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Supplementary Materials for

Integrated in vivo multiomics analysis identifies p21-activated kinase signaling as a driver of colitis

Jesse Lyons, Douglas K. Brubaker, Phaedra C. Ghazi, Katherine R. Baldwin, Amanda Edwards, Myriam Boukhali, Samantha Dale Strasser, Lucia Suarez-Lopez, Yi-Jang Lin, Vijay Yajnik, Joseph L. Kissil, Wilhelm Haas, Douglas A. Lauffenburger, Kevin M. Haigis*

*Corresponding author. Email: khaigis@bidmc.harvard.edu

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/11/519/eaan3580/DC1)

Table S7 (Microsoft Excel format). Statistics for trans-omics coexpression network analysis.
Table S8 (Microsoft Excel format). Phosphosite lists for each kinase used in GSEA.
Table S9 (Microsoft Excel format). Statistics for GSEA-based kinase enrichment.

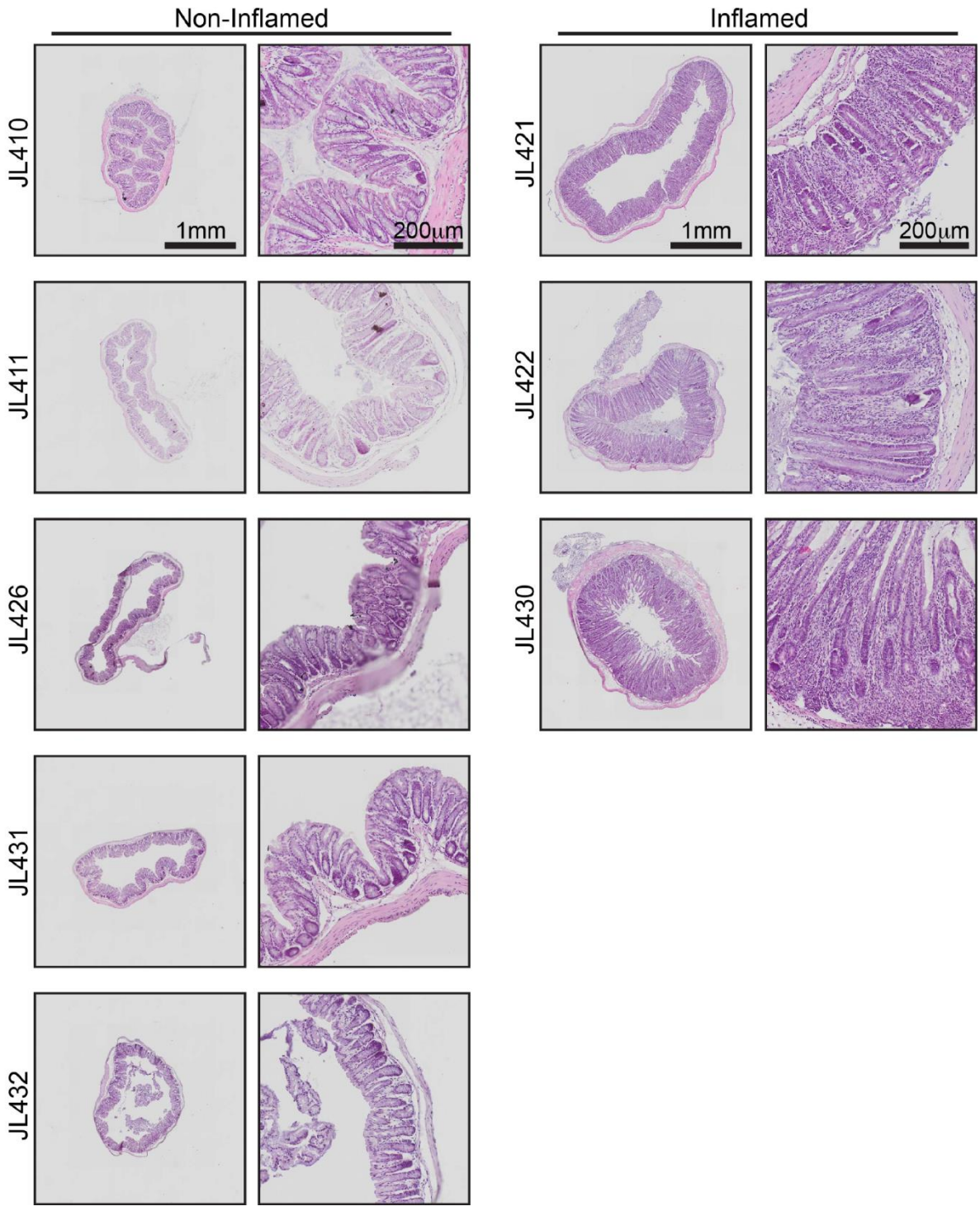

Fig. S1. Histological analysis of colon samples. Colons are from animals analyzed in the study. H&E–stained, formalin-fixed, paraffin-embedded sections from the proximal distal junction show increased crypt height and substantial immune cell infiltration in the animals injected with naïve T cells.

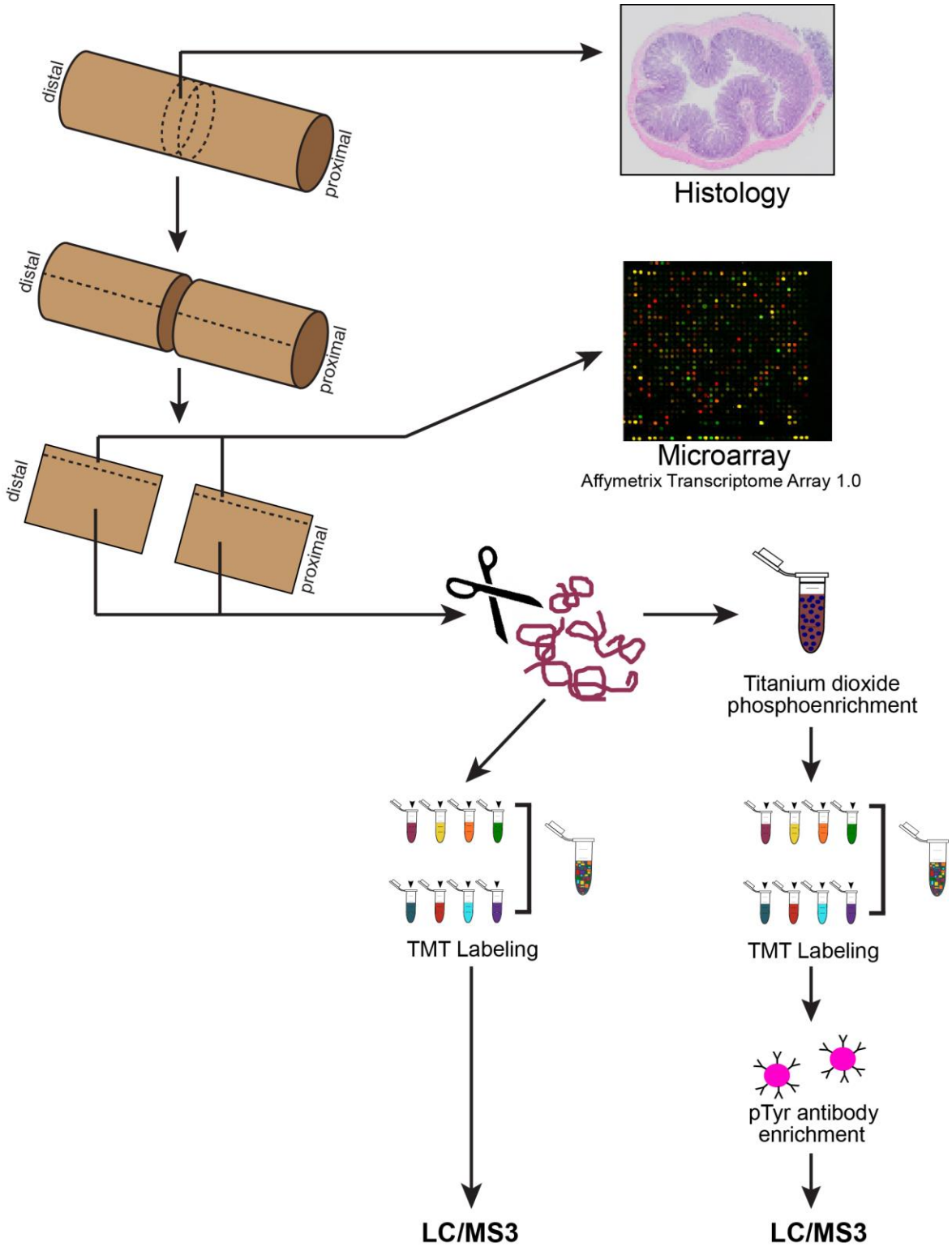

Fig. S2. Schematic of the experimental design. Upon sacrifice, colons were removed, medial colon was taken for histology, and the remaining tissue was divided for microarray and mass spectrometry. Tissue for these measurements was matched so that they are directly comparable.

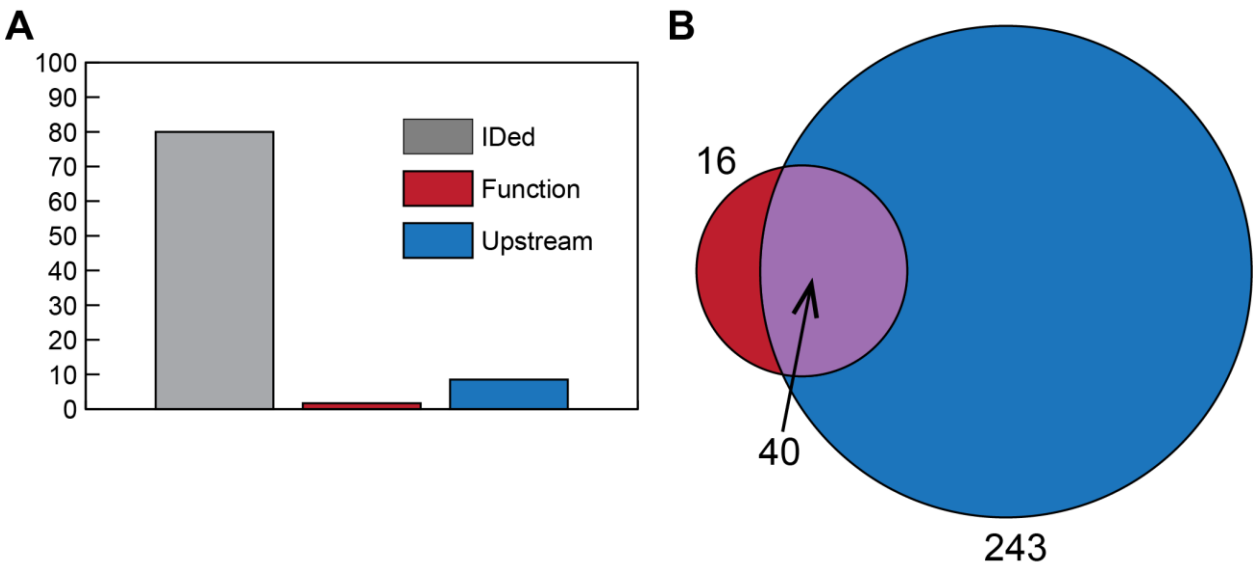

Fig. S3. Functional annotation of phosphorylation sites for mammalian proteins. (**A**) Lists of previously identified phosphorylation sites, functional annotation of phosphorylation sites, and phosphorylation sites with known upstream kinases were derived from Phosphosite. The percentage of unique measured phosphorylation sites that had been previously identified, have a known role in protein function, or have a known upstream kinase were calculated. (**B**) Overlap in functional and upstream kinase activation. Including upstream kinase information increases the number of phosphosites that have interpretable prior knowledge by 4.3-fold.

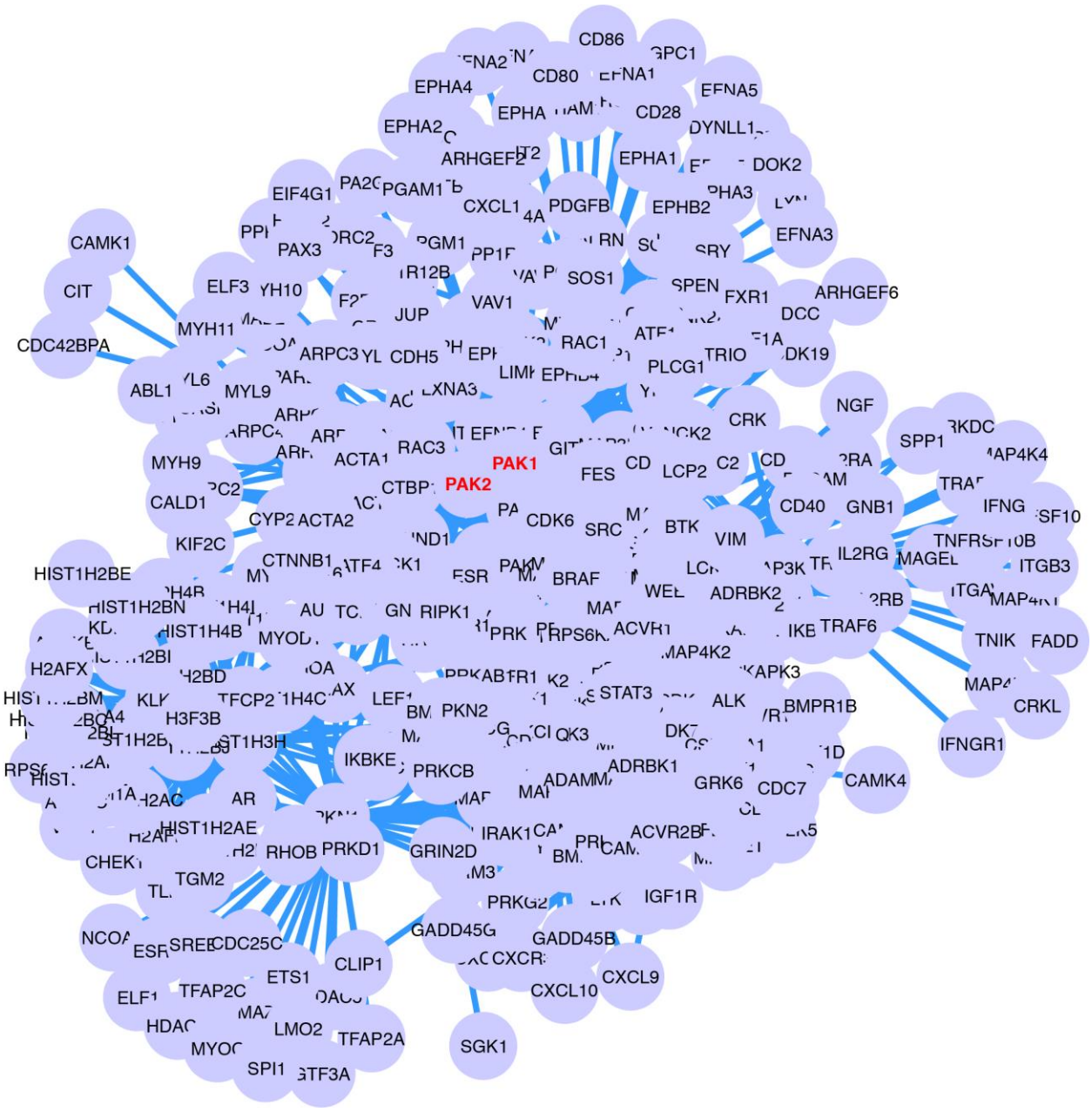

Fig. S4. Human PAK1 and PAK2 signaling network neighborhood. The neighborhood consists of 431 genes and 2534 interactions.

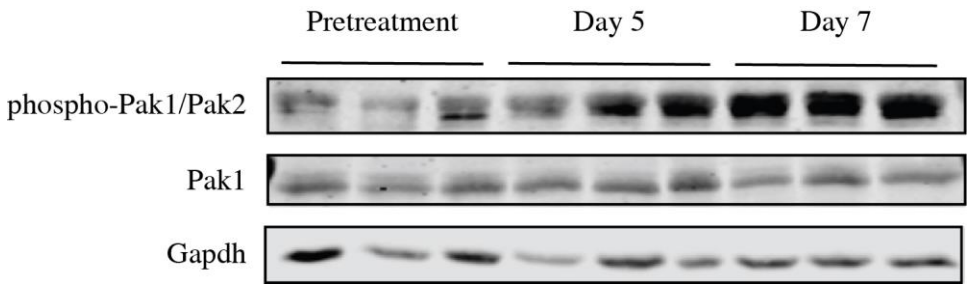

Fig. S5. Pak activation during acute colitis. WT C57BL/6J mice were treated with dextran sodium sulfate (2.5%) in the drinking water for 5 days. At day 5 or 7, the mice were sacrificed and Pak1/2 autophosphorylation was assessed by Western blotting analysis.

Table S1. Affymetrix microarray quantification of gene expression in individual samples. Each column corresponds to an individual animal. Animals without inflammation are highlighted in blue. Animals with colitis are highlighted in pink. Each row represents the expression data for an individual gene as measured by Affymetrix microarray.

Table S2. MS-based quantification of proteins in individual samples. Each column corresponds to an individual animal. Animals without inflammation are highlighted in blue. Animals with colitis are highlighted in pink. Each row represents the abundance data for an individual protein as measured by MS.

Table S3. MS-based quantification of phosphopeptides in individual samples. Each column corresponds to an individual animal. Animals without inflammation are highlighted in blue. Animals with colitis are highlighted in pink. Each row represents the quantification of an individual phosphopeptide as measured by MS.

Table S4. Differential expression analysis for each data set. For each data set, columns represent (i) the differentially expressed species, (ii) the *P* value, (iii) the FDR q value, and (iv) the fold-change in expression. Each row displays the data for an individual gene, protein, or phosphopeptide.

Table S5. Pathway analysis for each data set. Each row represents a pathway that was increased or decreased as identified by GSEA. The RNA, MS, and pMS data sets were analyzed separately. Columns display all the metrics from the GSEA analysis.

Table S6. Modules from trans-omics coexpression network analysis. Each column lists the genes, proteins, or phosphopeptides that comprise an individual module from the coexpression analysis.

Table S7. Statistics for trans-omics coexpression network analysis. Each row displays the statistics for a comparison between two coexpression modules.

Table S8. Phosphosite lists for each kinase used in GSEA. Each column lists the known substrates of the kinase listed at the top of the column. For each protein on the substrate list, the phosphorylated amino acid is listed. Substrate lists were obtained from the Phosphositeplus database.

Table S9. Statistics for GSEA-based kinase enrichment. Each row represents a kinase that was increased or decreased as identified by GSEA. Columns display all the metrics from the GSEA analysis. Kinases with statistically significant enrichment ($P < 0.05$, $q < 0.25$) are highlighted in red.