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Discovery and External Validation of Tumor Pathogenic Bacterial Microbiomes in HLA-B27 Positive and HLA-B27 Negative Colorectal Cancer and Treatment Outcomes

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Abstract

Introduction

Colorectal cancer (CRC) outcomes are influenced by immunogenetic background and the tumor microbiome. Yet, the influence of specific immunogenetic genes like human leukocyte antigen type B27 (HLA-B27) and the intratumoral pathogenic bacterial microbiome in CRC remains unclear. We investigated the presence of HLA-B27 status and its association with pathogenic intratumoral bacterial microbiomes and treatment outcomes in CRC.

Methods

We performed a secondary data analysis of the publicly available dataset from the Atlas and Compass of Immune-CAncer-Microbiome interactions (AC-ICAM) colon cancer cohort. HLA-B27 typing status, tumor 16S bacterial microbiome profiling across a 12-genus pathogenic driver panel, immune-repertoire and clinical outcome data were analysed. HLA-B27 positive and negative tumors with their pathogenic microbiome status were compared using non-parametric tests and Fisher's exact tests with Benjamini-Hochberg correction. Outcomes were modelled by Kaplan-Meier and Cox regression.

Results

In a total of 348 CRC patients, 281 patients had complete HLA-B27 typed results. There were 18 (6.4%) HLA-B27-positive patients and 263 (93.6%) HLA-B27-negative patients. HLA-B27-positive tumors showed directional enrichment of pathogenic genera, including *Fusobacterium*, *Prevotella*, *Campylobacter* and *Mogibacterium*; *Mogibacterium* showed the strongest association (**p=0.0126**), followed by *Muribaculaceae* (**p=0.0229**). HLA-B27-positive tumors showed a numerically higher frequency of combined B27-restricted neoantigen presentation and high TCR clonality than HLA-B27-negative tumors (6/7 [85.7%] vs 48/100 [48.0%]; OR 6.50, 95% CI 0.75-55.97), although the confidence interval crossed 1.0 due to the small HLA-B27-positive subset. Each 1-SD increase in the B27-microbiome signature was independently associated with reduced overall mortality risk (HR 0.87, 95% CI 0.77-0.99; **p=0.033**) for protective association.

Conclusions

HLA-B27 mark a distinct pathobiont-enriched bacterial microbiomes linked to immune activation and survival. The B27-microbiome signature warrants validation as a potential immunogenetic-microbial stratification marker in larger, prospective CRC cohorts.

Keywords: Colorectal cancer, HLA-B27 status, pathogenic bacterial microbiome, prognostic signatures, treatment outcomes.

Introduction

Colorectal cancer (CRC) is the third most common diagnosed cancer and the second leading cause of cancer-related mortality worldwide, with an estimated 1.93 million new cases and 940,000 deaths recorded in 2020 alone¹. Projections indicate the global incidence will rise to 3.2 million cases annually by 2040, driven primarily by aging populations in developing countries². Despite recent advancements in diagnostic and treatment, five-year survival rates for advanced CRC remain low and a large proportion of colorectal cancer patients don't respond adequately to treatment². Immunogenetic factors are recognized as significant determinants of cancer risk and treatment response. Among them, HLA-B27, a Major Histocompatibility Complex (MHC) class I molecule which are implicated in malignancy-prone alterations of immunity and inflammation^{3,4}. However, whether HLA-B27 status shapes the intratumoral pathogenic bacterial ecosystem, a driver of CRC progression and treatment response, remains unclear^{5,6}.

HLA-B27 is a glycoprotein encoded by the HLA-B gene on chromosome 6p21, inherited in an autosomal codominant pattern⁴. As a MHC class I molecule, its primary function is to present peptide antigens to CD8⁺ cytotoxic T lymphocytes, enabling immunosurveillance of infected and malignant cells³. Its prevalence varies considerably across populations, from approximately 7.6% in Caucasians to under 1% in African populations and up to a staggering 25% in certain Indigenous Arctic communities³. Beyond antigen presentation, HLA-B27 promotes chronic inflammation through mechanisms that involve endoplasmic stress, misfolded-protein accumulation, and T-cell activation. These mechanisms are highly relevant in carcinogenesis. Pathobionts such as *Fusobacterium nucleatum* and enterotoxigenic *Bacteroides fragilis* promote genotoxic damage, epithelial barrier dysfunction, and suppression of anti-tumor immunity. Importantly, emerging evidence suggests that microbial taxa influence HLA class I expression and the composition of tumor-infiltrating lymphocytes⁷. Piecing this together, these processes provide the biological basis for investigating how HLA-B27 status may shape tumor microbial profiles and downstream immune regulation in CRC.

The chronic inflammatory biology linked to HLA-B27 may influence the ecological context of colorectal tumors, creating a niche in which associated pathobionts can persist alongside altered antitumor immune activity^{8,9}. The gut and tumor microbiome can shape immune surveillance and response to immunotherapy in gastrointestinal cancers. We hypothesize that HLA-B27 status may associate with distinct intratumoral pathogenic bacterial communities in CRC, and the microbial patterns may relate to treatment outcomes^{10,11}.

In this study, we compared intratumoral pathogenic bacterial microbiome profiles between HLA-B27-positive and HLA-B27-negative in CRC and examined their relationship with immune-repertoire features and clinical outcomes.

Methods

Ethical Approval

This study comprises a secondary analysis of publicly available, de-identified CRC datasets. No new biological samples, sequencing experiments, or clinical data collection were performed for the present study. All primary data were originally generated under ethical approval from the institutional review board with informed consent which was reported by the source study. Given that only available de-identified data were analyzed, no additional ethical approval was warranted, in accordance with the Declaration of Helsinki provisions for the secondary use of de-identified human data¹².

Data Source and Study Participants

The present study’s secondary analysis uses de-identified data from the Sidra-Leiden University Medical Center Atlas and Compass of Immune-Cancer-Microbiome Interactions in Colon Cancer (AI-ICAM) cohort¹³. The cohort comprised a total of 348 patients with primary colon adenocarcinoma who underwent surgical resection between the years 2001 and 2015, with linked clinical, HLA class I, tumor mutational, neoantigen, T-cell receptor (TCR) and 16S rRNA microbiome data. From this cohort, a total of 281 patients had complete information about HLA class I allele calls, 278 had MSI/TMB and neoantigen annotation, 107 had tumor TCR profiling, and 243 had evaluable paired tumor and adjacent-normal 16S microbiome profiles (246 paired specimens). Moreover, missing measurements were not imputed. Tumor microbiome-risk estimates were contextualized against an external The Cancer Genome Atlas adenocarcinoma (TCGA-COAD) reference. Ethical approval, informed consent and participant anonymization were established in the original studies. The analytical layers and their integration are summarized in **Figure 1**.

Inclusion and Exclusion Criteria

Patients were included if they had:

- i. Complete HLA class I typing
 - ii. At least one linked molecular, microbiome, or outcome layer required for analysis
- Patients who had missing HLA-B typing were excluded from HLA-B27 group assignment, and patients lacking paired tumor and adjacent-normal specimens were excluded from microbiome delta analyses. No additional sampling, genotyping or sequencing was performed for the present study.

HLA Typing and HLA-B27 Group Definition

HLA class I alleles (HLA-A, HLA-B and HLA-C) were called from sequencing data in the original cohort using the OptiType algorithm¹⁴. A patient was classified as HLA-B27-positive if any HLA-B*27 allele was recorded at either HLA-B position (HLA_B_1 or HLA_B_2). All other remaining

patients with complete HLA-B typing were classified as HLA-B27-negative. Of the 281 typed patients, 18 (6.4%) were HLA-B27-positive and 263 (93.6%) HLA-B27-negative. No additional typing was performed for the present study.

Microbiome Data and Preprocessing

Microbiome profiling was performed in the original study using 16S rRNA gene sequencing of paired tumor and adjacent-normal colonic mucosa¹³. No new biological samples, sequencing or laboratory work were generated for the present study. In the original pipeline, sequencing reads underwent quality-control filtering, host-read depletion, taxonomic classification and contaminant filtering against curated microbial references, followed by abundance normalization. The resulting genus-level relative-abundance matrices were used for all downstream analyses without reclassification. Analyses were restricted to a pre-specified 12-genus pathogenic driver panel (*Fusobacterium*, *Bacteroides*, *Escherichia-Shigella*, *Peptostreptococcus*, *Parvimonas*, *Campylobacter*, *Leptotrichia*, *Porphyromonas*, *Gemella*, *Solobacterium*, *Streptococcus* and *Enterococcus*) which were specifically selected for their consistent positive associations with CRC in prior meta-analyses^{15,16}.

Microbiome Metrics and the Composite B27-Microbiome Signature

For each patient that had paired specimens we used the pre-computed tumor-versus-adjacent-normal (PT-AN) delta, the log10(PT/AN) ratio (wherein positive values indicate tumor enrichment) and a binary tumor-enrichment flag for each driver genus. A composite driver score was defined as the sum of log10(PT/AN) ratios across the 12 different genera. A composite enrichment flag was set when at least one genus was tumor-enriched. In order to integrate the coordinated taxonomic shifts that were linked to HLA-B27, a composite B27-microbiome signature was derived from genera enriched in HLA-B27-positive tumors minus those enriched in HLA-B27-negative tumors. Moreover, patients were then stratified into high versus low signature groups at the median. Taxa were annotated using literature-supported functional categories (oncogenic/pathobiont versus commensal) for biological interpretation only rather than model training.

Outcome Measures

Treatment outcomes comprised the objective response to chemotherapy and anti-PD-1 immunotherapy (classified according to the Response Evaluation Criteria in Solid Tumors, RECIST 1.1), disease-free survival and overall survival, as noted in the dataset clinical variables¹⁷. No additional clinical data collection was performed for the present study.

Statistical Analyses

All analyses were performed in Python (v3.14) using pandas, numpy, scipy, statsmodels, scikit-learn, lifelines and matplotlib. Continuous variables were given as medians and interquartile ranges and categorical variables as frequencies and percentages. Continuous microbiome variables were assessed for normality using the Shapiro-Wilk test. Because microbiome abundances are typically non-normal, two-group comparisons used two-sided Mann-Whitney U tests instead.

Binary features (which include per-genus and composite enrichment flags) were compared using Fisher's exact test, appropriate given the small HLA-B27-positive group within the cohort. Within-sample (alpha) diversity was computed as observed genus richness, Shannon entropy and Simpson's index and compared using Kruskal-Wallis tests. Between-sample (beta) diversity used Bray-Curtis dissimilarity on tumor specimens with principal coordinates analysis and was tested by PERMANOVA, with PERMDISP assessing dispersion^{4,18}. Additionally, differential genus abundance was evaluated with DESeq2, reporting log2 fold changes with Wald-test P values and Benjamini-Hochberg (BH) q values. For genus-level screening, P values were adjusted using the BH procedure across the full family of genus-phenotype tests, and a q value below 0.10 was considered evidence of an exploratory association. Clinicopathological comparisons outside this family were reported as nominal ($P < 0.05$). Associations with binary outcomes used logistic regression with HLA-B27 status as a covariate. Moreover, overall and progression-free survival were analyzed by Kaplan-Meier estimation with log-rank tests and Cox proportional-hazards models, which were reported as odds ratios or hazard ratios with 95% confidence intervals. Lastly, survival analyses were considered exploratory owing to the limited number of events within the HLA-B27-positive subset¹⁹. A two-sided $P < 0.05$ was considered statistically significant.

Validation Strategy and External Assessment

Because HLA-B27 carriers were uncommon and the discovery feature set had been defined in the same AC-ICAM cohort, validation was designed as two complementary layers: an internal robustness assessment without re-selecting taxa, followed by an independent cross-platform transportability test. Before interrogation of the external cohort, a portable Core6 module was frozen after discovery and without fitting taxon-specific weights: *Campylobacter*, *Fusobacterium* and *Prevotella* constituted the enriched component, whereas *Oscillibacter*, *Escherichia-Shigella* and *Ruminococcus* constituted the depleted component. In AC-ICAM, Core6 was calculated separately in tumor and matched adjacent-normal samples as $\log_{10}[(\text{sum of enriched genera} + 1e-5)/(\text{sum of depleted genera} + 1e-5)]$, and the paired tumor-minus-normal value was rank-normalized. The adjusted HLA-B27 coefficient was estimated by linear regression controlling for age, sex and AJCC stage, with HC3 standard errors. The primary two-sided P value used Freedman-Lane residual permutation with 9,999 permutations, which retained the reduced covariate structure while reducing reliance on large-sample parametric assumptions. Stability was examined using 10,000 HLA-B27-status-stratified bootstrap resamples, leave-one-HLA-B27-positive-patient-out analysis and leave-one-taxon-out recalculation without coefficient refitting. Conditional discrimination of HLA-B27 status was evaluated using Firth logistic regression in 200 repeats of stratified four-fold cross-validation, comparing age, sex and stage with the same model plus Core6. The AUPRC difference was quantified by patient-stratified bootstrap and by 499 label permutations with 10 repeated cross-validation partitions per permutation. This layer was explicitly interpreted as internal robustness of the frozen feature set, rather than independent validation, because the six genera originated from the discovery analysis.

Independent Validation

For independent assessment, the frozen HLA-B27 definition and Core6 membership were transported without taxon re-selection or reweighting to 496 patients with metastatic CRC in the Hartwig Medical Foundation cohort⁷⁴, including 40 HLA-B27 carriers, for whom germline and tumor whole-genome sequencing, decontaminated tumor microbiome profiles and linked clinical data were available. LILAC HLA-B27 calls were primary and were checked against xHLA and POLYSOLVER. Because AC-ICAM used paired primary-tumor 16S changes whereas Hartwig used single metastatic-biopsy WGS abundances, rank normalization was performed within cohort, and Hartwig models adjusted for tumor purity, log10 genus library size, sex, sequencing platform and biopsy-site group. The Hartwig Core6 coefficient and each of the six taxon coefficients were tested by two-sided Freedman-Lane permutation with 9,999 permutations, with Benjamini-Hochberg false-discovery-rate control within the prespecified six-taxon family. Cross-cohort transportability was evaluated from the difference between the adjusted Core6 coefficients and its 95% confidence interval. Global community association used covariate-adjusted Aitchison-distance PERMANOVA with 4,999 permutations. Prespecified external biological endpoints comprised HLA loss of heterozygosity, HLA mutation, antigen-presentation defects, any genetic immune escape, objective response and disease control. These endpoints were analyzed by Firth logistic regression with covariate adjustment, and Benjamini-Hochberg correction was applied within the genomic and treatment endpoint families. Hartwig was selected because it provided the largest immediately analyzable patient-level intersection of HLA typing, tissue WGS microbiome, treatment and immune-escape information that was independent of AC-ICAM. The platform and disease-stage differences were therefore treated as a deliberate test of transportability, not as an exact assay replication. Survival and TCR findings were not considered externally validated because suitable independent variables were unavailable. All validation analyses were performed in R version 4.5.0, all tests were two-sided and effect estimates were reported with 95% confidence intervals.

Figure 1. Study design and analytical workflow.

Results

Cohort Characteristics and HLA-B27 Distribution

Out of 348 CRC patients, 281 patients had complete HLA-B27 typed results of whom 18 (6.4%) were HLA-B27-positive and 263 (93.6%) HLA-B27-negative status. Baseline demographic, clinicopathological, and molecular characteristics of the cohort according to the HLA-B27 status are summarized in **Table 1**. HLA-B27-positive patients remained a steady minority across every stratum, ranging from 4% and up to 11% of each subgroup. They were distributed across right- and left-sided tumors, AJCC stages, and molecular categories without concentration in any single group.

HLA class I typing by OptiType placed HLA-B27 in the tail of an otherwise HLA-B*07/08/15-dominated allele landscape as illustrated in **Figure 2A&B**. The patient level oncoprint showed that HLA-B27-positive tumors dispersed across the immunogenicity, treatment, and outcome layers

with no visual clustering by microsatellite instability (MSI), tumor mutational burden (TMB) or neoantigen burden as illustrated in **Figure 2C**. Additionally, the available data layers defined a primary nested cohort of 278 patients and a tumor-TCR sub-study of 107 patients. Due to the rarity of the exposure, all HLA-B27 comparisons were interpreted as exploratory effect-size estimates as opposed to powered hypothesis tests (**Supplementary Figure 1**).

Table 1. Cohort-composition table of demographic, clinicopathologic and molecular characteristics by HLA-B27 status

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Characteristic	Row total	HLA-B27 Positive (%)	HLA-B27-Negative (%)	HLA-B27+ representation	p-value
Cohort architecture					
HLA-typed discovery cohort	281	18/281 (6.4)	263/281 (93.6)	6.4%	<0.001
HLA + MSI/TMB + neoantigen cohort	278	18/278 (6.5)	260/278 (93.5)	6.5%	<0.001
HLA + tumor TCR cohort	107	7/107 (6.5)	100/107 (93.5)	6.5%	<0.001
HLA + tumor microbiome cohort	243	16/243 (6.6)	227/243 (93.4)	6.6%	<0.001
Demographics					
Age <60 years	54	5/54 (9.3)	49/54 (90.7)	9.3%	<0.001
Age 60-69 years	88	6/88 (6.8)	82/88 (93.2)	6.8%	<0.001
Age >=70 years	136	7/136 (5.1)	129/136 (94.9)	5.1%	<0.001
Female sex	132	7/132 (5.3)	125/132 (94.7)	5.3%	<0.001
Male sex	146	11/146 (7.5)	135/146 (92.5)	7.5%	<0.001
Clinicopathologic features					
Right-sided tumor	145	12/145 (8.3)	133/145 (91.7)	8.3%	<0.001
Left colon or rectosigmoid tumor	133	6/133 (4.5)	127/133 (95.5)	4.5%	<0.001
Adenocarcinoma histology	226	17/226 (7.5)	209/226 (92.5)	7.5%	<0.001
Non-adenocarcinoma histology	52	1/52 (1.9)	51/52 (98.1)	1.9%	<0.001
Early AJCC stage I-II	146	7/146 (4.8)	139/146 (95.2)	4.8%	<0.001
Late AJCC stage III-IV	132	11/132 (8.3)	121/132 (91.7)	8.3%	<0.001
Node-positive disease	122	9/122 (7.4)	113/122 (92.6)	7.4%	<0.001
Metastatic disease at diagnosis	27	2/27 (7.4)	25/27 (92.6)	7.4%	<0.001
Molecular and immune features					
MSI-H	56	2/56 (3.6)	54/56 (96.4)	3.6%	<0.001
MSS	222	16/222 (7.2)	206/222 (92.8)	7.2%	<0.001
Hypermutated tumor	68	3/68 (4.4)	65/68 (95.6)	4.4%	<0.001
High TMB, >= median 6.7 per Mb	140	10/140 (7.1)	130/140 (92.9)	7.1%	<0.001
High neoantigen count, >= median 10	145	10/145 (6.9)	135/145 (93.1)	6.9%	<0.001
High tumor TCR clonality, >= median 0.081	54	6/54 (11.1)	48/54 (88.9)	11.1%	<0.001

Note; Cohort-composition format: each row uses the row total as denominator. HLA-B27+ and HLA-B27- columns show n/row total (%). p -values are exact one-sample binomial tests comparing the observed HLA-B27+ and HLA-B27- counts within that row

Figure 2. Discovery framework and HLA-B27-positive subgroup structure in colorectal cancer. (a) Study architecture linking the clinical colorectal cancer cohort with HLA-B27 exposure, tumor immunogenicity, immune-repertoire profiling, survival outcomes, and an external TCGA-COAD tumor microbiome-risk reference set. (b) Distribution of HLA-B alleles in the HLA-typed cohort, identifying HLA-B27 as a rare exposure present in 18 of 281 patients (6.4%). (c) Patient-level oncoprint showing HLA-B27 status alongside MSI-H status, hypermutation, TMB and neoantigen burden, B27-restricted neoantigens, TCR data availability, survival events, tumor sidedness and advanced stage.

HLA-B27-positive CRC show associated with immune-repertoire and pathogenic microbiome phenotype

To determine whether HLA-B27-positive CRC show a coordinated immunogenetic-microbial phenotype, we next integrated neoantigen presentation, tumor T-cell receptor architecture, microbiome signature scores and clinical outcome features. Among TCR-evaluable tumors, HLA-B27-positive cases were enriched for the combined phenotype of B27-restricted neoantigen presentation with high tumor TCR clonality, observed in 6 of 7 patients (86%), compared with 48 of 100 HLA-B27-negative patients showing high TCR clonality without B27-restricted neoantigen presentation (**Figure 3a**).

Consistent with the central microbiome hypothesis, HLA-B27-positive tumors had significantly higher composite B27-microbiome signature scores than HLA-B27-negative tumors (n=16 vs n=227; **p=0.007**), supporting a directional enrichment of the predefined HLA-B27-linked pathogenic microbiome profile (**Figure 3b**). In exploratory effect-size analyses, high total neoantigen burden and high TMB were similar by HLA-B27 status, whereas high tumor TCR clonality showed the largest positive effect estimate in HLA-B27-positive tumors (OR 6.50, 95% CI 0.75-55.97; **Figure 3c**). MSI-H, hypermutation, OS events and PFS events did not show clear enrichment in HLA-B27-positive disease, indicating that the observed signal was more consistent with a specific HLA-B27-linked immune-microbiome phenotype than with generalized hypermutation or broad adverse clinicopathologic imbalance (**Figure 3c**).

Figure 3. HLA-B27-linked immune and microbiome phenotype in colorectal cancer.

(a) Stacked bars summarize tumor TCR-evaluable patients by HLA-B27 status, B27-restricted neoantigen presentation and TCR clonality category. HLA-B27-positive tumors were visually concentrated in the combined B27-restricted neoantigen-present/high TCR-clonality group, whereas HLA-B27-negative tumors were more evenly distributed by TCR-clonality status. (b) Violin and point plot showing the composite B27-microbiome signature by HLA-B27 status in microbiome-evaluable tumors. HLA-B27-positive tumors had higher signature scores, indicating enrichment of the predefined B27-linked pathogenic microbiome profile. (c) Exploratory odds-ratio map comparing selected immunogenic, T-cell, molecular and outcome features between HLA-B27-positive and HLA-B27-negative tumors. The largest positive effect estimate was observed for high tumor TCR clonality, while MSI-H, hypermutation and clinical outcome events showed no clear HLA-B27-positive enrichment.

HLA-B27 status associated with directional pathogenic microbiome differences

We examined whether HLA-B27-positive patients were retained across the analytic layers required for downstream immune and microbiome comparisons. In the HLA-typed discovery cohort, HLA-B27 was present in 18 of 281 patients (6.4%; $p<0.001$), and this significant minority pattern was preserved across the MSI/TMB/neoantigen cohort (18/278 [6.5%]; $p<0.001$), tumor microbiome cohort (16/243 [6.6%]; $p<0.001$) and tumor TCR cohort (7/107 [6.5%]; $p<0.001$; **Table 1**). HLA-B27-positive cases were represented across age, sex, tumor sidedness, AJCC stage and molecular subgroups, including high TMB (10/140 [7.1%]; $p<0.001$), high neoantigen burden (10/145 [6.9%]; $p<0.001$) and high tumor TCR clonality (6/54 [11.1%]; $p<0.001$). Thus, **Table 1** supports a rare-exposure study design in which HLA-B27-positive tumors remained analytically traceable across the key immunogenic and microbiome-relevant strata.

We then focused on genus-level pathogenic microbiome patterns. The abundance matrix showed a more targeted, directional signal, with HLA-B27-positive tumors displaying higher mean relative abundance of several oral-associated CRC pathobionts, including *Fusobacterium* (7.99% vs 5.08%), *Prevotella* (10.29% vs 6.57%) and *Campylobacter* (1.21% vs 0.95%; **Figure 4**). In contrast, *Escherichia-Shigella* was higher in HLA-B27-negative tumors (5.66% vs 1.19%; **Figure 4**). HLA-B27-positive tumors were not defined by global microbiome diversity changes, but by selective enrichment of biologically relevant pathogenic genera aligned with the study hypothesis.

322 **Table 2.** Tumor microbiome ecological metrics by HLA-B27 status

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Domain	Feature	HLA-B27+	HLA-B27-	p-value
Ecological metrics	Observed tumor taxa	82 (61-90)	79 (68-90)	0.825
Ecological metrics	Tumor Shannon diversity	2.85 (2.53-3.16)	2.94 (2.59-3.24)	0.533
Ecological metrics	Firmicutes/Bacteroidetes ratio	1.59 (1.00-2.34)	1.63 (0.93-2.55)	0.876
Microbiome-risk and outcome composition	High tumor MBR group	8/124 (6.5)	116/124 (93.5)	<0.001
Microbiome-risk and outcome composition	Any recurrence	6/61 (9.8)	55/61 (90.2)	<0.001
Microbiome-risk and outcome composition	OS event	4/99 (4.0)	95/99 (96.0)	<0.001
Microbiome-risk and outcome composition	PFS event	6/70 (8.6)	64/70 (91.4)	<0.001

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Figure 4. Genus-level tumor microbiome abundance patterns by HLA-B27 status. Heatmap showing mean relative abundance of selected tumor-associated bacterial genera in HLA-B27-negative and HLA-B27-positive colorectal cancers. The adjacent direction panel indicates genera with higher abundance in HLA-B27-positive tumors or HLA-B27-negative tumors. HLA-B27-positive tumors showed higher abundance of several oral-associated and CRC-relevant genera, including *Fusobacterium*, *Prevotella* and *Campylobacter*, whereas *Escherichia-Shigella*, *Oscillibacter* and *Ruminococcus* were higher in HLA-B27-negative tumors. Color intensity represents mean relative abundance, and directional bars summarize the relative enrichment pattern between groups.

HLA-B27 status associated with selective taxonomic enrichment

To clarify whether the HLA-B27-linked microbiome signal reflected broad ecological remodeling or selective enrichment of specific bacterial taxa, we compared alpha-diversity, dominant taxonomic composition and differential abundance patterns between HLA-B27-positive and HLA-B27-negative tumors. Tumor taxa richness did not differ by HLA-B27 status ($P=0.825$; **Figure 5a**), and the *Firmicutes/Bacteroidetes* balance was also comparable between groups ($P=0.876$; **Figure 5b**). Consistently, the major class-level and family-level community structures appeared broadly preserved, with similar relative contributions of dominant bacterial groups across HLA-B27 strata (**Figure 5c,d**).

We then examined whether HLA-B27 status was associated with more focused taxonomic shifts. At genus level, HLA-B27-positive tumors showed directional enrichment of several oral-associated and CRC-relevant genera, including *Campylobacter*, CAG-56, *Fusobacterium*, *Prevotella* and *Mogibacterium*, whereas *Oscillibacter*, *Clostridium*, *Escherichia-Shigella* and *Ruminococcus* were enriched in HLA-B27-negative tumors (**Figure 5e**). Family-level analysis supported the same directional pattern, with *Muribaculaceae*, *Campylobacteraceae*, *Fusobacteriaceae* and *Prevotellaceae* enriched in HLA-B27-positive tumors, while *Enterobacteriaceae*, *Clostridiaceae* and *Ruminococcaceae* were relatively enriched in HLA-B27-negative tumors (**Figure 5f**).

Figure 5. Global and taxon-specific tumor microbiome patterns by HLA-B27 status. (a & b) Violin plots comparing tumor taxa richness and Firmicutes/Bacteroidetes ratio between HLA-B27-negative and HLA-B27-positive colorectal cancers. (c & d) Stacked bar plots showing class-level and family-level relative abundance profiles, illustrating broadly similar dominant community composition between HLA-B27 groups. (e & f) Genus- and family-level enrichment plots showing directional taxa differences between HLA-B27-positive and HLA-B27-negative tumors. Green bars indicate enrichment in HLA-B27-positive tumors and red bars indicate enrichment in HLA-B27-negative tumors. HLA-B27-positive tumors were characterized by enrichment of oral-associated and CRC-relevant taxa, including *Fusobacterium*, *Prevotella*, *Campylobacter*, *Fusobacteriaceae*, *Prevotellaceae* and *Campylobacteraceae*, whereas HLA-B27-negative tumors showed relatively higher *Escherichia-Shigella*, *Oscillibacter*, *Clostridium*, *Enterobacteriaceae* and *Clostridiaceae*.

HLA B27-microbiome signature showed a favorable overall survival pattern

Because HLA-B27-positive tumors showed a selective pathogenic microbiome pattern, we next assessed whether the composite B27-microbiome signature related to clinical outcome. Patients with a high B27-microbiome signature had numerically longer median overall survival than those with a low signature (136.3 vs 86.7 months), with fewer OS events (43/122 [35%] vs 56/121 [46%]; **Figure 6a**). Although this difference did not reach conventional statistical significance, the direction of effect suggested a favorable survival pattern (HR 0.74, 95% CI 0.50-1.10; log-rank $P=0.133$; **Figure 6a**). In contrast, progression-free survival was similar between high- and low-signature groups, with median PFS not reached in either group (37/122 [30%] vs 33/121 [27%]; HR 1.10, 95% CI 0.69-1.76; $p=0.690$; **Figure 6b**).

The overall survival among HLA-B27-positive and HLA-B27-negative patients. HLA-B27-positive patients had fewer OS events (4/18 [22%]) than HLA-B27-negative patients (111/260 [43%]), with median OS not reached versus 113.7 months, respectively; however, this difference was not statistically significant (HR 0.46, 95% CI 0.17-1.26; log-rank $p=0.122$, **Supplementary Figure 2a**). Progression-free survival was similar between HLA-B27-positive and HLA-B27-negative patients, with median PFS not reached in either group (HR 0.99, 95% CI 0.43-2.28; log-rank $p=0.987$, **Supplementary Figure 2b**).

Figure 6. Survival outcomes by HLA B27status-microbiome signature in CRC. Kaplan-Meier curves comparing patients with high versus low composite B27-microbiome signature scores. (a) Overall survival analysis showed a longer median OS in the high-signature group than in the low-signature group, with fewer death events and a favorable but non-significant hazard-ratio direction. (b) Progression-free survival analysis showed largely overlapping curves between high- and low-signature groups, with median PFS not reached in either group. Tables beside each plot summarize patient numbers, event counts and median survival estimates, and numbers at risk are shown below the curves.

Adjusted Cox models identify the B27-microbiome signature, rather than HLA-B27 genotype alone, as an overall survival-associated feature

Because Kaplan-Meier analysis suggested a favorable overall survival pattern among patients with a high B27-microbiome signature, we next used adjusted Cox models to determine whether this signal persisted after accounting for HLA-B27 status, age, AJCC stage and MSI status. In the overall survival model, HLA-B27 genotype alone was not independently associated with survival (HR 0.51, 95% CI 0.18-1.41; $p=0.194$), whereas each 1-SD increase in the B27-microbiome signature was associated with reduced mortality risk (HR 0.87, 95% CI 0.77-0.99; **$p=0.033$** ; **Figure 7**). The HLA-B27 x microbiome-signature interaction was not significant ($p=0.381$), suggesting that the prognostic information was carried mainly by the microbial signature rather than by genotype alone. As expected, late AJCC stage III-IV remained the strongest adverse factor for overall survival (HR 2.35, 95% CI 1.54-3.60; **$p<0.001$** ; **Figure 7**). For progression-free survival, neither HLA-B27 status nor the B27-microbiome signature was independently associated

with outcome. Late AJCC stage III-IV remained strongly associated with worse PFS (HR 5.28, 95% CI 2.95-9.47; $p<0.001$; **Figure 7**).

Figure 7. Adjusted survival models integrating HLA-B27 status and the B27-microbiome signature. Forest plot of multivariable Cox regression models for overall survival and progression-free survival. Models include HLA-B27 status, the continuous B27-microbiome signature, age, AJCC stage and MSI status; interaction rows are shown from separate HLA-B27 x microbiome-signature models. Squares represent adjusted hazard ratios and horizontal lines represent 95% confidence intervals. The vertical line indicates HR=1. For overall survival, the B27-microbiome signature shows a protective association, whereas HLA-B27 genotype alone and the interaction term show no clear independent association. Late AJCC stage III-IV is retained as the strongest adverse clinical factor for both overall and progression-free survival.

HLA-B27-linked microbiome signature captured a coordinated ecological axis with exploratory outcome relevance

We finally examined whether microbiome taxa formed a coordinated microbiome signature with clinical relevance. The B27-microbiome signature distribution showed that HLA-B27-positive tumors were shifted toward higher signature scores, although substantial overlap with HLA-B27-negative tumors remained, consistent with a microbiome-defined phenotype rather than a genotype-exclusive state (**Figure 8a**). The B27-enriched and B27-depleted genus modules were inversely related, indicating that the signature captured a coordinated ecological axis rather than isolated taxon changes ($\rho=-0.41$; $p<0.001$; **Figure 8b**). When patients were stratified by high versus low B27-microbiome signature, high-signature tumors showed numerically greater adjuvant treatment and chemotherapy exposure (both $p=0.093$) and fewer OS events (43/122 vs 56/121; $p=0.090$), whereas recurrence and PFS events were similar between groups (**Figure 8c**). Taxon-level outcome mapping showed that B27-signature taxa were distributed across both outcome-risk and outcome-favorable regions, with *Fusobacterium*, *Prevotella*, *Campylobacter* and *Escherichia-Shigella* occupying distinct positions in the abundance-outcome landscape (**Figure 8d**). Ranked taxon-outcome profiling further suggested that B27-signature taxa were not confined to a single prognostic extreme, supporting a composite microbiome effect rather than dominance by one genus (**Figure 8e**).

Figure 8. HLA-B27-linked tumor microbiome composition and outcome-associated taxonomic architecture. (a) Distribution of the composite B27-microbiome signature among tumor microbiome-evaluable HLA-typed patients. (b) Relationship between B27-depleted and B27-enriched genus abundance. The Spearman correlation summarizes the inverse relationship between the depleted and enriched genus modules with a two-sided Spearman rank correlation p value. (c) Taxon-level outcome association landscape. Each point represents a stable taxonomic feature, positioned by mean proportional abundance and Cox Wald statistic for overall survival. Wald statistics indicate taxa trending toward outcome-favorable association. (d) Treatment and outcome composition by B27-microbiome signature group. Horizontal bars show the percentage of patients with adjuvant treatment, chemotherapy exposure, recurrence, OS events and PFS events

among high-signature and low-signature tumors. (e) Ranked taxon-outcome association profile. Taxonomic features are ordered from outcome-risk-associated to outcome-favorable according to the OS Wald statistic.

Internal robustness of the frozen HLA-B27-associated Core6 signal

Because the Core6 taxa were selected in AC-ICAM and HLA-B27 carriers were uncommon, validation first tested whether the frozen association persisted without feature re-selection and whether it depended on any single carrier, taxon or data split. Among 243 HLA-typed patients with paired tumor and adjacent-normal microbiome profiles, including 16 HLA-B27-positive patients, the adjusted HLA-B27 effect on the rank-normalized tumor-minus-normal Core6 score was $\beta = 0.809$ (95% CI 0.355 to 1.263; HC3 Wald $P < 0.001$; permutation $P = 0.0013$; Figure 9a). All six taxon estimates were in the prespecified direction; *Campylobacter* and *Fusobacterium* were nominally significant by permutation ($P = 0.0400$ and $P = 0.0200$, respectively), but neither passed false-discovery-rate correction (both $q = 0.120$; Figure 9b). The HLA-B27-status-stratified bootstrap distribution remained positive in all 10,000 resamples (median $\beta = 0.815$, 95% percentile CI 0.388 to 1.232; Figure 9c). Across the 16 leave-one-HLA-B27-positive-patient-out models, β estimates ranged from 0.721 to 0.925 and every association remained significant (from $P < 0.001$ to $P = 0.0018$; Figure 9d). Removing each genus in turn also retained positive Core6 effects (from $P < 0.001$ to $P = 0.0126$; Figure 9e). In repeated stratified four-fold cross-validation, adding the frozen Core6 score to age, sex and stage increased AUPRC from 0.075 to 0.157, with a paired difference of 0.082 (95% bootstrap CI 0.024 to 0.207; conditional permutation $P = 0.0040$; Figure 9f).

Figure 9. Internal robustness validation of the AC-ICAM HLA-B27-associated paired Core6

microbiome signal. This figure validates the stability of the frozen AC-ICAM Core6 association under resampling, patient deletion, taxon deletion and conditional cross-validation; it does not constitute independent external validation. (a) Patient-level paired Core6 change by HLA-B27 status among 243 HLA-typed patients with paired tumor and adjacent-normal profiles, including 16 HLA-B27-positive and 227 HLA-B27-negative patients. Core6 was calculated as $\log_{10}[(\text{Campylobacter} + \text{Fusobacterium} + \text{Prevotella} + 1e-5)/(\text{Oscillibacter} + \text{Escherichia-Shigella} + \text{Ruminococcus} + 1e-5)]$ in tumor minus the corresponding value in matched adjacent normal tissue. Violin envelopes show the distribution, boxes show the median and interquartile range, whiskers extend to 1.5 times the interquartile range and points represent patients. The displayed β is the adjusted HLA-B27 effect on the rank-normalized Core6 outcome from a linear model controlling for age, sex and stage with HC3 standard errors ($\beta = 0.809$, 95% CI 0.355 to 1.263; two-sided Wald $P < 0.001$; two-sided Freedman-Lane permutation $P = 0.0013$, 9,999 permutations). (b) Adjusted HLA-B27 effects and 95% confidence intervals for the six prespecified paired genus changes. Each outcome was the rank-normalized tumor-minus-normal $\log_{10}$ abundance change with pseudocount $1e-5$. Models controlled for age, sex and stage with HC3 standard errors. Two-sided Freedman-Lane permutation P values were adjusted across the six-taxon family using the Benjamini-Hochberg procedure. All estimates were in the prespecified

direction, but no taxon passed $q < 0.05$. **(c)** Distribution of the adjusted Core6 effect from 10,000 HLA-B27-status-stratified bootstrap resamples. The diamond indicates the bootstrap median, the thick interval indicates the empirical 95% percentile interval and the vertical navy line indicates the original estimate. The bootstrap median was 0.815, the empirical 95% interval was 0.388 to 1.232 and 100% of bootstrap estimates were positive. $P_{perm} = 0.0013$ is the primary Freedman-Lane permutation P value. **(d)** Leave-one-HLA-B27-positive-patient-out influence analysis. Each row excludes one of the 16 positive patients and refits the adjusted model without changing the score definition. Estimates ranged from 0.721 to 0.925, and two-sided HC3 Wald P values ranged from $P < 0.001$ to $P = 0.0018$. The gold dashed line indicates the full-cohort estimate. Display indices P01 to P16 are linked to study identifiers in the source-data workbook. **(e)** Leave-one-taxon-out analysis of the complete Core6 module and six reduced modules, recomputed without coefficient refitting. All adjusted estimates remained positive, with two-sided HC3 Wald P values from $P < 0.001$ to $P = 0.0126$. **(f)** Conditional internal out-of-fold discrimination of HLA-B27 status using Firth logistic models in 200 repeats of stratified four-fold cross-validation. The clinical model included age, sex and stage; the augmented model added the frozen Core6 score. Precision-recall curves use each patient's mean out-of-fold prediction. AUPRC was 0.075 for clinical covariates and 0.157 after Core6 addition. The paired difference was 0.082 (95% patient-stratified bootstrap CI 0.024 to 0.207). $P_{cond} = 0.0040$ was obtained from 499 label permutations with 10 repeated cross-validation partitions per permutation; the inset shows the permutation distribution and the teal line indicates the observed difference.

Independent Hartwig assessment of HLA-B27 ascertainment and microbiome transportability

Because an independent public cohort with paired primary-tumor and adjacent-normal 16S profiles was unavailable, the frozen HLA-B27 definition and Core6 membership were transported without taxon re-selection or reweighting to 496 patients with metastatic CRC profiled by tumor whole-genome sequencing, including 40 HLA-B27 carriers. This strategy tested independent genotype ascertainment and cross-platform transportability rather than exact assay replication. HLA-B27 status was highly reproducible across callers: LILAC and xHLA agreed in 496 of 496 patients ($\kappa = 1.000$, 95% CI 1.000 to 1.000), and LILAC and POLYSOLVER agreed in 495 of 496 patients ($\kappa = 0.986$, 95% CI 0.958 to 1.000). Carrier prevalence was comparable between Hartwig and AC-ICAM (40 of 496 versus 18 of 281; Fisher exact $P = 0.478$; **Figure 10a**). The adjusted Hartwig Core6 effect was $\beta = 0.028$ (95% CI -0.291 to 0.348; permutation $P = 0.8621$), compared with $\beta = 0.809$ in AC-ICAM; the cross-cohort effect difference was -0.781 (95% CI -1.336 to -0.226; interaction $P = 0.0058$; **Figure 10b**). No individual Hartwig taxon passed false-discovery-rate correction (**Figure 10c**), and the adjusted whole-community association was not significant (partial $R^2 = 0.00231$; permutation $P = 0.2628$; **Figure 10e**). No genomic immune-escape or treatment-response endpoint passed the prespecified within-family false-discovery-rate correction. Disease control was nominally lower among HLA-B27 carriers (OR = 0.368, 95% CI 0.149 to 0.912; $P = 0.0309$; $q = 0.186$; **Figure 10f**).

Figure 10. Independent Hartwig validation of HLA-B27 ascertainment and assessment of microbiome transportability. This figure validates the reproducibility of HLA-B27 carrier ascertainment across three algorithms in the external cohort and tests whether the frozen AC-ICAM Core6 and associated biological signals transport to an independent metastatic CRC cohort profiled by tumor whole-genome sequencing. It does not show external replication of the AC-ICAM microbiome, immune-escape or treatment-response associations. Hartwig included 496 patients, including 40 HLA-B27 carriers. **(a)** Independent verification of HLA-B27 ascertainment and carrier prevalence. Hartwig LILAC calls agreed with xHLA in 496 of 496 patients ($\kappa = 1.000$, 95% CI 1.000 to 1.000; exact McNemar $P = 1.000$) and with POLYSOLVER in 495 of 496 patients ($\kappa = 0.986$, 95% CI 0.958 to 1.000; exact McNemar $P = 1.000$). Carrier prevalence was 18 of 281 in AC-ICAM and 40 of 496 in Hartwig (two-sided Fisher exact $P = 0.478$); prevalence error bars show exact 95% binomial confidence intervals. **(b)** Harmonized Core6 HLA-B27 effects in AC-ICAM and Hartwig. AC-ICAM used the paired tumor-minus-normal Core6 change and Hartwig used the WGS Core6 validation score; outcomes were rank-normalized within cohort. AC-ICAM was adjusted for age, sex and stage ($\beta = 0.809$, 95% CI 0.355 to 1.263; permutation $P = 0.0013$). Hartwig was adjusted for tumor purity, log₁₀ genus library size, sex, sequencer type and biopsy-site group ($\beta = 0.028$, 95% CI -0.291 to 0.348; permutation $P = 0.8621$). The Hartwig-minus-AC-ICAM effect difference was -0.781 (95% CI -1.336 to -0.226; two-sided interaction $P = 0.0058$). **(c)** Harmonized adjusted HLA-B27 effects and 95% confidence intervals for the six prespecified taxa. AC-ICAM outcomes were rank-normalized paired genus changes and Hartwig outcomes were rank-normalized centered-log-ratio WGS abundances. Cohort-specific models used the covariates specified in (b). Two-sided Freedman-Lane permutation P values were adjusted separately within each six-taxon cohort family using the Benjamini-Hochberg procedure. No Hartwig taxon passed $q < 0.05$. **(d)** Cross-cohort concordance of the six adjusted taxon effects. The dotted line indicates identity. Spearman ρ was -0.486, with an exact two-sided permutation $P = 0.356$ across all 720 taxon-label permutations. Two of six taxa had the same effect direction in both cohorts (exact two-sided binomial $P = 0.688$). **(e)** Global community association with HLA-B27. Partial variance explained was estimated using covariate-adjusted Aitchison-space PERMANOVA with 4,999 permutations. AC-ICAM paired tumor-minus-normal community change had partial $R^2 = 0.00438$ and permutation $P = 0.3154$; Hartwig metastatic WGS community composition had partial $R^2 = 0.00231$ and permutation $P = 0.2628$. **(f)** Independent Hartwig genomic immune-escape and treatment-response endpoints. Points show adjusted Firth odds ratios with 95% confidence intervals. Immune-escape models included tumor purity, liver-biopsy status, sex and sequencer type. Response models included Core6 score, genetic immune escape, tumor purity, liver-biopsy status, sex and treatment class; 310 patients were response-evaluable, including 26 HLA-B27 carriers. Benjamini-Hochberg q values were calculated within the prespecified endpoint families. No endpoint passed $q < 0.05$. Disease control was nominally lower in HLA-B27 carriers (OR = 0.368, 95% CI 0.149 to 0.912; $P = 0.0309$; $q = 0.186$).

Discussion

To the best of our knowledge, this is the first study to examine whether HLA-B27 status is associated with the intratumoral pathogenic bacterial microbiome and clinical outcomes in CRC. Among 348 CRC patients, 281 had complete HLA-B27 typing, of whom 18 (6.4%) were HLA-B27-positive and 263 (93.6%) were HLA-B27-negative. Although HLA-B27 status did not appear to globally restructure tumor microbial diversity, it marked a selective and biologically coherent enrichment of oral-associated CRC pathobionts, including *Fusobacterium*, *Prevotella* and *Campylobacter*. This finding suggests that HLA-B27 may not act as a direct determinant of overall microbial richness, but rather as an immunogenetic context within which specific pathogenic taxa become more prominent. Importantly, the derived B27-microbiome signature, rather than HLA-B27 genotype alone, suggested stronger relevance to overall survival, supporting the concept that host immunogenetics and tumor microbial ecology may jointly shape clinically meaningful CRC phenotypes. These results add a new layer to CRC microbiome research by linking a defined MHC class I allele with intratumoral pathobiont composition, tumor immune architecture and outcome patterns. Translationally, this framework may help identify microbiome-informed immune-risk strata and supports future prospective studies testing whether HLA-informed microbial signatures can refine patient stratification, prognosis or microbiome-directed therapeutic approaches in CRC.

The crux and main implication of our findings is that the immunogenetic background and tumor microbiome are both intertwined as opposed to being independent axes of CRC biology^{20,21}. The oral-associated pathobionts enriched in HLA-B27 enriched-positive tumors (*Fusobacterium*, *Prevotella* and *Campylobacter*) are among the most consistently CRC-linked taxa in metagenomic meta-analyses²²⁻²⁴. They are also increasingly understood to translocate from the oral cavity to colonize the tumor niche²⁵⁻²⁸. Particularly, *Fusobacterium nucleatum* promotes genotoxic damage, immune evasion, and chemoresistance^{29,30}. Hence, its abundance predicts poorer survival outcomes^{30,31}. Moreover, it has a distinct clade that is known to dominate the colorectal niche^{32,33}. It is biological coherent that these taxa have a predilection to accumulate in HLA-B27-positive tumors. HLA-B27 misfolding brings about endoplasmic reticulum stress and an IL-23/Th17-skewed inflammatory expression which is the same environment that has been shown to favor pathobiont expansion in HLA-B27 driven gut dysbiosis models³⁴⁻³⁶. Our combined signature association with the overall survival rates concurs with the growing pool of literature that exemplifies that microbial community structure carries far more prognostic weight compared to any single taxon individually³⁷. Furthermore, it also aligns with the established role of the gut microbiome as a determinant of anti-PD-1 response in gastrointestinal tumors^{38,39}. Additionally, current interventional evidence highlight that microbiome modulation can overcome immunotherapy resistance⁴⁰⁻⁴³. Mechanistically, pathobiont-driven suppression of antitumor CD8⁺ immunity offers a plausible route by which a microbiome signature could potentially influence outcomes independently of the HLA-B27 genotype⁴⁴. Collectively, when we view this together, we can piece that these data support a model in which HLA-B27 sets an inflammatory stage that selects a prognostically significant microbial community, integrating along with it host genetics, microbial ecology, and antitumor immunity into a single translational framework.

Moving on, several of our observations concur with prior work. The absence of a global diversity shift between the different HLA-B27 strata mirrors reports that tumor-site alpha diversity is often conserved even when specific taxa differ^{15,16,45}. Additionally, our taxon-level enrichments recapitulate the oral-pathobiont signal repeatedly described in CRC^{37,46-49}. Our finding that a microbiome signature, rather than HLA genotype, carries prognostic value is consistent with evidence that HLA class I loss and downregulation drive immune escape and outcome in cancer patients^{3,50,51}. Hence, this contrasts with the notion that germline allele identity has an understated prognostic value. Conversely, some of our findings diverge from what is expected. Whereas HLA-B27 has been associated with modestly increased malignancy risk in spondyloarthritis cohorts, we observed no independent prognostic effect of HLA-B27 on survival⁵²⁻⁵⁵; this likely reflects differences in population (CRC patients versus inflammatory-disease cohorts), the small HLA-B27-positive sub group, and the intratumoral rather than systemic nature of our exposure. Current reports that linked *Fusobacterium* to poor prognosis contrast with our observation that high-signature tumors that are enriched for such taxa tend to trend towards better survival^{52,56-58}. This rather odd apparent discrepancy may arise given our composite signature integrated both risk- and protective-leaning taxa into a net axis. This is because relative abundance within a driver panel tends to behave differently from absolute *Fusobacterium* load and also because stage dominated prognosis in our adjusted models⁵⁹⁻⁶². Lastly, differences in the sequencing modality (16S genus-level versus metagenomic species-level), cohort ancestry, and the exploratory nature of a rare-exposure analysis explain the divergency from studies powered for common exposures.

These findings elucidate several outlets for possible future work. First, the composite B27-microbiome signature should be validated in larger, prospectively sampled CRC cohorts that have a balanced HLA-B27 representation. Ideally, it should also employ shotgun metagenomics to resolve species- and strain- level drivers such as the CRC-adapted *Fusobacterium* clade and also to capture functional gene content beyond 16S genus profiles^{32,63-65}. Second, because the gut microbiome is a modifiable determinant of immunotherapy response, HLA-B27-anchored signatures could be tested as stratification biomarkers in anti-PD-1 trials^{10,38,66,67}. Microbiome targeted interventions (such as dietary, prebiotic, or fecal microbiota transplantation) can also be evaluated for their capability to remodel the pathobiont-weighted niche in patients that are genetically predisposed⁶⁹⁻⁷¹. Third, our study is of a cross-sectional, retrospective design that precludes casual inference and, thus, cannot establish directionality between genotype, microbiome, and outcome⁷². Fourth, treatment exposures were rather heterogeneous and incompletely annotated, limiting immunotherapy-specific analyses⁷³. Each of these was crucial to a hypothesis-generating reuse of existing structured data. No new sampling, sequencing, or randomization was possible as a result. Therefore, we can only frame the results as exploratory rather than confirmatory.

Conclusions

In this cohort, HLA-B27 status did not globally restructure the colorectal tumor microbiome; however, it marks a coherent enrichment of oral-associated pathobionts. It identified a small subgroup with a directional enrichment of oral-associated pathogenic taxa, including *Fusobacterium*, *Prevotella* and *Campylobacter*. A composite B27-microbiome signature, as

opposed to the genotype itself, is independently showed a modest association with overall survival after adjustment. These findings suggest that HLA-B27 may contribute to a tumor immune-microbial context rather than act as an independent outcome biomarker. It is certain that there is a need to validate this genotype-anchored signature in larger prospective studies with metagenomically characterized cohorts.

Ethics Declaration

This retrospective secondary analysis used processed public data from previously published human studies. Original recruitment, consent and ethics arrangements were reported by the source investigators. No new recruitment, intervention or access to directly identifying participant information was undertaken. The authors conducted this secondary data use in accordance with the principles of the Declaration of Helsinki for secondary data use in health research⁷⁴.

Data Availability Statement

The present study did not generate any new biological materials or experimental reagents. All analyses were conducted using publicly available CRC datasets.

Clinical Trial Number

Clinical trial number: not applicable.

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Declaration of Competing Interests

The authors declare that they have no competing interests that could be construed as a potential conflict of interest.

Generative AI Statement

Artificial intelligence tools were used to support language editing, manuscript organization, refinement and clarity of presentation. All AI-assisted content was reviewed, verified and revised by the authors, who take full responsibility for the integrity, accuracy and final content of the manuscript.

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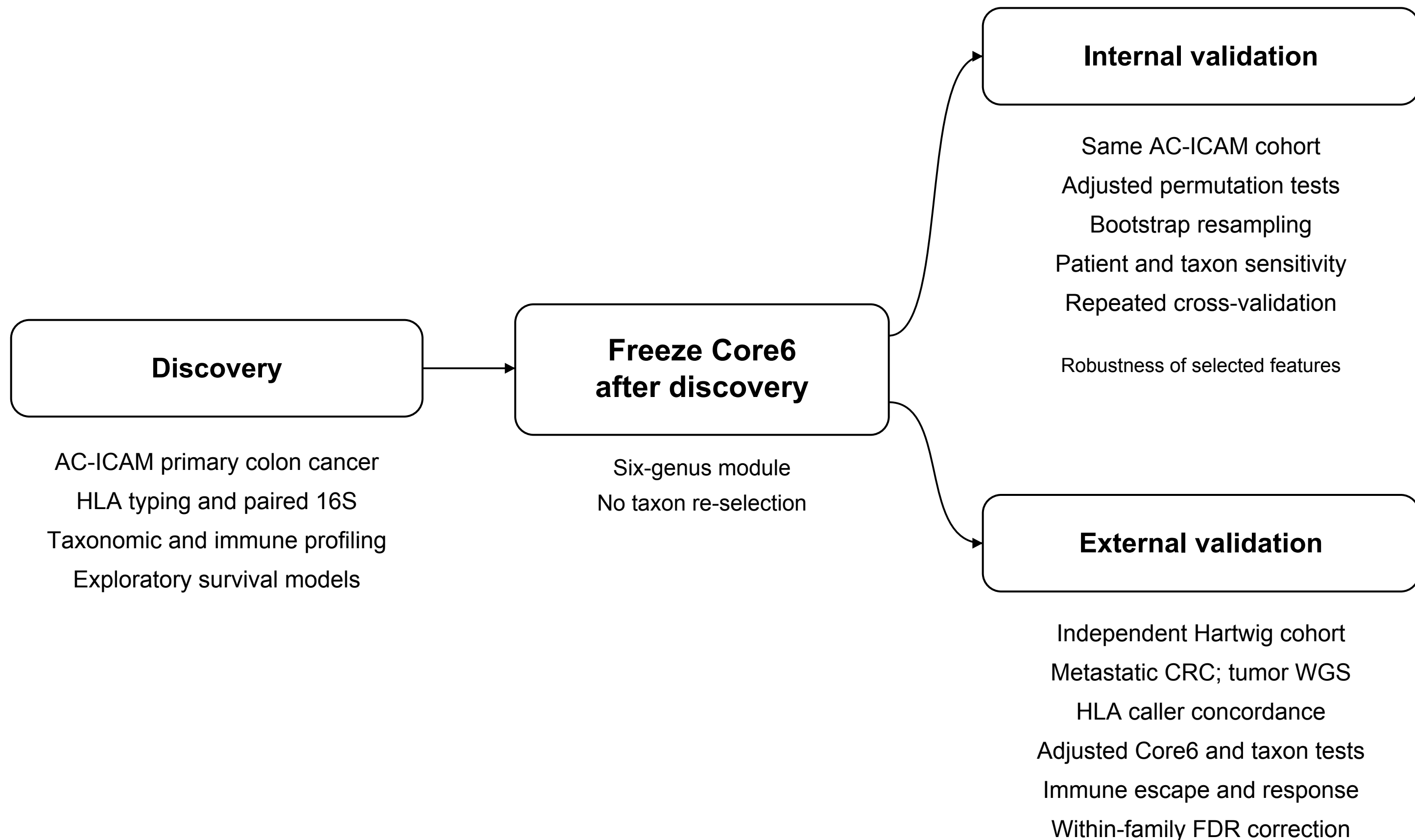
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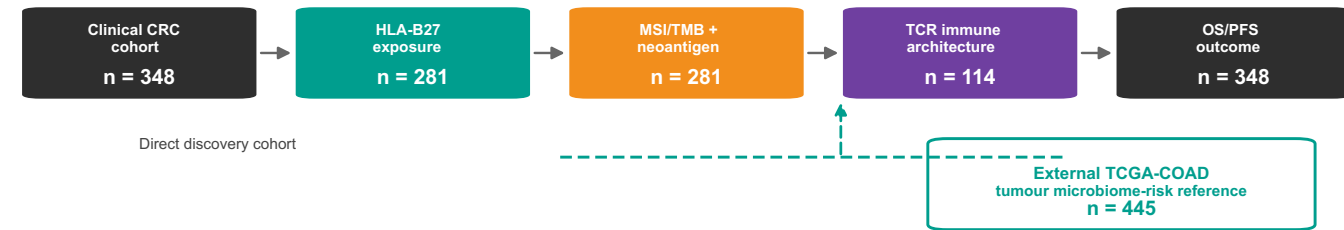
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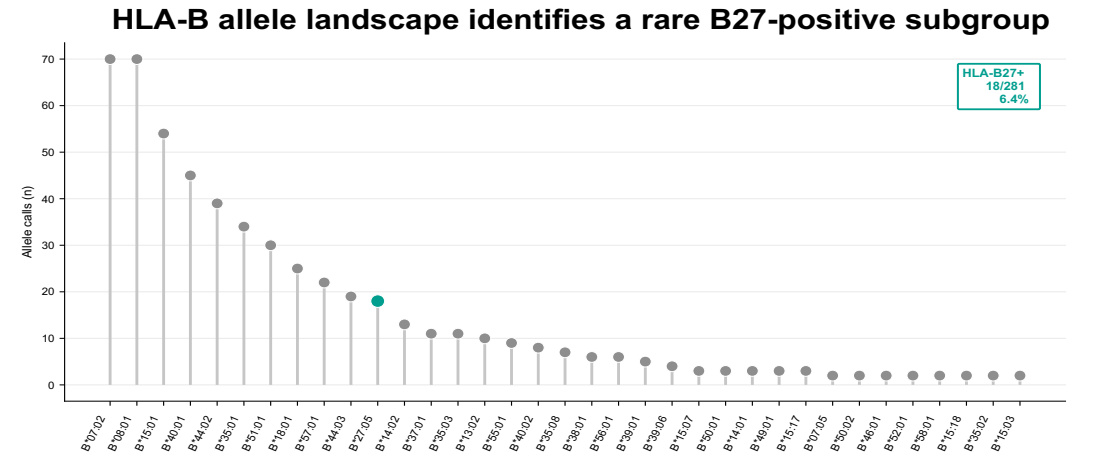


a.

Discovery architecture linking HLA-B27, tumour immunogenicity, immune repertoire and microbiome-risk reference

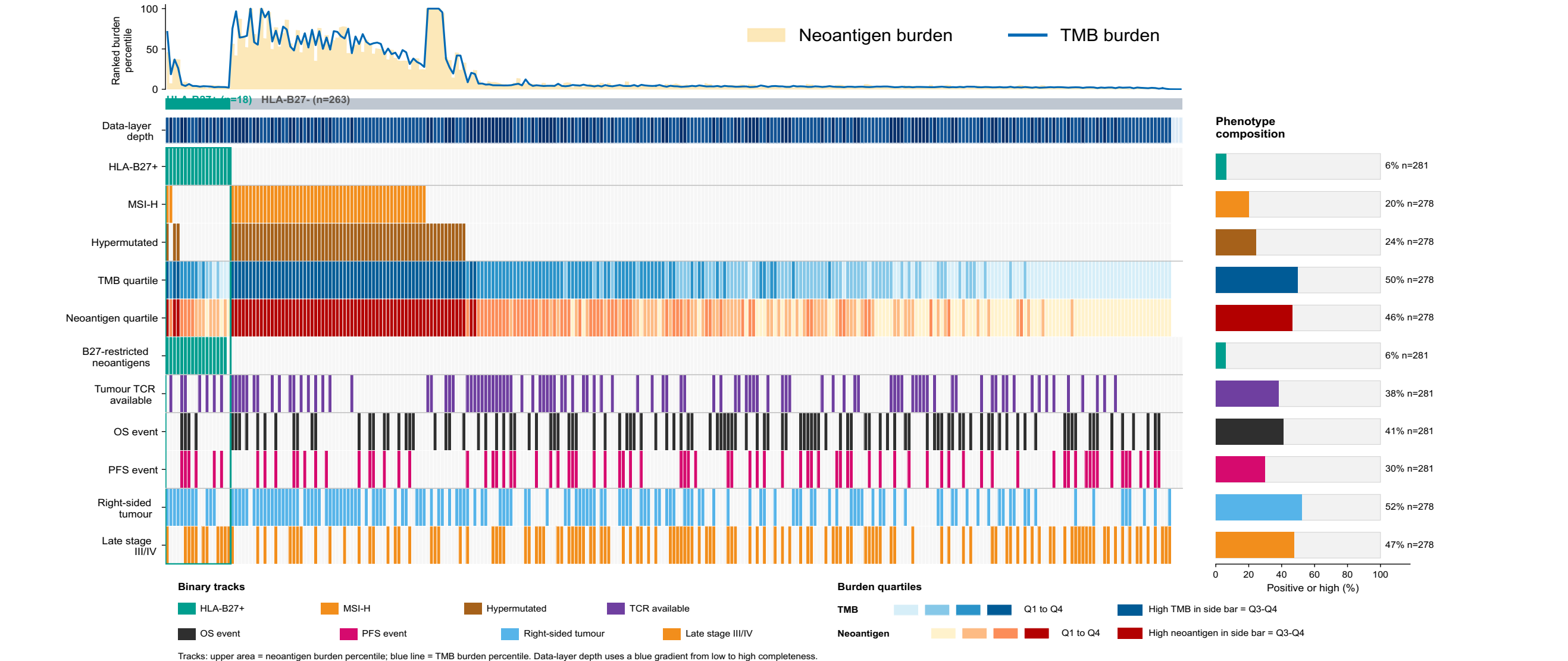


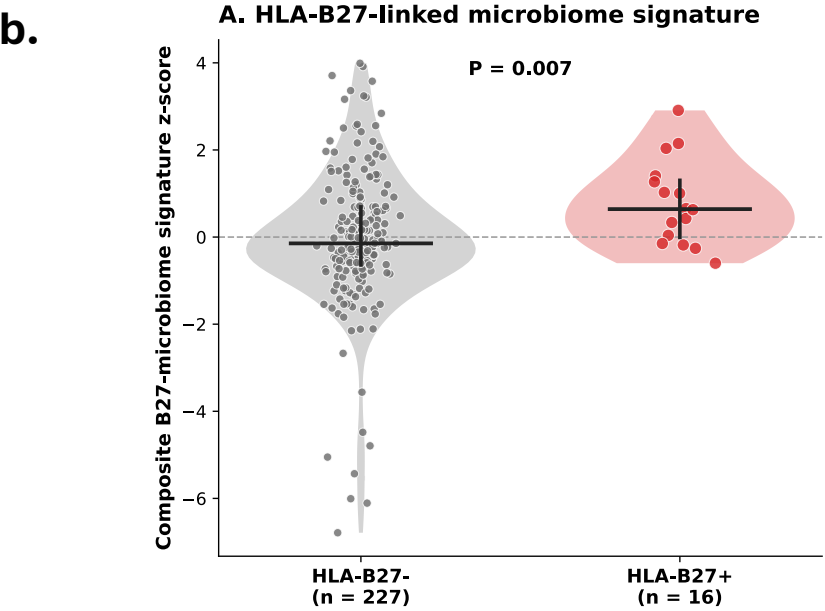
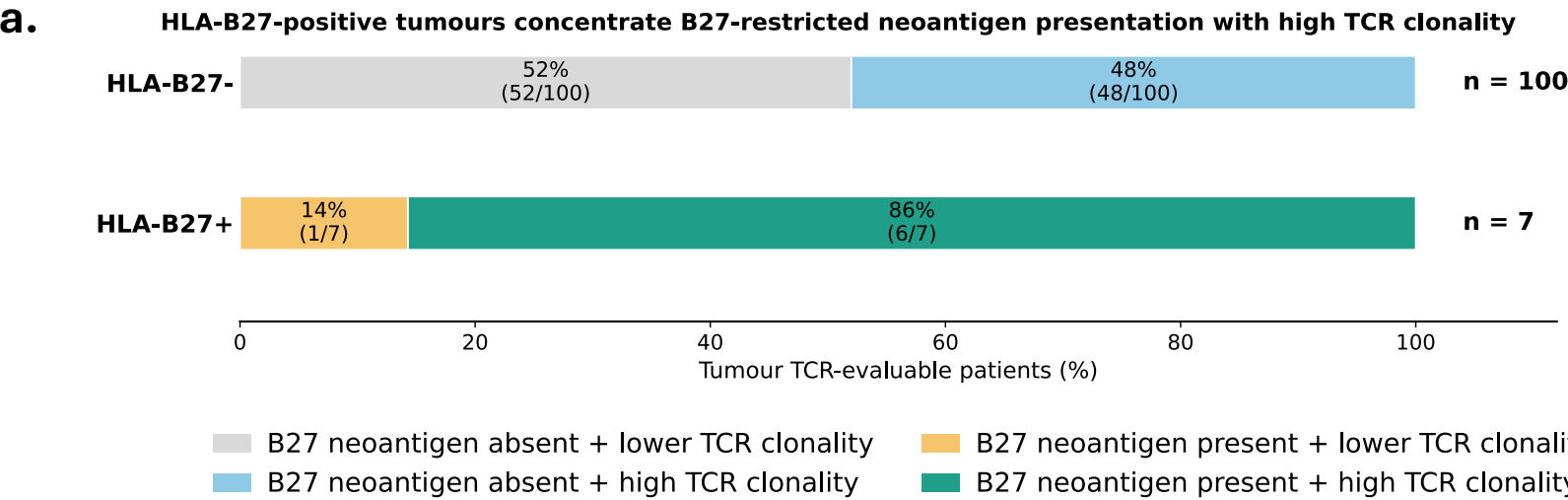
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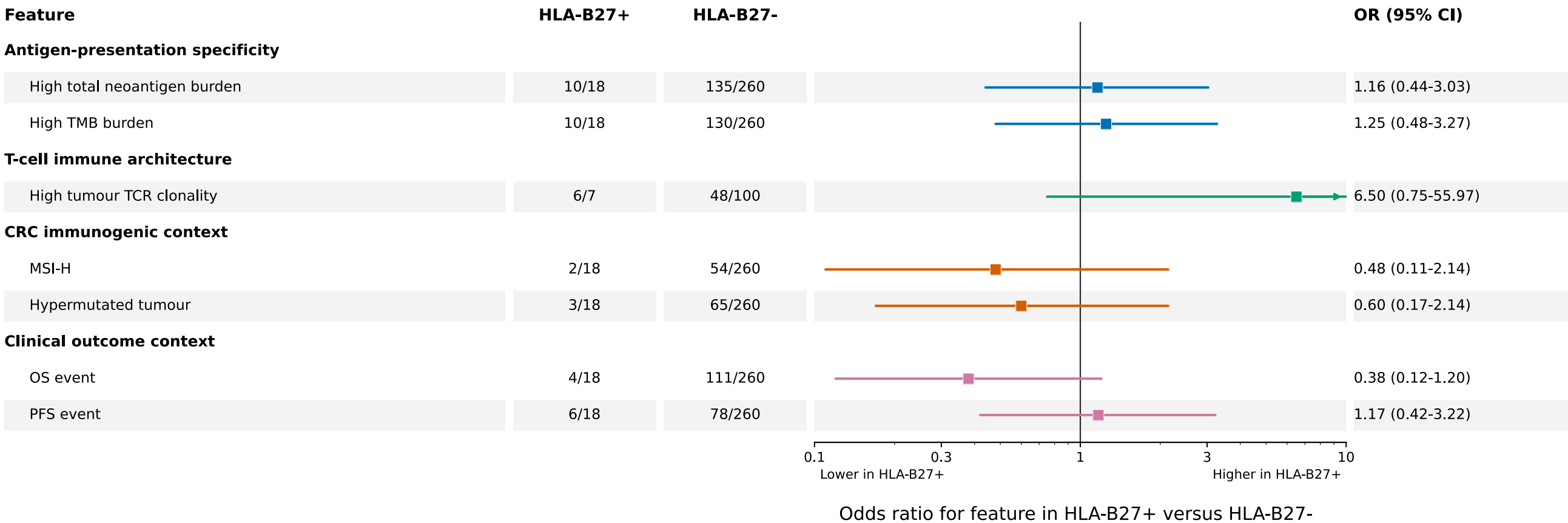
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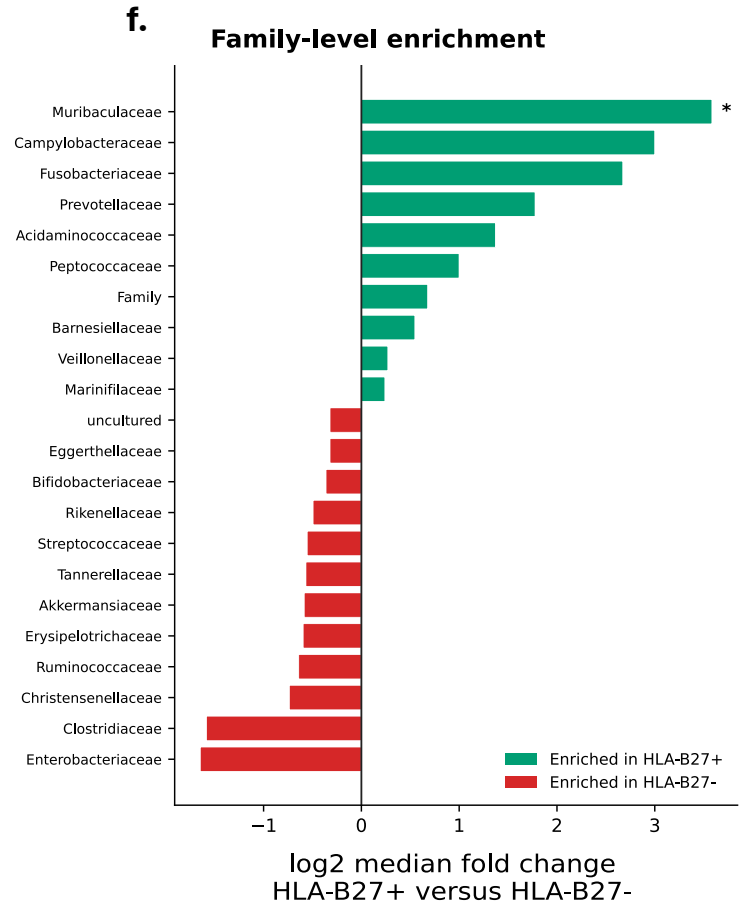
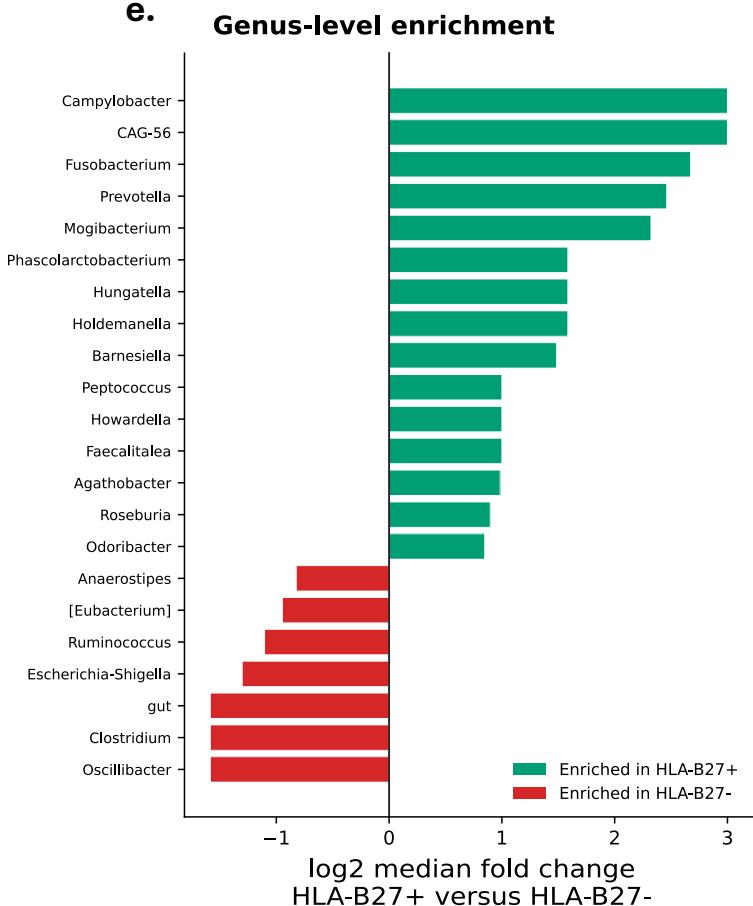
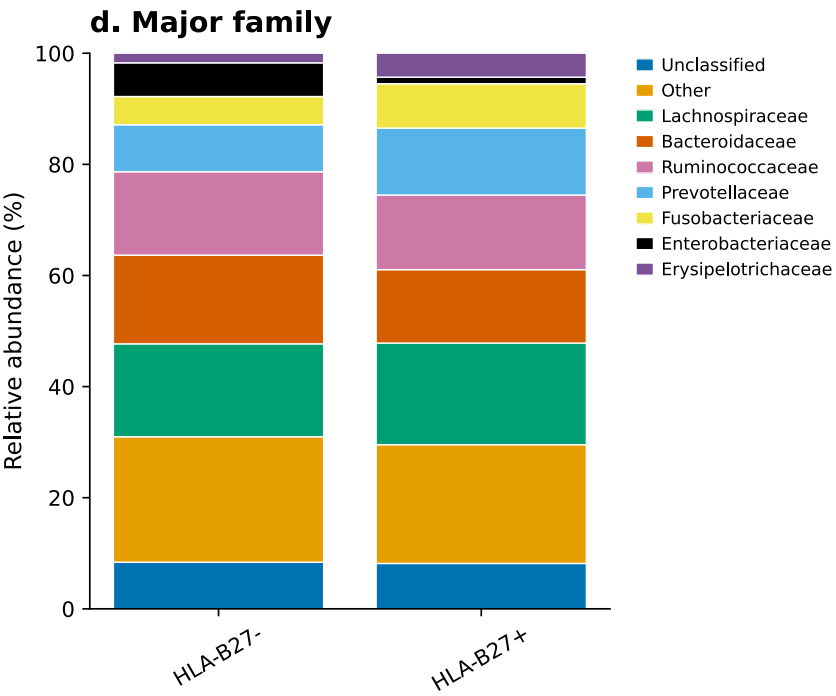
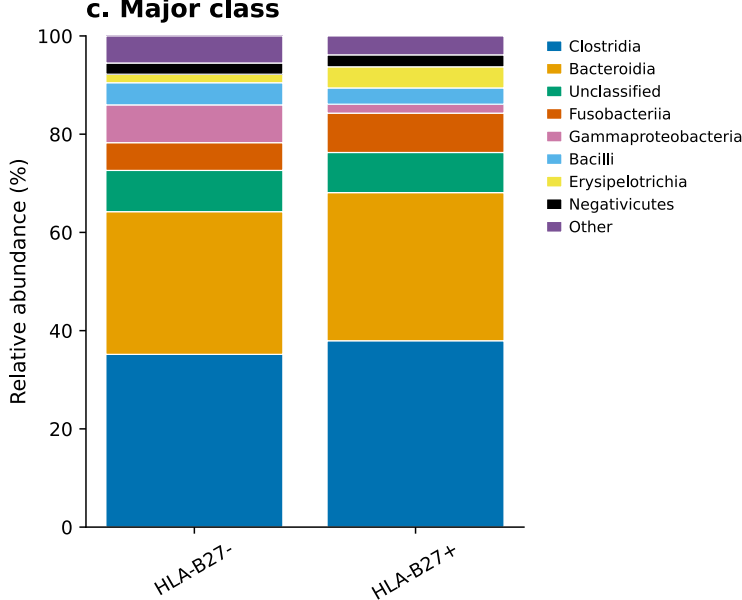
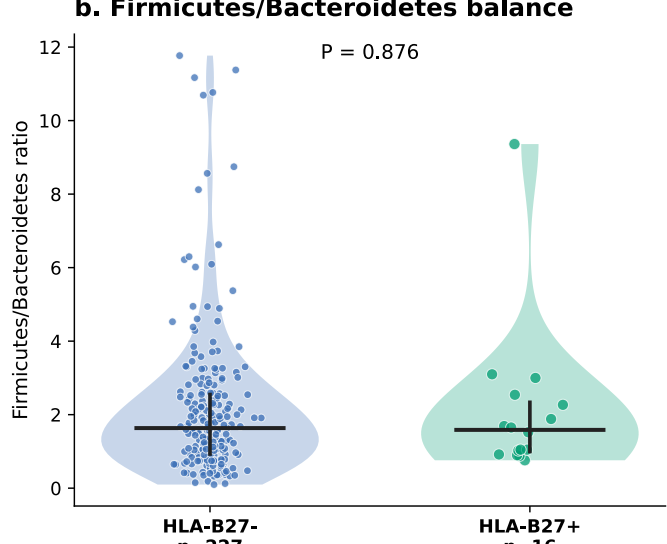
Patient-level HLA, immunogenicity, clinical outcome and data-depth oncoprint





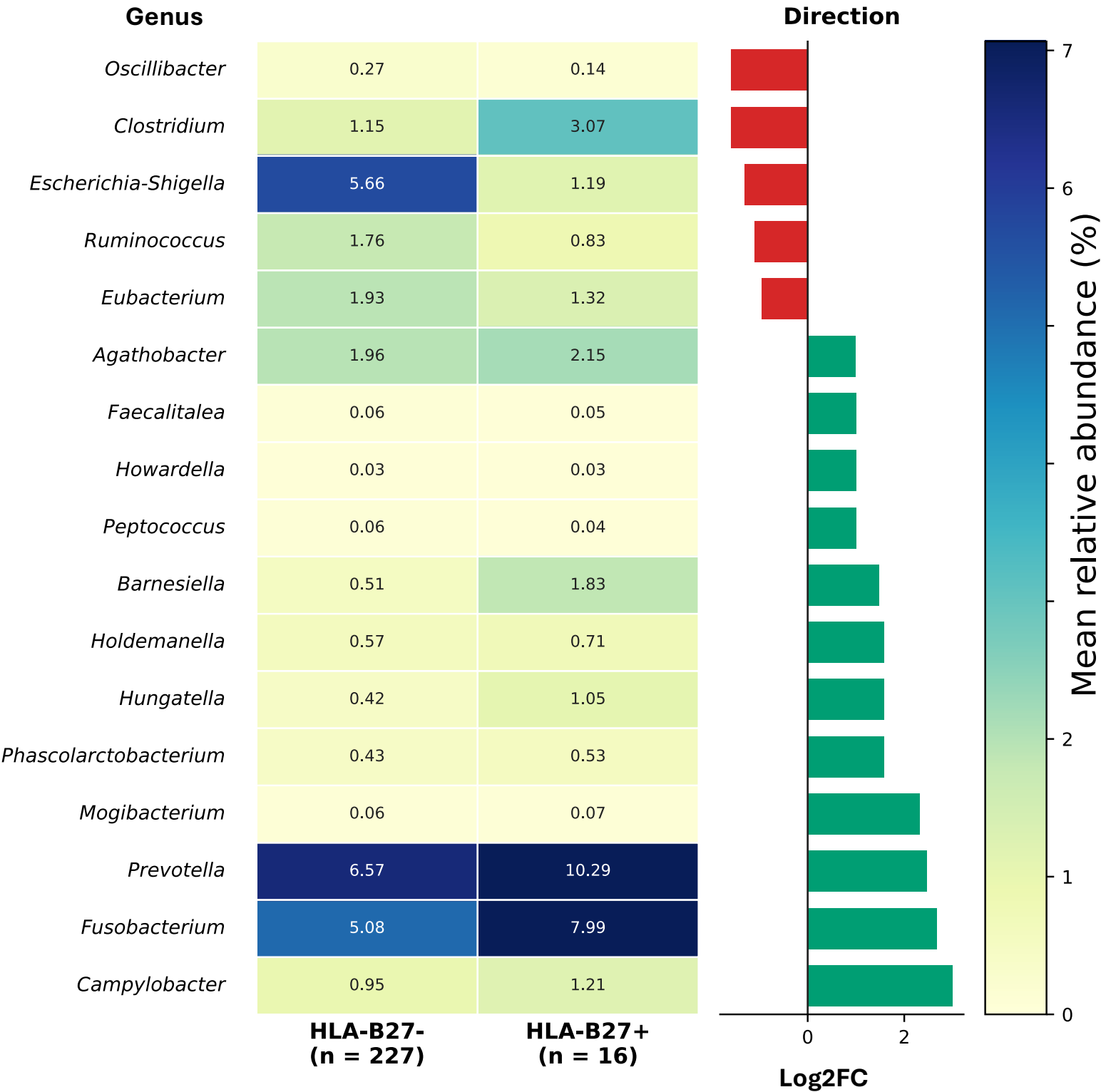
C. Exploratory effect-size map for immunogenic, TCR and outcome features



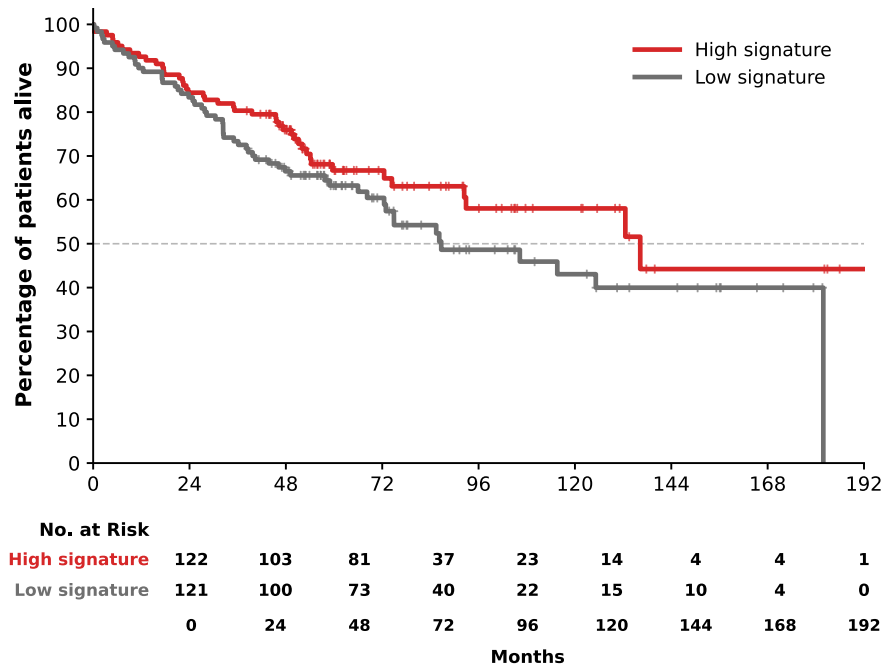


Top genus-level tumour microbiome abundance matrix

Higher in HLA-B27+ Higher in HLA-B27-



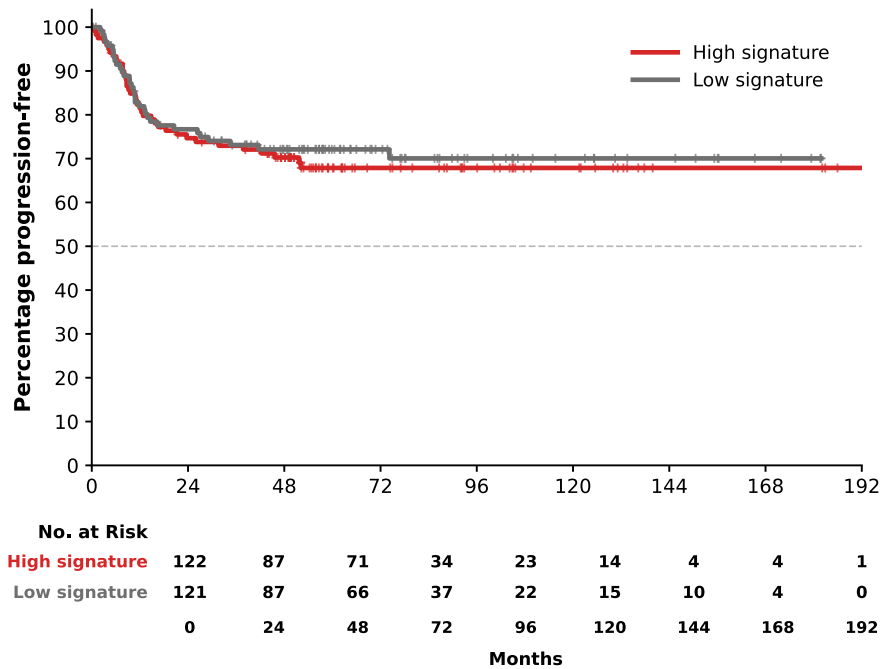
a Overall survival by B27-microbiome signature



Group	No. of patients	No. of events (%)	Median OS (mo)
High signature	122	43 (35)	136.3
Low signature	121	56 (46)	86.7

Hazard ratio, 0.74 (95% CI, 0.50-1.10)
log-rank P=0.133

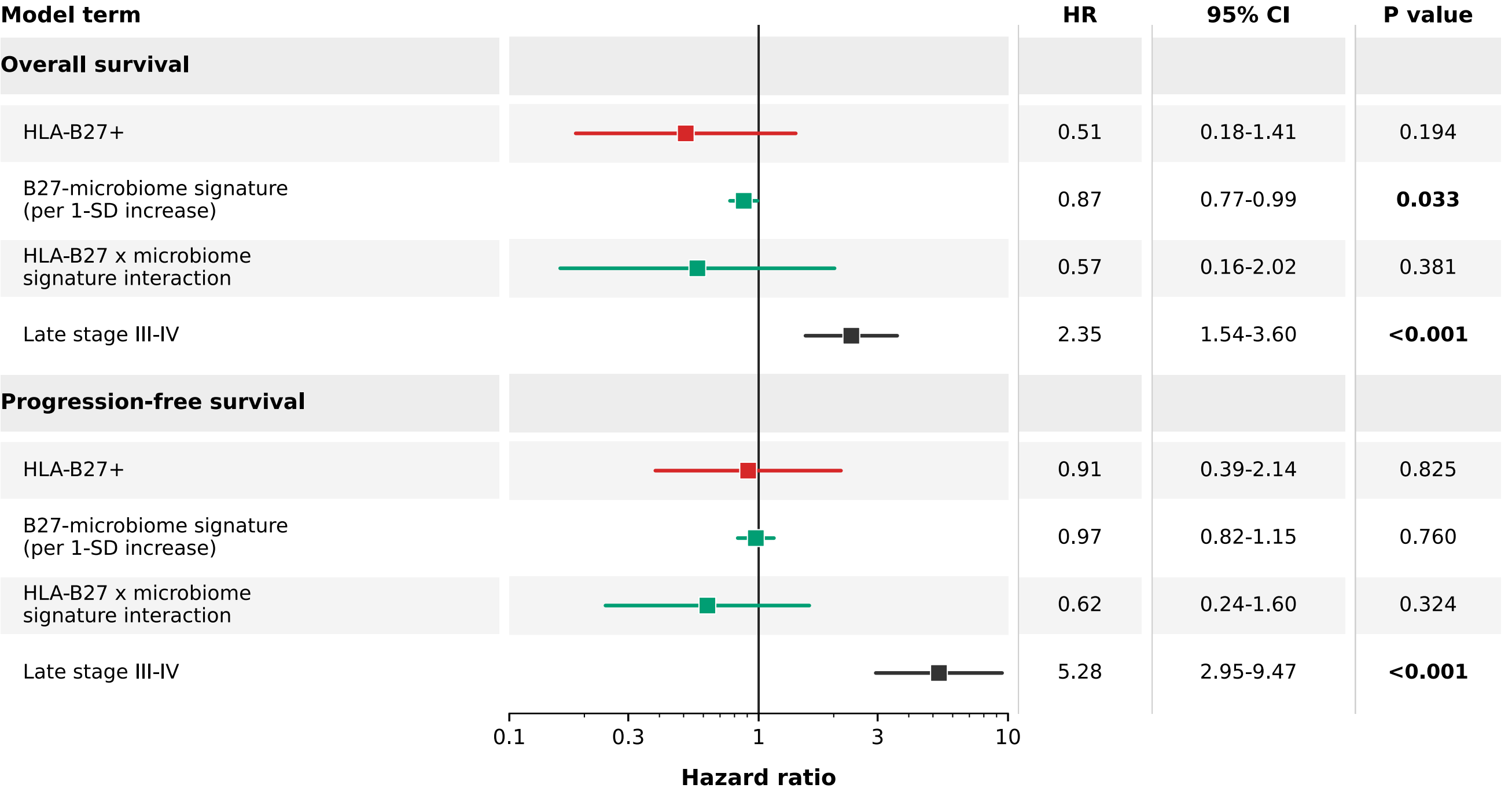
b Progression-free survival by B27-microbiome signature



Group	No. of patients	No. of events (%)	Median PFS (mo)
High signature	122	37 (30)	NR
Low signature	121	33 (27)	NR

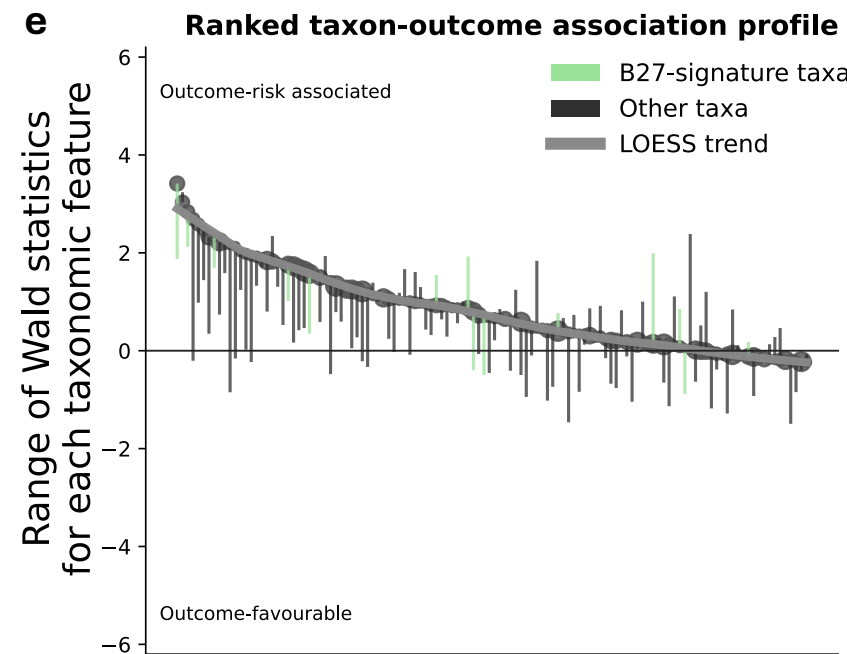
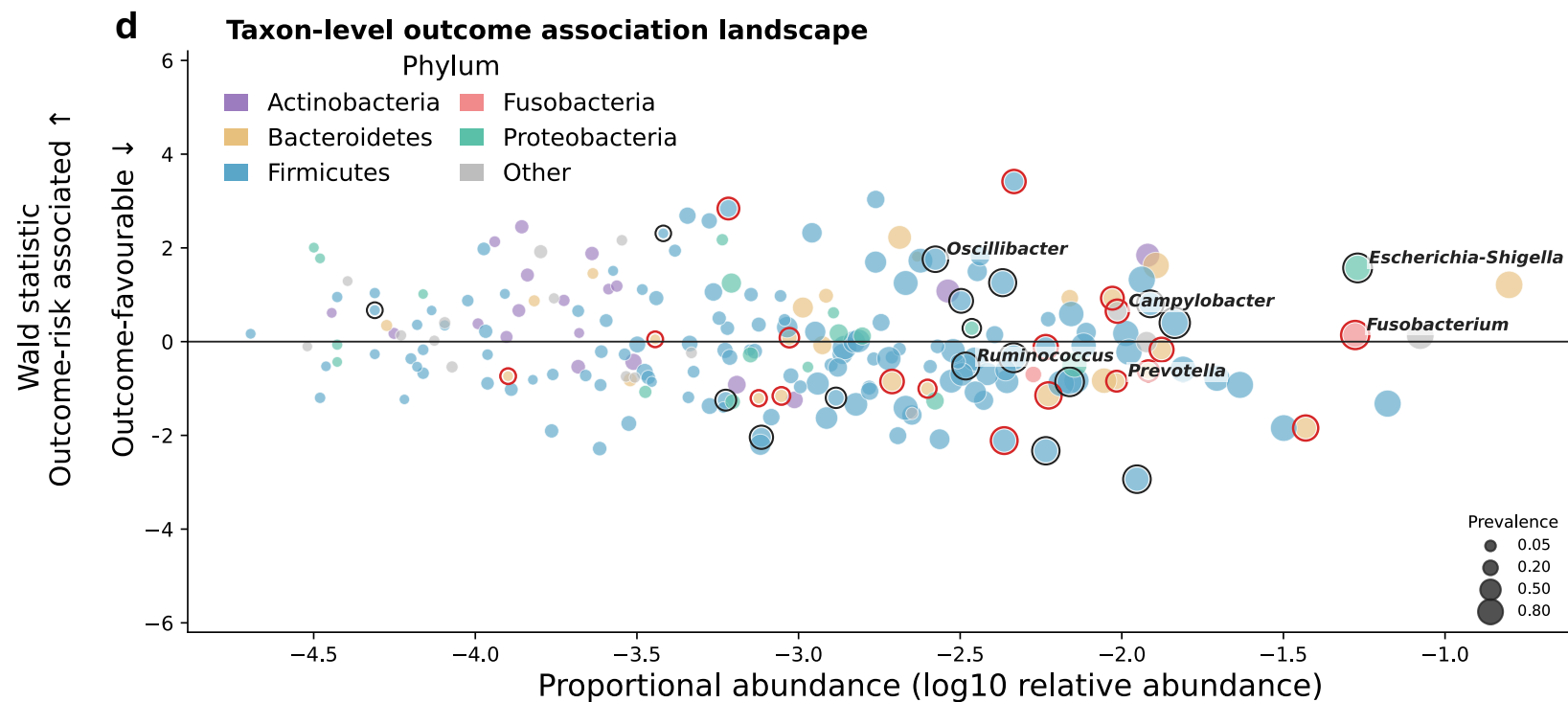
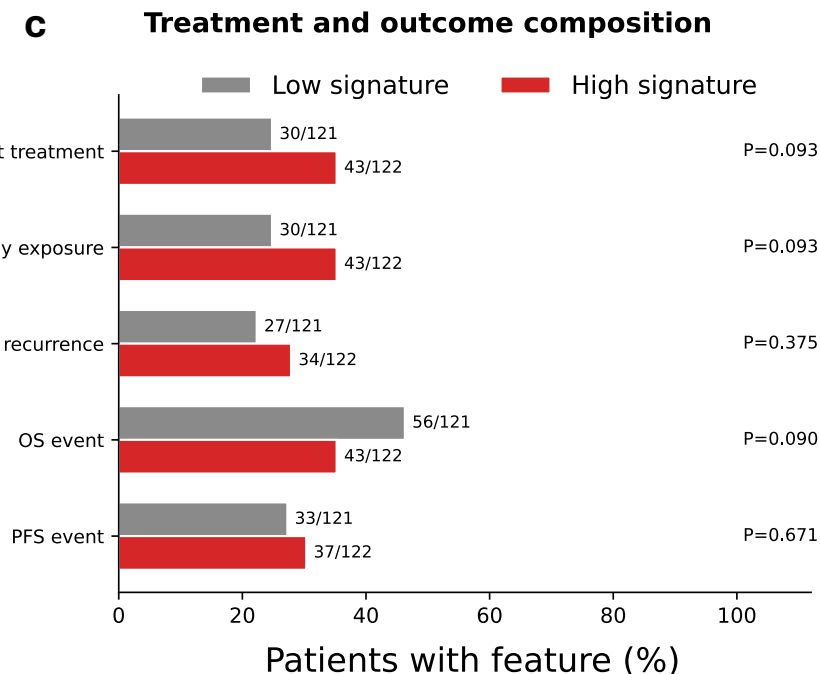
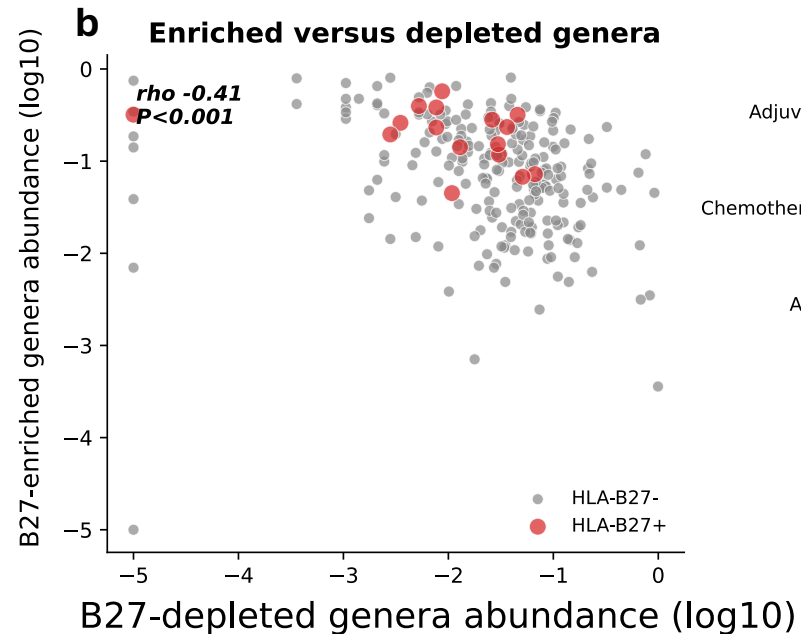
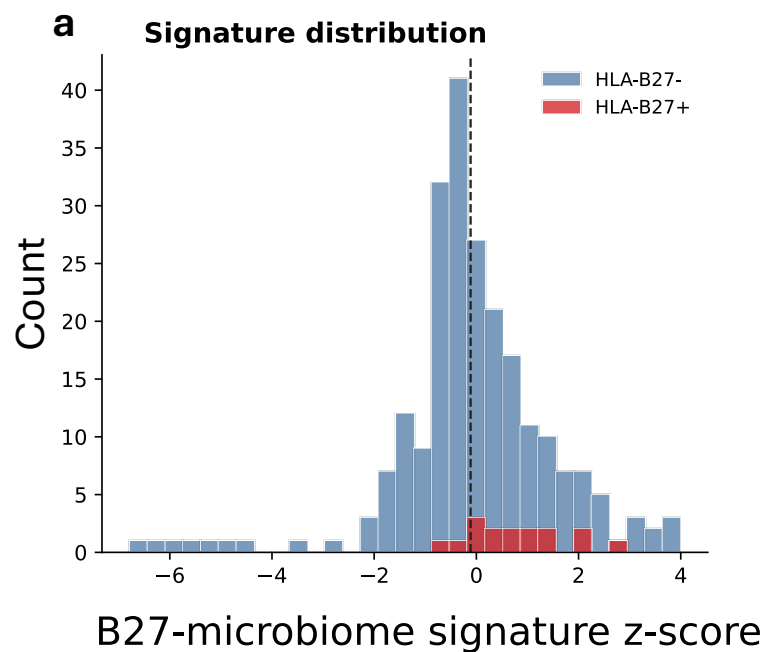
Hazard ratio, 1.10 (95% CI, 0.69-1.76)
log-rank P=0.690

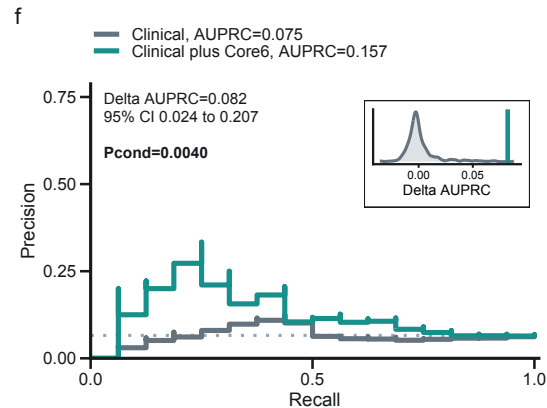
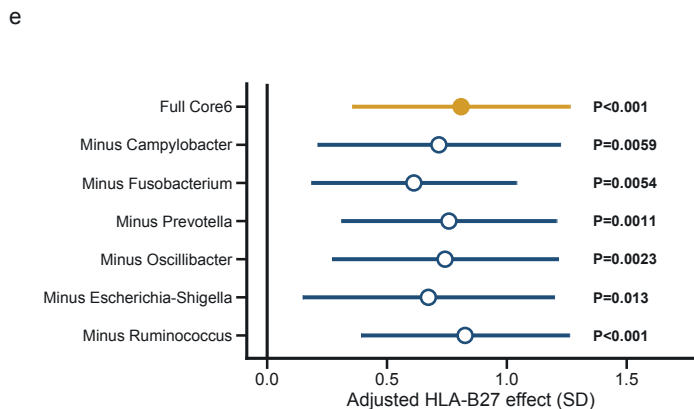
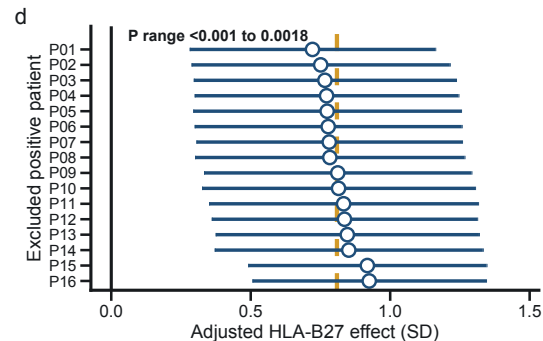
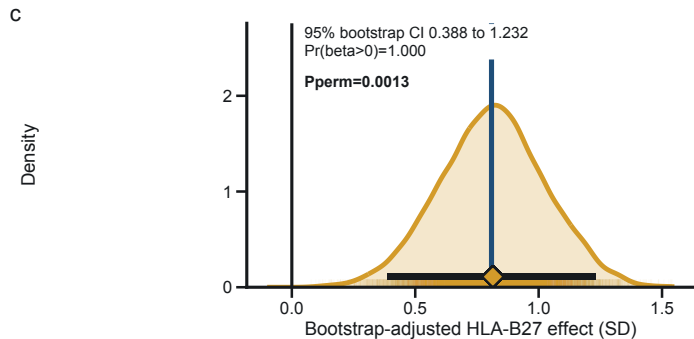
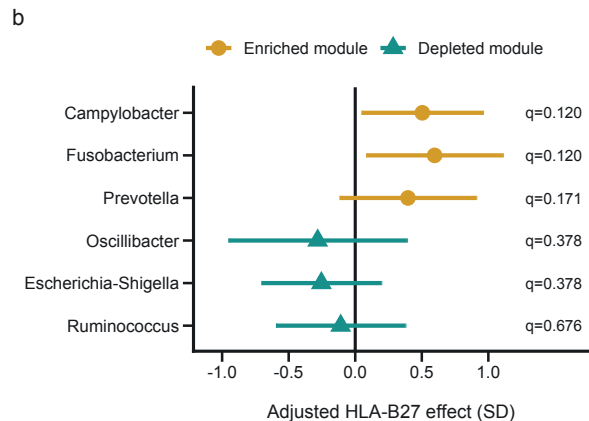
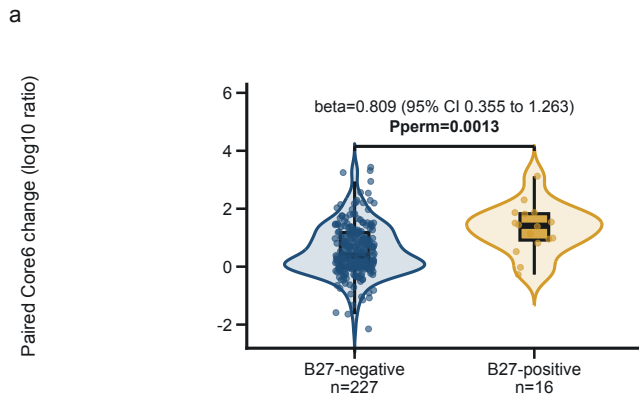
Cox models integrating HLA-B27 and microbiome signature

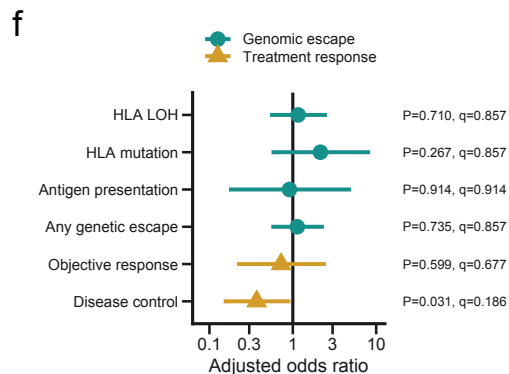
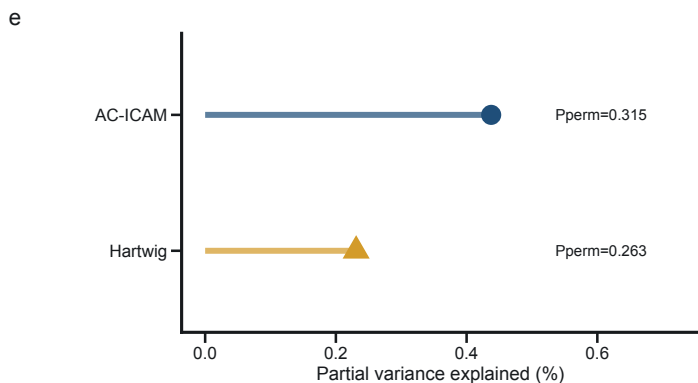
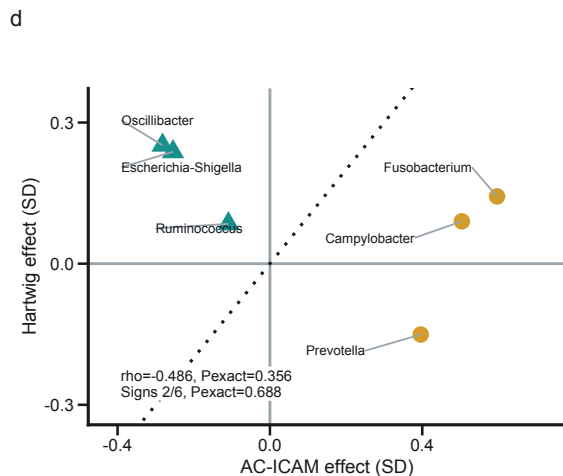
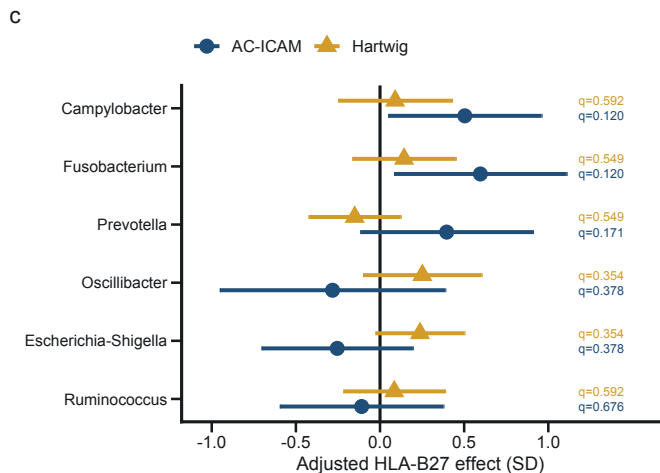
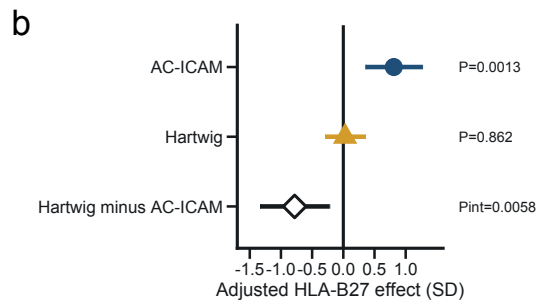
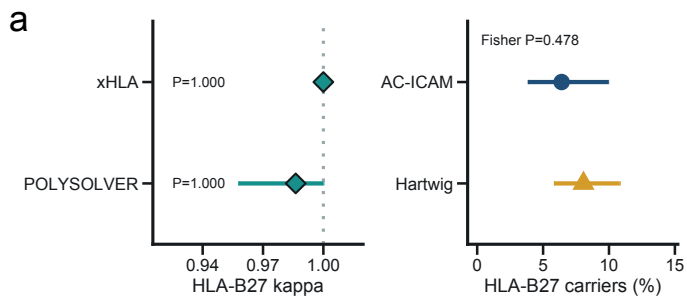


Adjusted models include HLA-B27 status, B27-microbiome signature, age, AJCC stage and MSI status. Interaction rows are from separate HLA-B27 x microbiome-signature models.

HLA-B27-linked tumour microbiome composition and outcome







Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [DatasetDiscovery.xlsx](#)
- [DatasetValidation.xlsx](#)
- [Graphicalabstract.pdf](#)