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Supplemental information

Experimental bacterial dysbiosis with consequent immune alterations increase intrarectal SIV acquisition susceptibility

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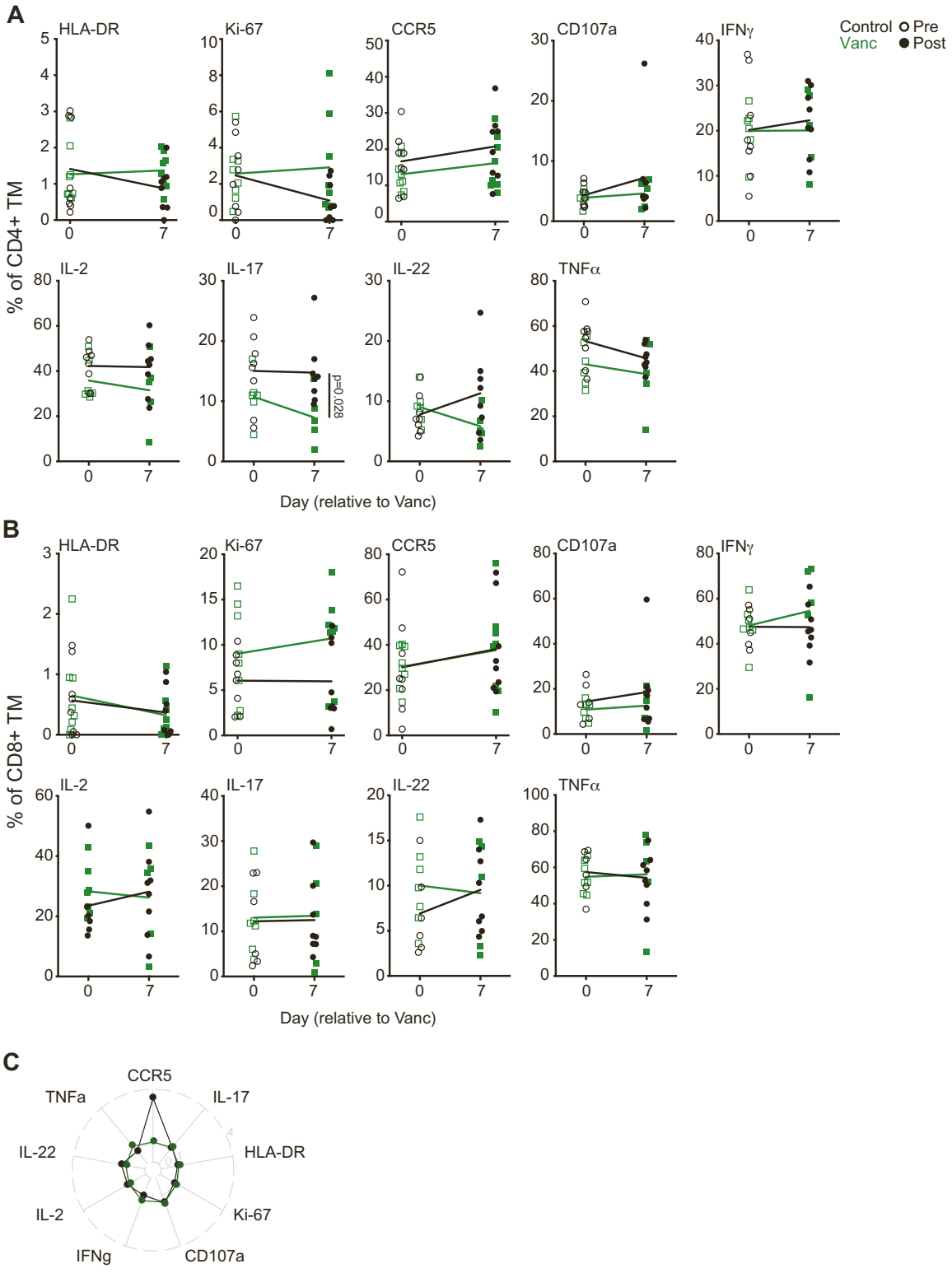

Figure S1. Vancomycin treatment does not promote overt immune activation in RMs.

(A-B) Frequencies of rectal activation markers and stimulation-induced cytokines in CD4+ TM (A) and CD8+ TM (B) across the pre-challenge phase. **(C)** Radar plot depicting fold-change from baseline (post- versus pre-Vanc timepoint) expression of rectal CD8+ TM activation markers and stimulation-induced cytokines. All lines represent mean per group. Data points are derived from 1 biological replicate, n=5-8 per group. Significance assessed by unpaired or paired two-way t-test. Vertical p-value denotes significance post-treatment.

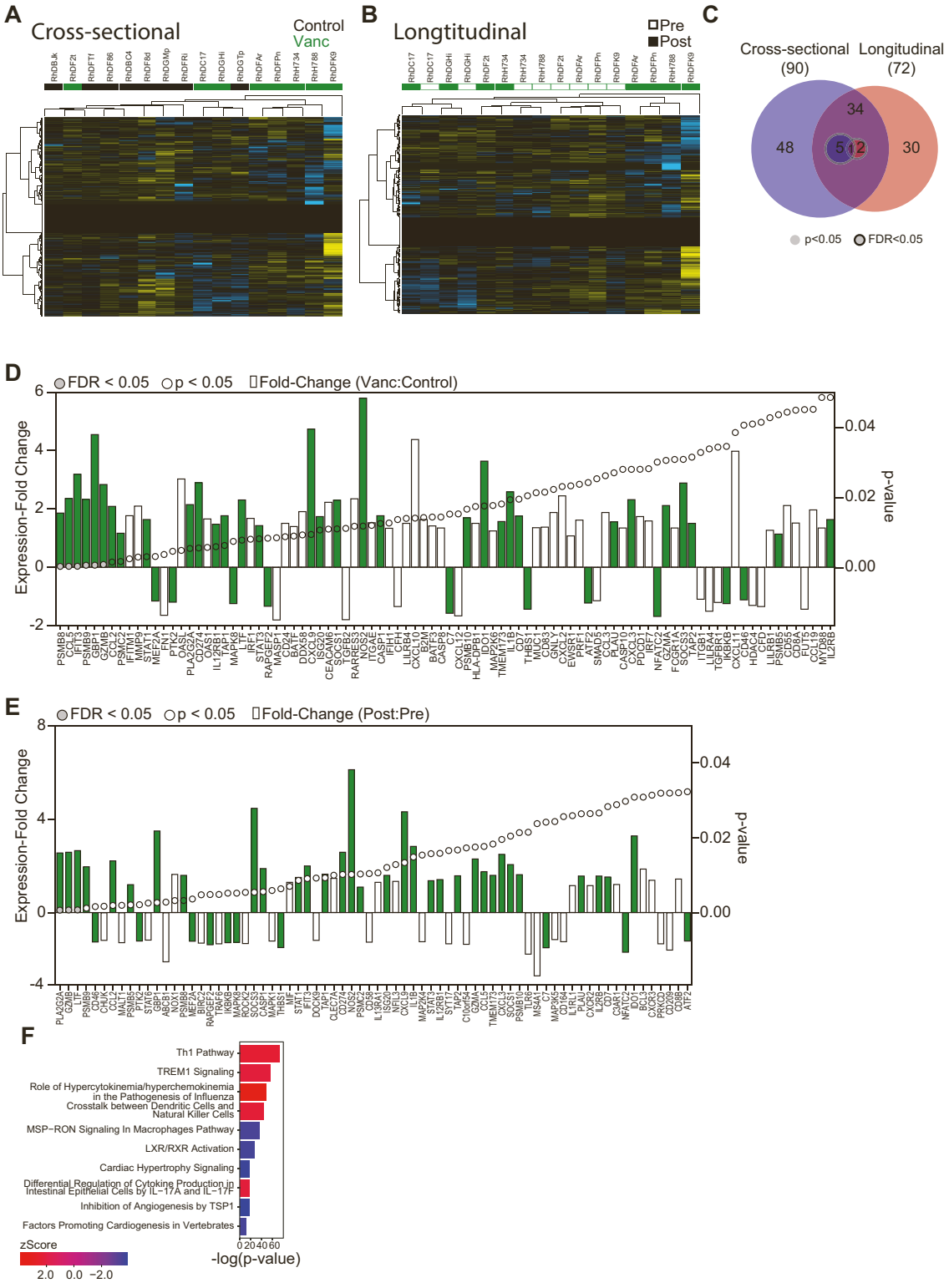

Figure S2. Vancomycin treatment is associated with a differentially regulated transcriptome.

(A-B) Heatmap depicting relative rectal transcript abundance as assessed by NanoString in cross-sectional samples at day 7 post-treatment (A) and in longitudinal samples pre- and post-treatment (B). Transcript values and samples are clustered by Euclidian distance with animal treatment and acquisition identifiers indicated below the heatmap. **(C)** Venn-diagram depicting the number of Nanostring-quantified, differentially-abundant genes in cross-sectional and longitudinal comparisons as in A-B. **(D-E)** Differentially abundant transcripts identified by NanoString in cross-sectional (D) and longitudinal (E) analyses as in A-B. Transcripts are ordered by ascending p-value, with significance values shown in circles, aligned to the right axis. Transcript expression fold-change values are shown by bars, aligned to the left axis. **(F)** p-values and z-scores of the top 5 cross-sectionally enriched and diminished pathways (z-score >|±2.0|) as identified by IPA, from NanoString-quantified transcript counts in rectal homogenates. Data points are derived from 1 biological replicate, n=8 per group. Significance methods as follows: Welch's t-test with Benjamini-Yekutieli FDR (C-E), and Fisher's Exact Test (F).

Supplementary Table 1. Animal Characteristics

Animal	Group	Age at Study Initiation (years)	Testosterone at Study Initiation (pg/mL)	T/F variants	Challenge Number
RhDBC4	Control	14.1	1228.38	1	3
RhDBJk	Control	14.3	2093.40	1	1
RhDF86	Control	7.0	1484.52	2	3
RhDF8d	Control	6.3	910.98	1	5
RhDFRi	Control	6.0	1423.86	1	11
RhDFTf	Control	6.0	1023.03	1	1
RhDGMp	Control	4.3	927.49	1	7
RhDGTp	Control	3.0	832.65	1	2
RhDC17	Vanc	14.3	1636.14	1	2
RhDF2t	Vanc	6.3	3280.69	2	4
RhDFAr	Vanc	7.3	1332.20	1	3
RhDFK9	Vanc	5.0	1807.80	6	2
RhDFPn	Vanc	6.0	1980.75	1	2
RhDGHi	Vanc	4.3	816.43	1	2
RhH734	Vanc	17.1	1688.38	10	6
RhH788	Vanc	14.3	4480.30	5	1