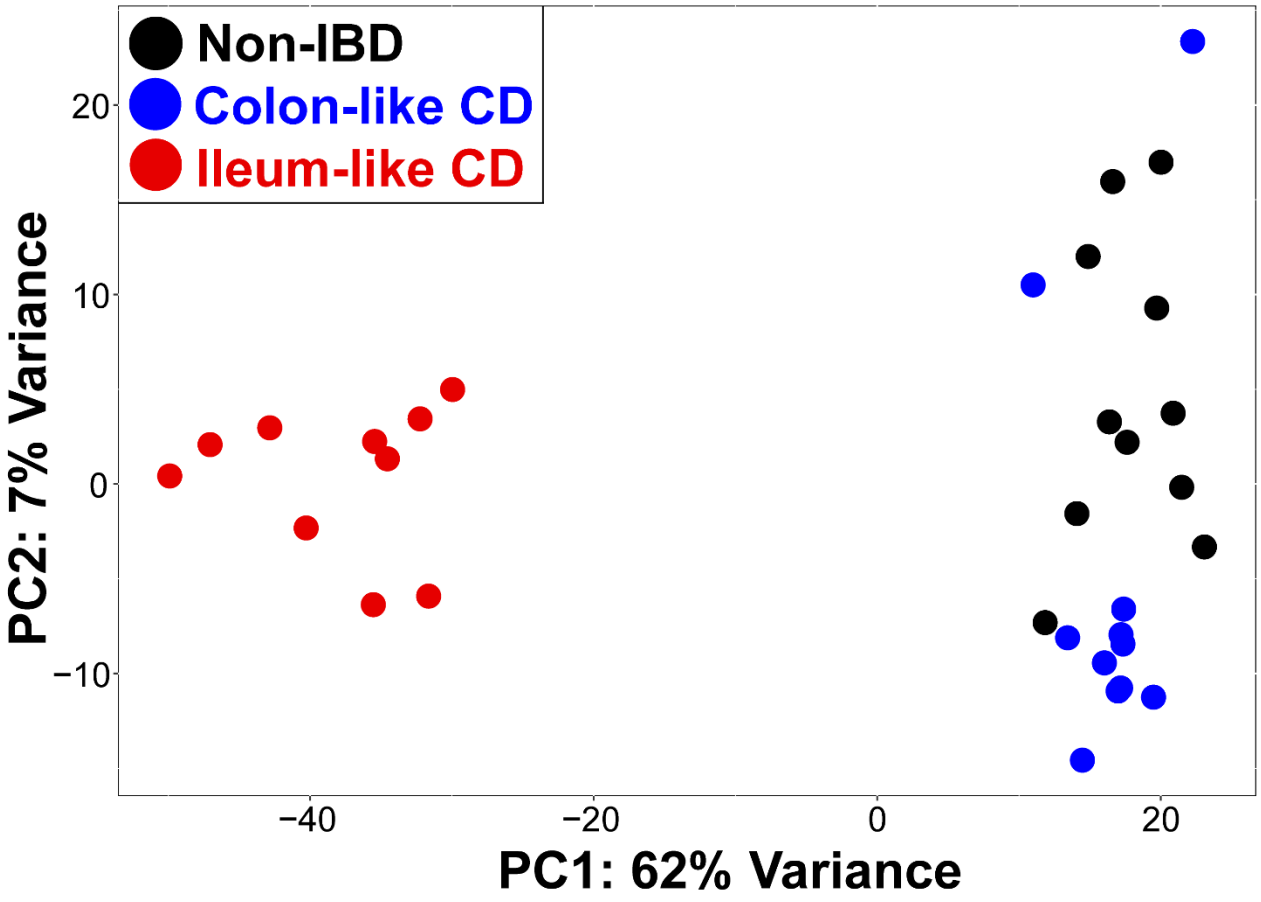
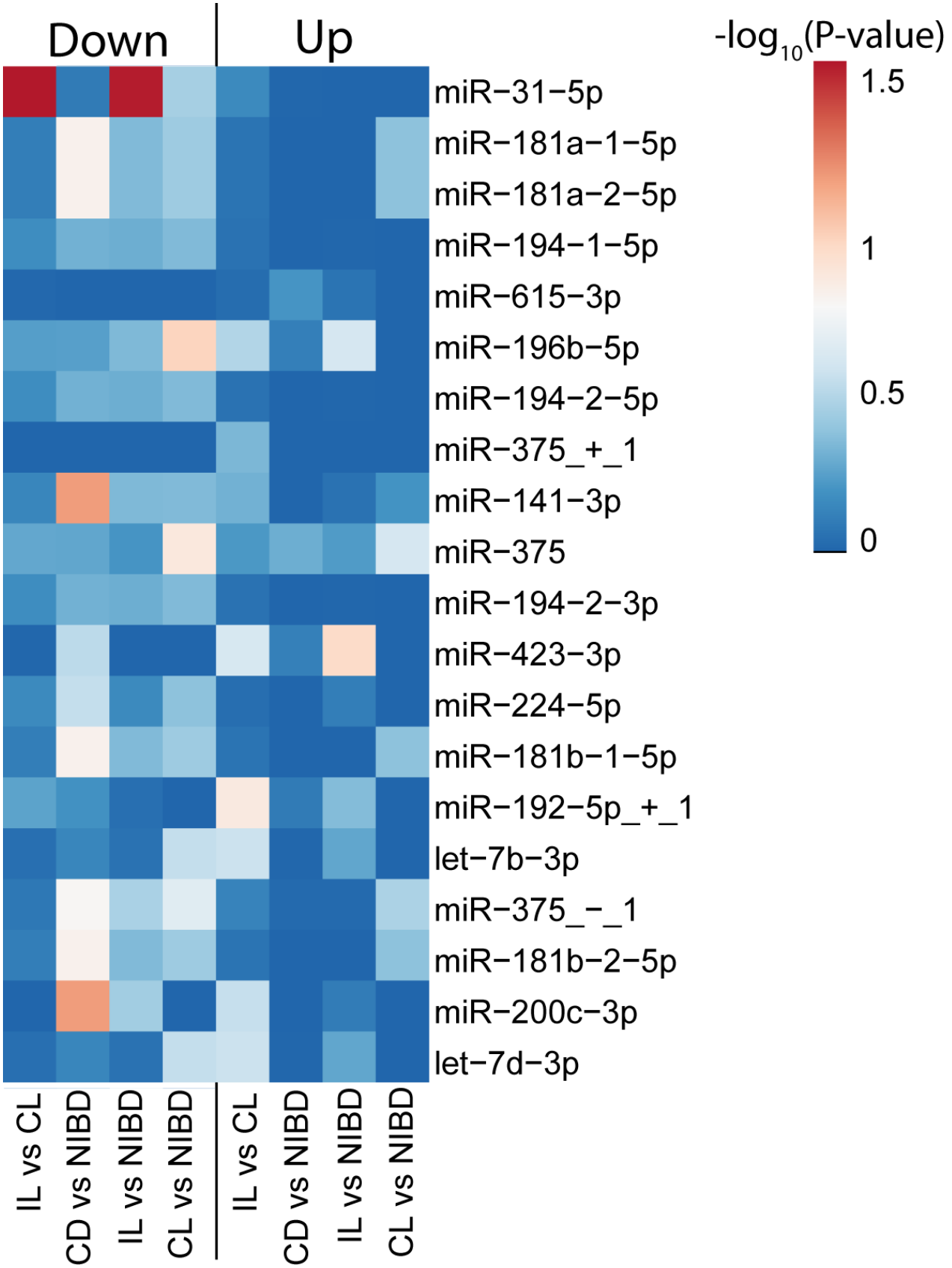
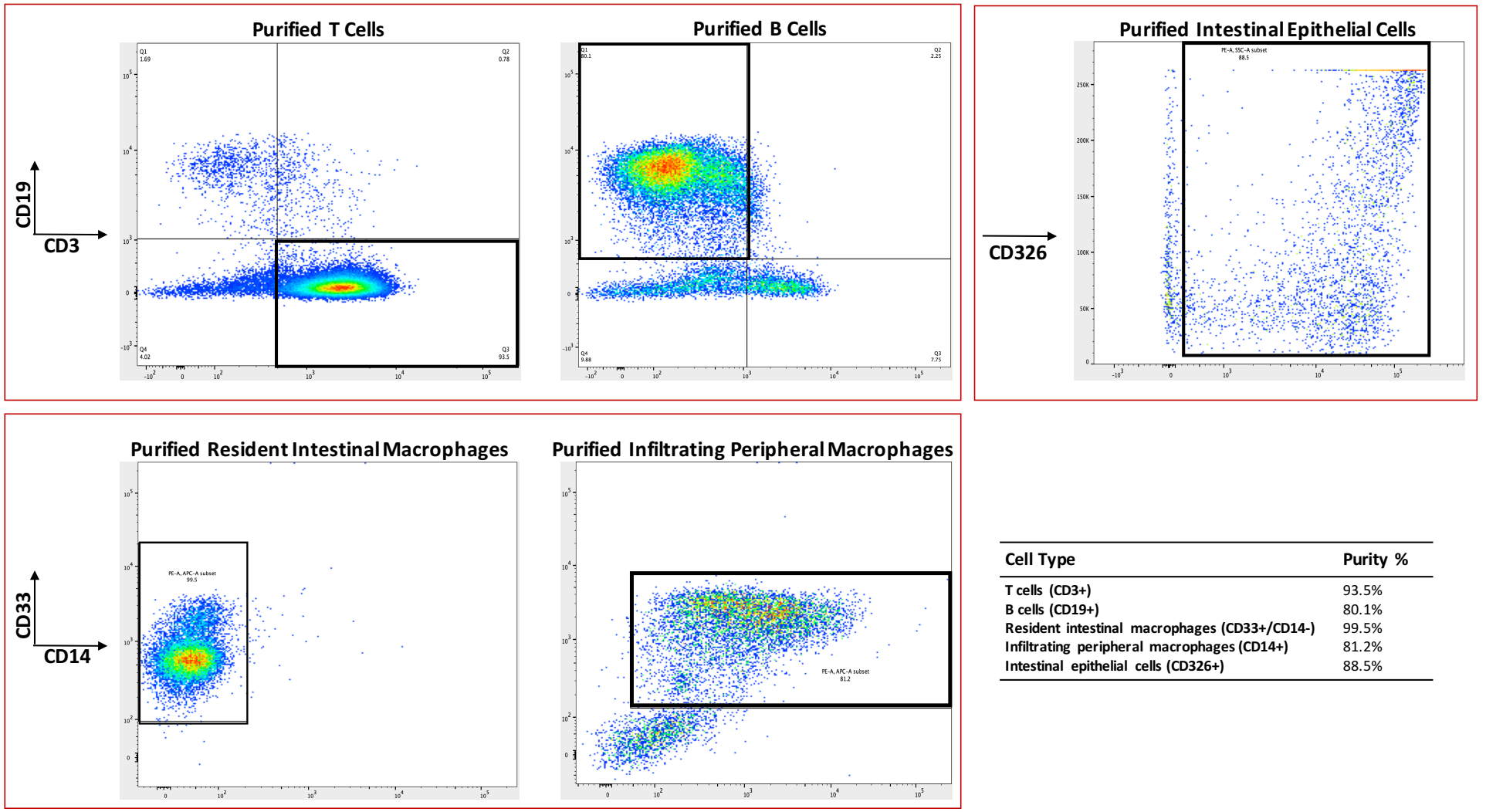


Supplementary Figure 1. mRNA expression profiles segregate Crohn's disease (CD) samples into two distinct molecular subtypes. Using updated gene annotations from GENCODE, we recapitulated our previous analysis[1] to show that clustering of colon-like CD patients (blue, n=11) with non-IBD patients (black, n=11) is distinct from ileum-like CD patients (red, n=10). PCA loadings for the above plot can be found in Supplementary Table 3.

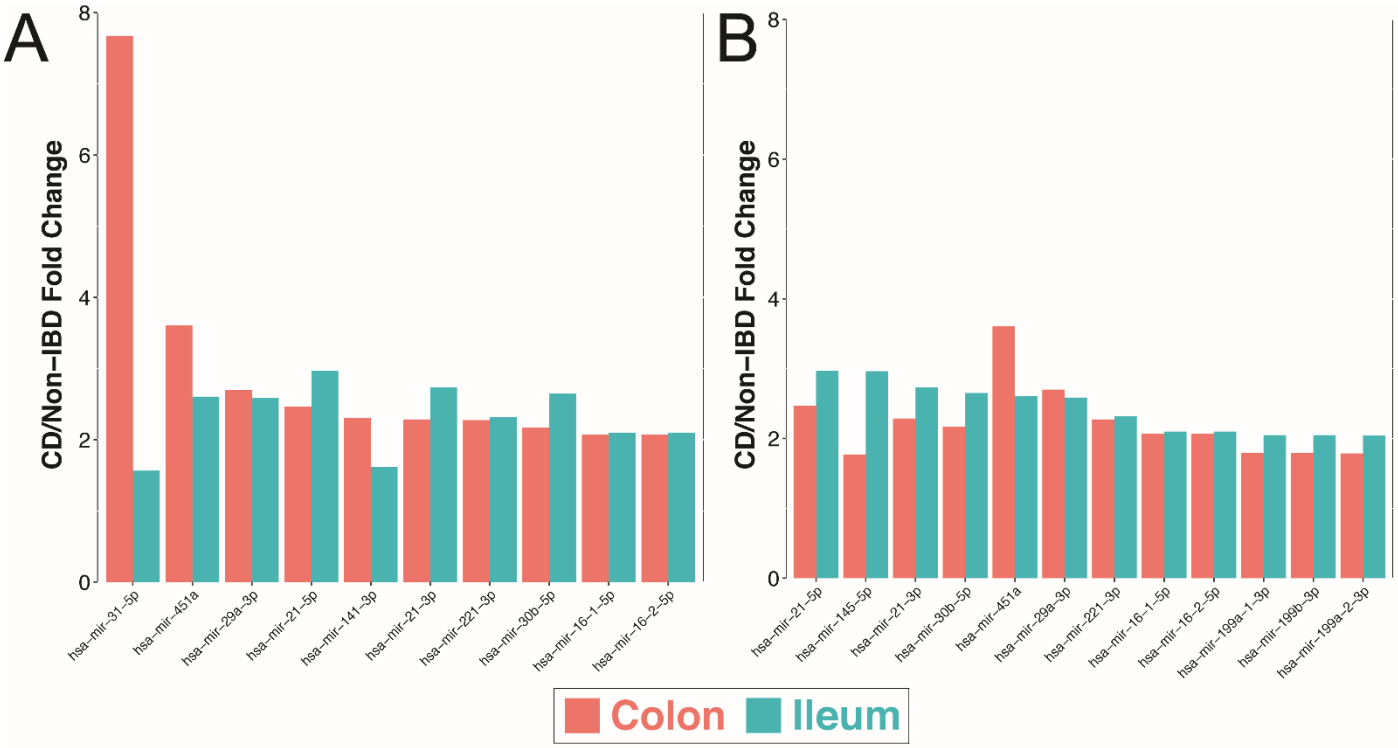


Supplementary Figure 2. microRNA-31 is potential master regulator of pathways disrupted in CD pathogenesis. Using genes that were differentially expressed between IL-CD and CL-CD samples, CD and NIBD samples, IL-CD and NIBD samples, and CL-CD and NIBD samples, we used miRHub to test whether the top differentially expressed microRNAs significantly targeted differently expressed genes within four different conditions. miR-31 significantly targets genes that are downregulated in IL-CD.

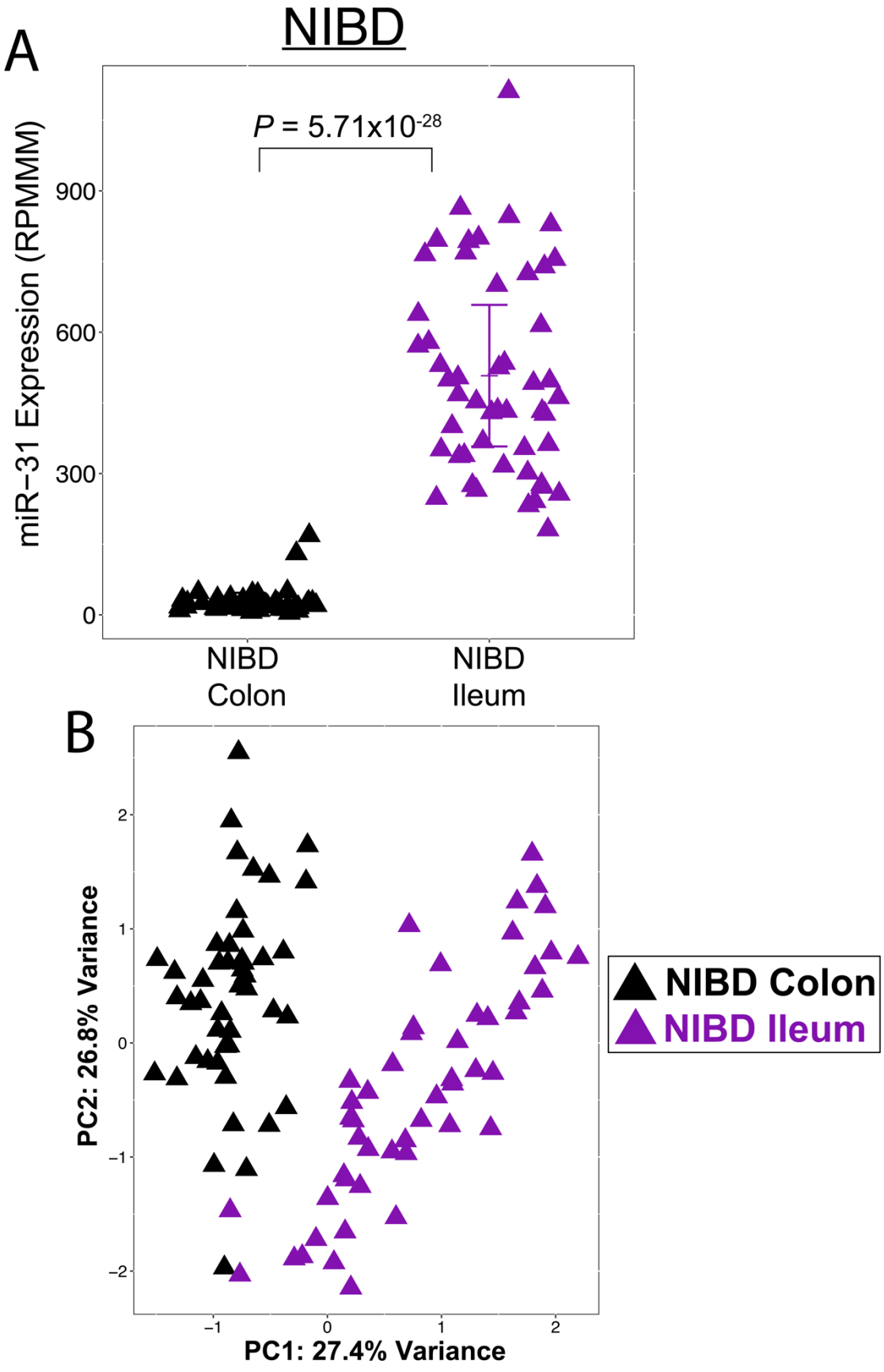


Cell Type	Purity %
T cells (CD3+)	93.5%
B cells (CD19+)	80.1%
Resident intestinal macrophages (CD33+/CD14-)	99.5%
Infiltrating peripheral macrophages (CD14+)	81.2%
Intestinal epithelial cells (CD326+)	88.5%

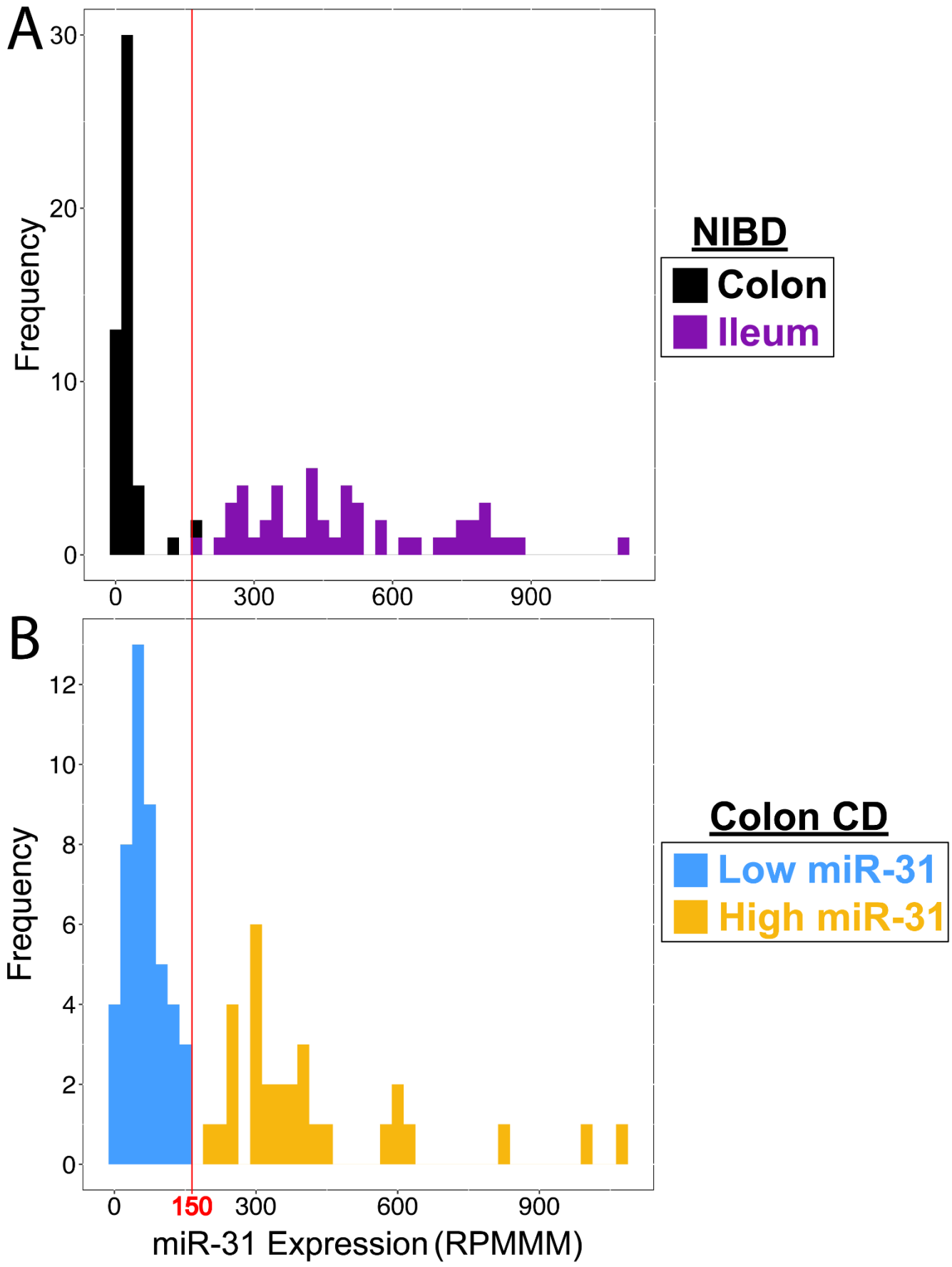
Supplementary Figure 3. Confirmation of successful purification of LPMC subsets by flow cytometry. Isolated LPMC were purified by magnetic separation using reagents from Miltenyi. Purified subsets were stained with fluorescently conjugated antibodies against CD3, CD19, CD14, CD33, and CD326. Purity of T and B cells was determined based on expression of CD3 or CD19. Purity of resident intestinal macrophages and infiltrating peripheral macrophages was determined based on the presence or absence of CD14 expression. Purity of intestinal epithelial cells was determined based on CD326 expression.



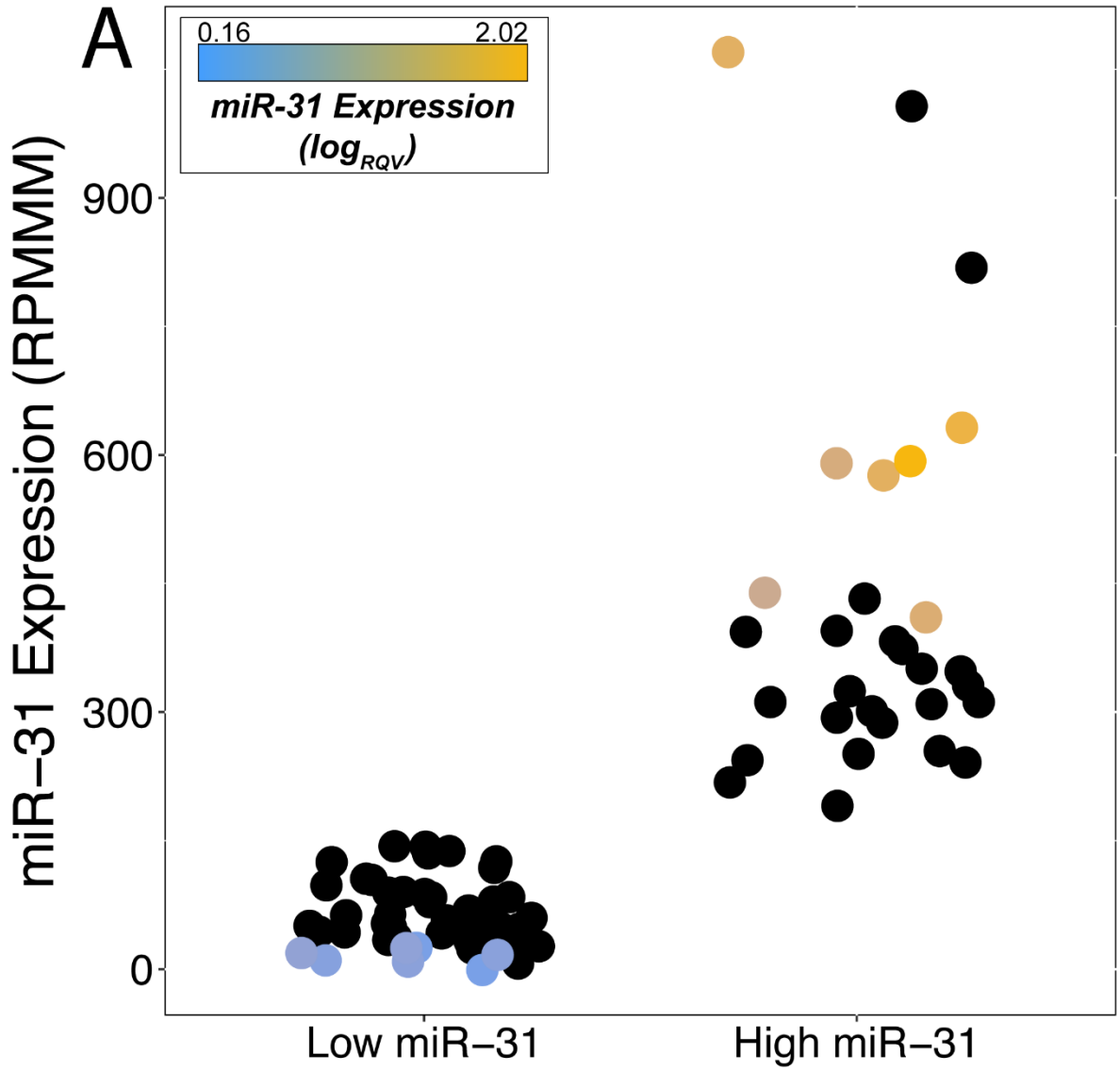
Supplementary Figure 4. miR-31 is uniquely upregulated in the colon of pediatric CD patients relative to NIBD patients. The average expression of the 82 microRNAs used for principal component analysis were compared between CD and NIBD patients for colon samples (CD, n=76; NIBD=48) and ileum samples (CD, n=60; NIBD, n=50). microRNAs with a fold change greater than 2 when comparing CD expression with non-IBD expression are shown above for colonic microRNA expression (A), and ileal microRNA expression (B).



Supplementary Figure 5. Pediatric NIBD microRNA expression profiles are significantly different between colon and ileum tissue. (A) miR-31 is significantly differentially expressed between NIBD colon samples (n=48) and NIBD ileum samples (n=50). (B) More broadly, PCA using microRNA expression profiles reveals that PC1 splits NIBD colon and ileum samples, with miR-31 being the highest contributor to the variance explained along this axis. Data is mean RPMMM ± SEM with significance determined by 2-tailed unpaired Student's test.



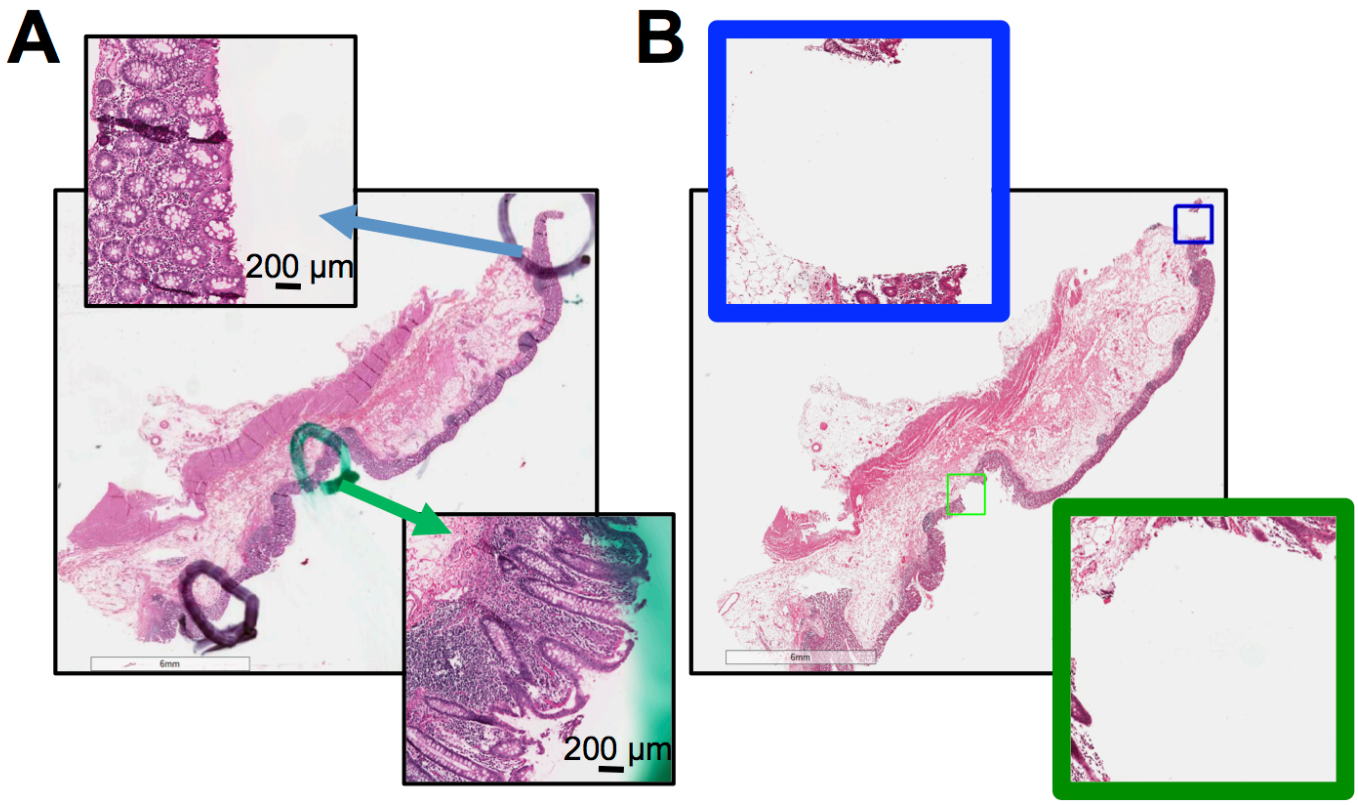
Supplementary Figure 6. Normalized miR-31 expression of 150 reads per million mapped to miRs (RPMMM) can distinguish pediatric NIBD colon (n=48) and ileum samples (n=50). (A) Pediatric miR-31 expression is lower in the colon compared with miR-31 expression the ileum. The lowest ileal miR-31 expression that we observed in the ileum was 181 RPMMM. (B) Using a threshold of 150 RPMMM, we segregate pediatric CD samples into a low miR-31 group (<150 RPMMM; n=46), and a high miR-31 group (≥150 RPMMM; n=30).



B

Sample	Subgroup	miR-31 smRNA-seq	miR-31 qPCR Ct	miR-31 qPCR RQV
PB305	Low	0	37.78	0.43
PB223	Low	10.48	35.54	2.38
PB109	Low	11.92	33.39	1.70
PB102	Low	17.98	32.31	2.20
PB205	Low	19.65	38.95	3.59
PB227	Low	23.6	33.76	3.11
PB140	Low	25.37	32.77	0.56
PB107	High	411.6	28.68	47.32
PB103	High	439.45	28.90	30.29
PB126	High	575.55	29.05	59.96
PB115	High	590.29	29.36	41.27
PB213	High	592.02	27.47	103.03
PB132	High	632.62	27.03	78.32
PB209	High	1070.25	25.88	58.07

Supplementary Figure 7. RT-qPCR confirmation of miR-31 expression in FFPE pediatric colonic mucosal samples. (A) Pediatric miR-31 expression of FFPE colon samples according to small RNA-sequencing is confirmed through RT-qPCR of a subset of miR-31 low (n = 7) and miR-31 high (n = 7) samples from each group ($r = 0.94$, $p = 3.89 \times 10^{-7}$). Points are colored according to RQV obtained through RT-qPCR of the same samples (blue-gold, low-high). (B) Table of expression values of small RNA-sequencing and RT-qPCR matched samples. Significance determined through a test for association between paired samples using Pearson's product moment correlation coefficient.



Supplementary Figure 8. Hematoxylin and eosin (H&E) staining of isolated tissue from FFPE histological sections for an FFPE sample from a pediatric Crohn's disease patient, and mucosal region selected for miRNA isolation and qRT-PCR. A) Section before and B) after selection of the mucosal region. Circles indicate the selected area selected for RNA extraction for small RNA-sequencing and qRT-PCR.