Gut microbiome remains stable following COVID-19 vaccination in healthy and immuno-compromised individuals

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Abstract

The bidirectional interaction between the immune system and the gut microbiota is a key contributor to various host physiological functions. Immune-associated diseases such as cancer and autoimmunity, as well as the efficacy of immunomodulatory therapies, have been linked to microbiome variation. While COVID-19 infection has been shown to cause microbial dysbiosis, it remains understudied whether the inflammatory response associated with vaccination also impacts the microbiota. Here, we investigate the temporal impact of COVID-19 vaccination on the gut microbiome in healthy and immuno-compromised individuals; the latter included patients with primary immunodeficiency and cancer patients on immunomodulating therapies. We find that the gut microbiome remained remarkably stable post-vaccination irrespective of diverse immune status, vaccine response, and microbial composition spanned by the cohort. The stability is evident at all evaluated levels including diversity, phylum, species, and functional capacity. Our results indicate the resilience of the gut microbiome to host immune changes triggered by COVID-19 vaccination and suggest minimal, if any, impact on microbiome-mediated processes. These findings encourage vaccine acceptance, particularly when contrasted with the significant microbiome shifts observed during COVID-19 infection.

43 **Introduction**

44 In the first 30 months of the pandemic, there have been reported to be almost 800 million PCR
45 confirmed cases of COVID-19 infection and approaching 7 million related deaths globally[1]. To
46 reduce this severity, vaccines were deployed with the aim of promoting anti-SARS-CoV-2
47 immunity, with almost 13.5 billion vaccine doses administered globally, 150 million of which
48 were administered in the United Kingdom[1]. Yet, continued COVID-19 transmission remains of
49 concern[2] with one of the reasons being vaccine hesitancy[3]. Thus, data helping to understand
50 holistic effects of vaccination will have a profound impact on the public health management.

51 The SARS-CoV-2 mRNA and viral vector vaccines induce a strong immune response through
52 the promotion of both innate and adaptive immunity against the spike protein[4]. Of note, there
53 has been reported to be a promotion of inflammatory cytokines IFNy, IL-15 and IL-6 secretion
54 in response to vaccination[5] each known to impact intestinal epithelial architecture and mucosal
55 immunity[6–8]. The sequestration of the spike mRNA by antigen-presenting cells aims to limit the
56 spread into systemic circulation, however the SARS-CoV-2 spike protein was also found in
57 blood plasma following vaccination[9], potentially leading to inflammation at different sites of the
58 body other than the site of vaccination, including that of the gut. This led us to hypothesize
59 that the systemic immune response to the SARS-CoV-2 vaccination may impact the gut
60 microbiota.

61 Numerous studies have analysed the gut microbiome during COVID-19 infection[10], finding
62 notable depletion in both commensal bacterial, such as *Bacteroides* and *Bifidobacterium spp*.
63 and beneficial *Lachnospiraceae,* coupled with increased abundances of opportunistic
64 pathogens such as *Streptococcus* and *Clostridium hathawayi*; this indicates a marked dybiosis
65 induced by COVID-19 infection. Yet since the initiation of the vaccination programme against
66 COVID-19, very few studies have addressed the impact of the vaccination on the gut
67 microbiome[11–14]. Previous work has addressed the link between the gut microbiome and
68 vaccine immunogenicity in which, baseline abundances of certain bacterial species before the

69 first vaccine dose have been correlated with a defined end point of vaccine efficacy, typically
70 a vaccine-related readout, such as virus neutralisation or spike-specific antibody titres.

71 Yet, two open questions remain: how the gut microbiome is affected by COVID-19 vaccination
72 in the days following vaccination when the inflammatory response is at its highest, and are
73 any immediate changes in the gut microbiome maintained or resolved once humoral immunity
74 has been initiated. Addressing this knowledge-gap could help understand the extent and the
75 nature of reciprocal links between the gut microbiome and systemic immunity in the context of
76 vaccination. We therefore sought to analyse the gut microbiome of patients receiving doses
77 of the COVID-19 vaccines to decipher whether there were any notable, characteristic changes
78 in the gut microbiome in either healthy or immunocompromised individuals [Table 1]. The
79 immunocompromised patients we recruited were cancer patients receiving immune
80 checkpoint modulating therapies and patients with primary immunodeficiencies. These
81 cohorts consist of patients with impaired immunity resulting from either therapeutic
82 intervention or monogenic defects in immunoregulatory genes (CTLA4, NFKB1, CD40L),
83 respectively.

84 This presented us with the unique opportunity to elucidate whether the COVID-19 vaccines
85 alter the gut microbiome in the absence of complete, functional immunity and subsequent
86 impairments of the regulation of the gut microbiome.

Methods

Study recruitment and ethics

Participants volunteered for an observational study and were enrolled to one of 3 cohorts, healthy controls, cancer patients which presented with either melanoma or renal malignancies, or primary immunodeficient patients with defined mutations in key immunoregulatory genes, as well as patients with clinical presentation aligning to that of defined primary immunodeficiency, including antibody deficiency [Table 1]. Almost all participants in each cohort received BNT162b2 Pfizer vaccine, aside from one patient at second dose receiving AstraZeneca, and 6 patients receiving Moderna at the third [Table 1]. Patients were excluded if presenting with positive COVID-19 serology or if presenting in hospital with clinal symptoms/features related to their disease which may influence the physiological response to the COVID-19 vaccination. The research was conducted in accordance with the principles of Good Clinical Practice and following approved protocols of the NIHR National Bioresource. Samples were collected at NIHR Cambridge Clinical Research Facility, Addenbrookes Hospital, Cambridge, UK. Samples were provided with the written informed consent of all study participants under the NIHR National BioResource - Research Tissue Bank (NBR-RTB) ethics (research ethics committee (REC):17/EE/0025). All participants were consented under the East of England Cambridge South national consent (REC:13/EE/0325).

Participant sampling

Participant samples were anonymised by clinical staff prior to sample delivery to the research lab. Peripheral blood and fecal samples were collected longitudinally over the course of up to three doses of the vaccines against COVID-19 (sample coverage varied across doses [Supplementary Figure 1]). Samples were taken from 9th February 2021 to the 22nd February 2022. Peripheral blood mononuclear cells (PBMCs) were extracted from blood samples using density gradient centrifugation, stored temporarily at -80°C, before being transferred to long-term storage in liquid nitrogen. Serum was isolated from peripheral blood via centrifugation

114 and stored at -80°C until required. Fecal samples were collected at 3 timepoints around each
115 vaccine dose: pre-dose (94% of samples taken within 3 days prior to vaccination, the
116 remaining 3 samples taken up to 14 days prior), acute (day 2 or 3 after vaccination) or late
117 (day 16–28 after vaccination). Fecal matter was collected in OMNIgene•GUT kits (DNA
118 Genotek, Canada) whereby samples are stored in a stabilizing, inactivating solution. Samples
119 were transported to the lab and homogenized upon arrival before being stored at -80°C until
120 required.

121

122 ***Fecal DNA extraction and sequencing***

123 DNA was extracted from fecal samples using QIAamp® PowerFecal® Pro DNA kits (Qiagen).
124 Samples were thawed and approximately 250mg of fecal sample was lysed via bead beating.
125 According to the kit protocol, the sample was then cleaned of non-DNA organic and inorganic
126 material, then washed using ethanol. DNA was eluted into 10mM Tris and quantified using the
127 Qubit™ 1X dsDNA High Sensitivity (HS) Assay Kit (Thermo Fisher Scientific, UK) using the
128 Qubit™ fluorometer (Thermo Fisher Scientific, UK). DNA at a concentration of 10mg/uL was
129 sent for sequencing. Samples were sent in bulk for sequencing blinded to sample timepoints
130 of each patient. Shotgun metagenomic sequencing was performed with Illumina NextSeq 2000
131 sequencing platform using paired-end reads of 150bp in length, resulting in a median of
132 approximately 28 million reads per sample.

133

134 ***Shotgun metagenomic analysis***

135 Raw sequencing data was pre-processed with PRINSEQ++[15] (v.1.2.4) in paired read mode,
136 quality trimming to a minimal Phred score of 30 in a window of 15 bases and removing reads
137 of less than 75 bp length after trimming. Additionally, host contamination was removed by
138 mapping against the GRCh38 reference human genome using Bowtie2[16] (v.2.4.5) and
139 removing any mapped reads from the dataset.

140 Raw, trimmed and filtered reads were checked for quality using FastQC (v.0.11.9)
141 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). From the remaining read pairs,
142 taxonomic profiling was determined using mOTUs3[17] (v.3.0.1) profiler. For functional profiling,
143 the remaining read pairs after filtering were assembled using metaSPAdes[18] (v.3.15.4) with a
144 k-mer size of 55. The resulting scaffolds were filtered for at least 200 bp length and weighted
145 by their average coverage of the filtered reads to enable quantitative analysis. The remaining
146 scaffolds were aligned to the EggNOG database[19](downloaded on 2022/04/08) using
147 DIAMOND[20] (v.2.0.13). Microbiome analysis was performed in R (v.4.2.2) using relative
148 abundances, within the phyloseq[21] (v.1.42.0), microbiome (v.1.20.0)[22] and vegan[23] (v.2.6-4)
149 packages. Differential abundance analysis was performed using DESeq2[24] (v.1.38.3).

150

151 ***Serological assessment of immune response to COVID-19 vaccines***

152 Serum samples were thawed, heat-inactivated at 56°C for 30 mins and measured for the
153 dilution of serum that reduces viral activity by 50% (NT_{50}); the method for which has been
154 previously described[25]. For anti-SARS-CoV-2 specific IgG antibodies, we utilised a previously
155 described method[26,27], in which Luminex bead sets are covalently coupled to the recombinant
156 SARS-CoV-2 proteins nucleocapsid protein, spike and receptor-binding domain (RBD) to
157 quantify antibody levels.

158

159 ***Statistical analysis***

160 Wilcoxon tests with multiple testing correction using the bonferroni were deployed throughout,
161 using pairwise comparison where appropriate. Mixed effect linear modelling was performed
162 using lmer4[28] in R. Correlation was determined using Spearman's Rank coefficient.

Results

The composition of gut microbiome is not altered by vaccination against COVID-19

To investigate the impact of the COVID-19 vaccines on the gut microbiome, shotgun metagenomic sequencing was performed on a total of 239 fecal samples from 59 patients from our three cohorts (43 healthy control, 160 cancer and 36 primary immunodeficient patient samples) [Supplementary Figure 1]. Samples were taken over the course of 3 vaccine doses, at one of three vaccine timepoints: pre-dose (before vaccination), acute (2–3 days after vaccination) or late (16–28 days after vaccination) for each vaccine dose [Fig1A]. Pre-dose sampling provides a baseline assessment of the gut microbiome prior to vaccination, whilst acute samples provide the opportunity to assess the effect of vaccination on the gut microbiome at the height of the initial inflammatory response, and late samples allow a determination of any resolution or maintenance of acute effects.

When first observing the alpha diversities of all samples taken from each of our cohorts, we noted significant differences between the cohorts [Supplementary Figure 2A], however when assessing samples taken at different vaccine timepoints from within each cohort, we did not observe any significantly differences [Figure 1B]. In agreement with this, when assessing samples from the same patient at different vaccine timepoints using a paired sample analysis (tracking individual patient samples across multiple timepoints, e.g. pre-dose & acute) approach, we also did not see any significant differences in the alpha diversities of our patient samples [Supplementary Figure 2B][Supplementary Table 1]. This indicates that the COVID-19 vaccine is not affecting the diversity of the gut microbiome, despite the distinct microbial diversity between the cohorts and individuals.

We next applied principal component analysis (PCA) to visualize the beta-diversity of our microbiome composition data using the abundance of all detected operational taxonomic units (OTUs) [Fig1C]. The principal components (PCs) describe the largest variation components in the dataset, representing shifts in microbiome composition and potentially reflecting to the abundance changes of bacterial species between the samples. The first 5 principal

190 components were responsible for the 2.9%, 2.4%, 2.1%, 2.0% and 1.9% of variation in the
191 data respectively, and were further analysed using mixed effect linear models with multiple
192 input variables from our available metadata. In our linear models we asked whether the
193 vaccination timepoint of the samples in each cohort could improve the explained variance of
194 the PCs when compared to a baseline model describing the explained variance using patient
195 samples as the grouping variable. We found that there was no significant improvement on the
196 baseline model [Supplementary Figure 2C]. To further, a PERMANOVA analysis did not find
197 the covariate of the timepoints from which the samples were taken to be significantly affecting
198 the microbial composition [Supplementary Table 2]. These analyses together support that the
199 variance we see in our samples is not a signature of the COVID-19 vaccines, rather of the
200 patients presenting with different microbiome compositions themselves.

201 Next, we asked whether the COVID-19 vaccines induce any changes in the phylum-level
202 composition of the gut microbiome and profiled the relative abundance of phyla across all
203 samples, [Fig1D] observing variation in our patient samples. Moreover, when comparing the
204 top six most prevalent phyla, no significant differences were observed between vaccine
205 timepoints [Fig1E] despite significant differences in these phyla between cohorts and
206 individuals [Supplementary Figure 2D]. When using a paired analysis approach, we observed
207 only marginal, small effect size, differences (after multiple testing correction) in seven (out of
208 231) timepoint comparisons in the cancer cohort [Supplementary Figure 2E] [Supplementary
209 Table 3]. Of those 7, however, only one had a log2FC greater than 1 in either direction (+1 or
210 -1) (Supplementary Figure 2F), indicative of notable change in median relative abundance of
211 Lentisphaerae.

212 This demonstrates that the COVID-19 vaccines do not appreciably alter the composition of
213 the gut microbiome, irrespective of the unique compositions found in our cohort samples.

214

215 ***COVID-19 vaccination does not induce species level changes in the gut microbiome***

216 We next sought to analyse differentially abundant microbial species between vaccine
217 timepoints, i.e. pre-dose, acute, and late, using DESeq2[24]. All cohorts were analysed
218 independently for the abundance changes in samples taken at each timepoint with the most
219 differentially abundant species in a representative heatmap. For the cancer cohort, when
220 assessing the abundance of these top differential responding bacterial species between
221 samples taken pre-dose and acutely, unsupervised clustering does not demonstrate evident
222 grouping of timepoints [Fig2A]. Among all the species, only two were significantly increased in
223 acute samples compared to pre-dose samples, *Klebsiella pneumoniae* and *Butyrivibrio*
224 *crossotus* found in 15 [p = 1.01e-24] and seven samples [p = 7.63e-12] out of the 97 cancer
225 patient samples respectively [Fig2B]. The former is only representative in a quarter of the
226 cohort (11 patients), only melanoma patients, and within those has an average relative
227 abundance of 0.7% [Fig2C]; the latter in two renal cancer patients, representing on average
228 3% of the relative abundance [Fig2D]. Considering approximately 2500 species are
229 represented across all patient samples, change in two low-abundant and sparsely represented
230 species signifies negligible changes. Similar findings were seen for our other two cohorts,
231 healthy controls and primary immunodeficient patients [Supplementary Figure 3A and 3C, and
232 3B and 3D respectively]. When performing paired sample differential abundance analysis in
233 all three cohorts using DESeq2, we find that no significantly altered species between pre-dose
234 and acute, or pre-dose and late sample in the healthy control and cancer cohorts. Samples
235 from only one primary immunodeficient patient showed a significant reduction in *Enterobacter*
236 *sp.* in a late sample compared to pre-dose [Supplementary Figure 3E]. Thus, our findings
237 demonstrate that on a species level there is no unified, biologically relevant change in
238 abundance of microbial species induced by the COVID-19 vaccines.

239 As the differential abundance analysis considers the change in abundance of all species
240 irrespective of their relative abundance within each sample, we were curious whether there
241 were any noticeable changes in the most abundant species found within each patient cohort

242 that could be attributed to the vaccine timepoints. There was no significant difference in any
243 of the most abundant 15 species in samples taken at any of the three vaccine timepoints
244 [Fig2E], representing on average 47% of the relative abundance of the species within patient
245 samples in the cancer cohort, 50% within the healthy controls and 53% within the primary
246 immunodeficient patient cohort. This indicates that we see no effect of the vaccine on the
247 species occupying the highest proportion of the microbial niche.

248 There was considerable concern both at the time of the initial vaccine programme, and to this
249 day, on the safety of COVID-19 vaccines. Given that, we sought to observe if there was any
250 differential outgrowth of bacterial species that have been associated to various immune-
251 related diseases, including gastric cancer and autoimmunity, as well as metabolic and
252 neurological diseases. Although our study cannot address long-term outgrowth of bacterial
253 associated to these diseases, we are able to highlight if there is any temporal, acute changes
254 in these species which is still of physiological relevance. *Faecalibacterium prausnitzii,* which
255 is reported to be reduced in both gastric cancers, autoimmunity, and Crohn's disease[29],
256 showed no significant abundance changes at the vaccine timepoints in our cohorts [Fig2F].
257 We also found no presence of *Helicobacter pylori*, which is strongly associated with the
258 initiation and development of gastric cancers[30]. *Akkermansia muciniphila,* found to be
259 increased in obesity[31] and correlated with response rates to immune checkpoint blockade
260 therapies in various malignancies[32], was not altered by vaccination in all three cohorts [Fig2G].
261 In studies of Alzheimer's, *Escherichia coli* has been demonstrated to promote
262 neurodegeneration[33], in our samples we did not see significant difference induced by the
263 vaccine in any of our cohorts [Fig2H]. This supports that the COVID-19 vaccine does not
264 promote the change in abundance of microbes that are associated with various immune-
265 related diseases within any of the three cohorts and is indicative of no greater risk of the
266 aforementioned diseases as a result of COVID-19 vaccination.

267

268 Gut microbiome diversity is not correlated with the magnitude of the response to the COVID-19 vaccines

269 Within the current literature, a few studies have reported changes in the gut microbiome that
270 correlate with vaccine efficacy[12–14], so we sought to determine whether the gut microbiome
271 composition was related to the magnitude of the COVID-19 vaccine response. We performed
272 an assessment of vaccine efficacy using a live-virus neutralisation assay, as a predictive
273 measure of vaccine protection[32], to assess whether vaccine efficacy had correlation with
274 microbial diversity.

275 When taking the neutralising capacity of patient serum at both second dose [Fig3A] and third
276 dose [Fig3B], we asked whether the Shannon diversity of gut microbiome at different vaccine
277 timepoints, was affected by or correlated with neutralisation. We did not see any correlation
278 between diversity and vaccine efficacy in any of our patient cohorts; the same is true for the
279 quantity of anti-spike IgG antibodies [Supplementary Figure 4]. This indicates that the gut
280 microbiome diversity was not correlated with the magnitude of the immune response in our
281 patient cohorts, thus suggesting that improved efficacy of the vaccine does not come at a cost
282 of microbial disturbance.

283

284 ***The gut microbiome functional capacity was not affected by COVID-19 vaccines***

285 Having investigated the composition and relative abundance of the microbial species that
286 constitute the gut microbiome, we next sought to investigate whether the functional capacity
287 of the microbial species was altered by the COVID-19 vaccines. Using the EggNOG database,
288 we assigned functional annotations to the sequenced metagenomes. The highest level of
289 functional annotation depicts three functional groups, cellular processes and signalling,
290 information storage and processing, and metabolism. In these, we did not see any significant
291 differences between the vaccine timepoints within our cohorts [Fig4A]; similar to taxonomic
292 data presented earlier, when assessing cohort samples separately , there are significant
293 changes [Supplementary Figure 5A].

294 We next observed the abundance of the 22 defined functional groups in the next functional
295 annotation level down in the separate vaccine timepoints within each patient cohort [Fig4B].
296 Representative graphs of the most abundant functional annotations within each of the highest
297 three level functional levels remained unchanged after vaccination within our patient cohorts
298 [Fig4C], the same is true for the remaining 19 (Supplementary Figure 5B].

299 At the lowest functional annotation level, we interrogated the abundance of cluster of
300 orthologous genes (COGs) at separate vaccine timepoints within our cohorts. Remarkably, no
301 COGs out of a possible 2142 presented in our patient samples, were significantly altered
302 (when corrected for multiple testing) as a result of the COVID-19 vaccines in any of the three
303 cohorts. This demonstrates that the functional annotations of the gut microbiome are not
304 altered by the administration of the COVID-19 vaccines.

305

306 **Discussion**

307 To our knowledge, this study is the first to assess the gut microbiome composition in response
308 to the COVID-19 vaccines across multiple doses and at multiple timepoints with samples taken
309 pre-dose, acutely and late following vaccination. The study is also the first to assess the effect
310 of the COVID-19 vaccines on the gut microbiome in cancer patients and in patients with inborn
311 errors of immunity associated with severe immune dysregulation. As sampling across the
312 cohorts varied throughout, we opted to combine the three vaccine doses and assess vaccine
313 timepoints or samples from within each cohort. This allowed us to better observe the influence
314 of the COVID-19 vaccines in these contexts.

315 The relative abundance of microbes within the gut microbiome has more recently been
316 assessed with vaccine immunogenicity including that of vaccines against SARS-CoV-2 virus.
317 The first reported study of the gut microbiome in COVID-19 vaccinated patients correlated
318 vaccine immunogenicity of the inactivated virus, CoronaVac, and the mRNA vaccine encoding
319 spike protein, BNT162b2 vaccine, with the baseline abundance of gut *Bifidobacterium*

320 *adolescentis* and *Roseburia faecis* respectively[12], as well as noting shifts in microbiome
321 composition. We did not observe changes in these bacterial species, nor the composition, as
322 our study design was to look at change over vaccine doses individually rather all together. A
323 previous study investigated the variability of the gut microbiome response to the COVID-19
324 vaccine by correlating RNAseq data with microbial abundance using 16S rRNA gene amplicon
325 sequencing[14]. They identified several differentially abundant taxa between high- and low-
326 antibody responders and high- and low-T-cell responders. In the context of
327 immunocompromised cohorts, a previous study assessed patients with inflammatory bowel
328 disease[13] well known to be characterised by gut microbiome dysbiosis[34], who were receiving
329 anti-TNF immunomodulators. Their study did not demonstrate changes in diversity in above
330 geometric mean vaccine responders but found *Bilophila* abundance correlated to an improved
331 response. What these studies have in common is associating microbiome composition to
332 vaccine immunogenicity, however these studies analysed single post-vaccination time point
333 with potential for confounding effects of various lifestyle and other factors known to profoundly
334 affect microbiome composition such as diet, medications, sampling time of the day etc.[35–38].
335 Our multi-time-point study around individual vaccine dose allowed us to more robustly assess
336 the microbiome changes (or the lack thereof). As the patients enrolled in our study were not
337 all healthy, our cohort samples span a much wider and higher diversity compared to the other
338 study, and all our samples were taken in UK, it remains a possibility that country-specific
339 differences explain the variance in the findings across the studies.

340 In our patient cohorts, we did not find any significant effect on the diversity of the gut
341 microbiome following COVID-19 vaccination despite considerable differences between the
342 cohorts. In line with the studies investigating the gut microbiome of patients with primary
343 immunodeficiencies[39], we also observed decreased diversity in our cohort compared to control
344 samples. These patients have been demonstrated to exhibit increased gut permeability with
345 higher rates of bacterial translocation[40], perhaps indicative that there is bidirectional
346 permeability of systemic immunity affecting the gut microbiome. In our study, we find no

347 influence on microbiome variation after vaccination in patients at genetically determined
348 persistent state of immune dysregulation.

349 In melanoma patients, the presence of species bacteria species from the *Actinobacteria* and
350 *Firmicutes* phyla have been associated with better responses to immune checkpoint blockade
351 therapies[41–43]. An *Akkermansia muciniphilia* signature was also found in renal cancer patients
352 responding better to immune checkpoint blockade therapy[44,45]. While cancer progression is
353 reported to be linked to gut microbiome composition and its derived metabolites, these
354 associations vary between cancer types[46]. We saw wide compositional variation within our
355 cancer cohort samples, perhaps due to the wide range of disease presentation and treatment
356 included in our patients. Our study did not consider factors such diet and medications known
357 to affect the gut microbiome[47]. Nevertheless, this is not critical as our analysis suggests
358 stability as opposed to specific changes and post-hoc power calculation indicates sufficient
359 power against false negatives when taking paired samples (Pre-dose-Acute, Pre-dose-Late
360 and Acute-Late) (for effect size Cohen's d = 0.55, estimated power = 0.8). Cancer patients'
361 response to vaccination depend significantly on cancer type, for example antibody-related
362 immune responses in solid cancers are better than in haematological cancers[48]. It is therefore
363 notable that in an immunologically diverse cohort of individuals, with varied vaccine responses,
364 we did not observe any effect of the COVID-19 vaccination on the gut microbiome, indicating
365 stability irrespective of pre-dose composition.

366 While we did not observe changes at any taxonomic level or functional capacity, we cannot
367 rule out genetic changes at mutational levels that may alter the microbiota function. An
368 independent functional validation such as metabolomics to look for bacterial derived short-
369 chain fatty acids, tryptophan and bile acid metabolites known to mediate microbiome-host
370 interactions[49] could be utilised to assess this.

371 Sampling from the PID cohort was limited reflecting the rarity of the individuals within the
372 general population. Despite this, we were able to recruit patients characterised by monogenic
373 defects in both intrinsic and extrinsic B-cell aetiologies, thus representing multiple facets within

374 the rare PID population. Further, we were unable to sample all patients at all vaccine
375 timepoints across all vaccine doses. Nevertheless, our findings still bare relevance as we
376 assess patient cohorts individually and where possible the paired sample data analysis aligns
377 with the overall findings.

378 Although the global vaccination efforts have controlled the spread of the SARS-CoV-2 virus,
379 there were still reported to be 2.6 million new cases within the past month[1], highlighting that
380 prevention of disease through vaccination is still relevant for public health. Considering the
381 measurable impact of common life factors such as alcohol consumption, meat intake and
382 commonly used medications on the microbiota[49], our study finds that the vaccination has
383 negligible, if any, impact on microbiome-mediated processes. The contrast is even starker
384 when considering large microbiome changes have been reported for COVID-19 infection[10].
385 Our findings indicates that the gut microbiome remains stable post-vaccination and provides
386 an additional reassurance towards promoting vaccine uptake.

387

388 **Data availability**

389 Sequencing data will be uploaded to the European Genome-phenome Archive, whilst code
390 will be available on GitHub: https://github.com/RHBoston/COVID-19_Vaccination_GM.

391

392 **Acknowledgements**

393 We are grateful to the clinical staff at Addenbrookes hospital who facilitated patient recruitment
394 and sample collection, and the members of the Thaventhiran and Patil lab for help in
395 processing the samples.

396

397 **Author Information**

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Contributions

R.H.B performed sample collection and performed data analysis and interpretation of results. R.G., S.B., and L.K. performed data analysis and interpretation of results. E.C.H., N.B-C., J.C.Y-C., P.P.G., L.F., A.K., A.E.L., and S.B., performed sample collection and experimental assays, as well as providing technical support. A.C.-N., A.E., C.S., A.B., S.J., N.K., S.G., E.S. recruitment of participants of the study and sample collection. M.S.B., C.P., S.L., N.J.M., J.E.D.T. provided oversight on recruitment of participants, sample collection and experiments. V.B. provided technical support. R.H.B., R.G., J.E.D.T., and K.R.P prepared the figures and wrote the manuscript. R.H.B., J.E.D.T., and K.R.P designed and supervised the study. J.E.D.T., and K.R.P contributed equally.

Competing interests

The authors declare no competing interests.

Funding declaration

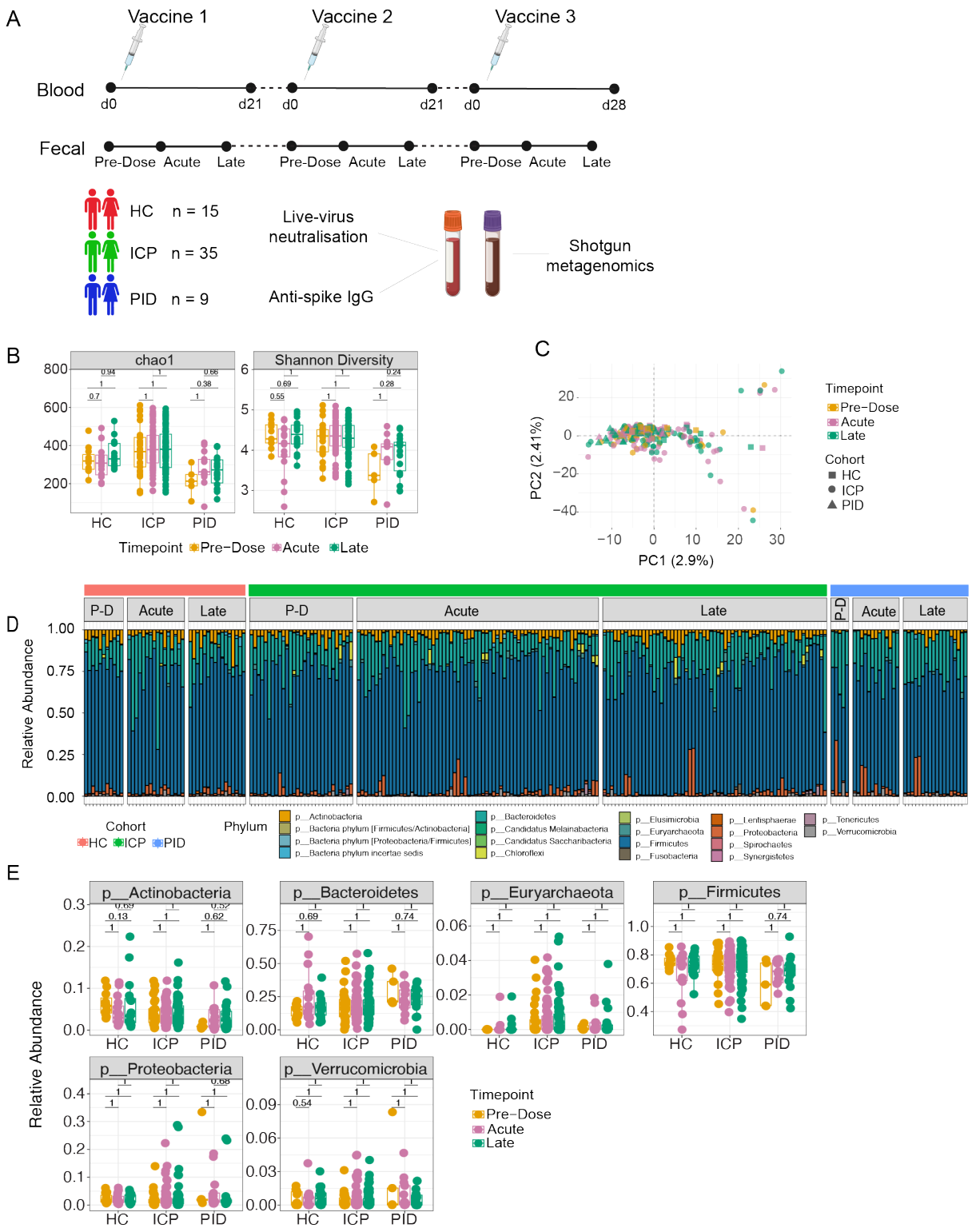
This work was funded by the UK Medical Research Council (project numbers MC_UU_00025/11 and MC_UU_00025/12).

421 **Table 1.** Characteristics of the participants in this study.

422

Cohort	*Participants*	*Age*	*Vaccine*	*Condition*	*Treatment*
Healthy Controls	6F 9M	28–59 Mean = 43.7	Vaccine doses, n = 20 90% Pfizer doses 10% Moderna doses	NA	NA
Immune checkpoint treated cancer patients (ICP)	9F 26M	39–86 Mean = 61.7	Vaccine doses, n = 70 97% Pfizer doses 3% Moderna doses	11 Metastatic Melanoma, 10 Adjuvant Melanoma, 5 Melanoma controls, 6 Metastatic Renal, 3 Renal controls	3 Nivolumab, 13 Pembrolizumab, 10 Ipilimumab + Nivolumab, 1 Ipilimumab + Pembrolizumab
Primary Immunodeficient patients (PID)	4F 5M	19–61 Mean = 41.1	Vaccine doses, n = 19 95% Pfizer doses 5% AstraZeneca doses	1 CD40L deficiency, 2 CTLA4 deficiency, 4 NFKB1 deficiency, 2 Undiagnosed condition	5 intravenous immunoglobulin, 3 Antibiotics

423

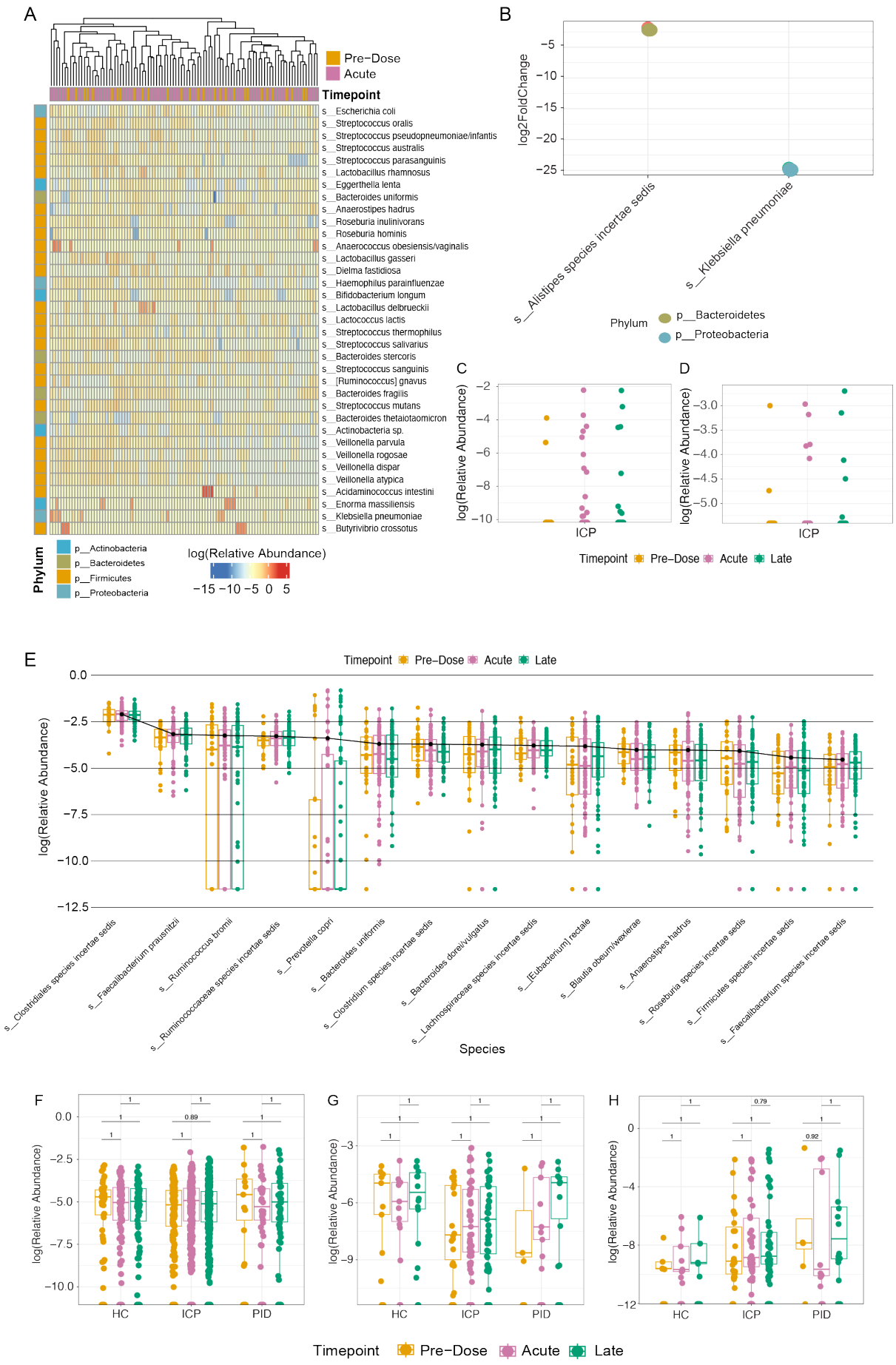

A
Vaccine 1
Vaccine 2
Vaccine 3
Blood
d0
d21
d0
d21
d0
d28
Fecal
Pre-Dose Acute Late
Pre-Dose Acute Late
Pre-Dose Acute Late
HC n = 15
ICP n = 35
PID n = 9
Live-virus neutralisation
Anti-spike IgG
Shotgun metagenomics
B
chao1
Shannon Diversity
HC ICP PID
Timepoint Pre−Dose Acute Late
C
PC2 (2.41%)
PC1 (2.9%)
Timepoint
Pre−Dose
Acute
Late
Cohort
HC
ICP
PID
D
Relative Abundance
P-D Acute Late
Cohort
HC ICP PID
Phylum
p__Actinobacteria
p__Bacteroidetes
p__Euryarchaeota
p__Firmicutes
p__Proteobacteria
p__Verrucomicrobia
E
Relative Abundance
p__Actinobacteria
p__Bacteroidetes
p__Euryarchaeota
p__Firmicutes
p__Proteobacteria
p__Verrucomicrobia
HC ICP PID
Timepoint
Pre−Dose
Acute
Late

424

425 ***Figure 1.*** *The composition of the gut microbiome remains unaltered following COVID-19 vaccination.*

426 *(A) 59 patients were recruited for longitudinal analysis of the effect of the vaccines against COVID-19.*
427 *Samples were assigned to one of three patient cohorts, healthy control (HC), immune-checkpoint*
428 *therapy treated cancer patients (ICP), or patients with primary immunodeficiencies (PID). Blood*
429 *samples were analysed for their live-virus neutralisation capacity and quantifying the amount of anti-*
430 *spike IgG antibodies, whilst fecal samples were analysed with shotgun metagenomics for taxonomic*
431 *and functional annotations. (B) Diversity measures of chao1 and Shannon assessed in fecal samples*
432 *taken from different vaccine timepoints, from within healthy control (HC), immune-checkpoint therapy*
433 *treated cancer patients (ICP) and patients with primary immunodeficiencies (PID). Statistical testing*
434 *performed using Wilcoxon test and adjusted for multiple testing using FDR correction. (C) Principal*
435 *component (PC) analysis at the operational taxonomic unit level. Each dot represents a unique sample*
436 *from within each cohort (shapes) taken at unique timepoints following vaccination (colours). (D) Relative*
437 *abundance at the phyla taxonomic level depicted by colours of each of the bars, from samples taken*
438 *from each of the cohorts (HC, ICP and PID), separated by the vaccine timepoints from which the sample*
439 *was taken; PD = Pre-Dose, Acute and Late. (E) Relative abundance of the 6 most prevalent phyla in*
440 *patient samples from within each of the cohorts and separated by the vaccine timepoint from which the*
441 *sample was taken. Statistical testing performed using Wilcoxon test and adjusted for multiple testing*
442 *using FDR correction. (N = 43 HC, 160 ICP, and 36 PID).*

443


A
Pre−Dose
Acute
Timepoint
s__Escherichia coli
s__Streptococcus oralis
s__Streptococcus pseudopneumoniae/infantis
s__Streptococcus australis
s__Streptococcus parasanguinis
s__Lactobacillus rhamnosus
s__Eggerthella lenta
s__Bacteroides uniformis
s__Anaerostipes hadrus
s__Roseburia inulinivorans
s__Roseburia hominis
s__Anaerococcus obesiensis/vaginalis
s__Lactobacillus gasseri
s__Dielma fastidiosa
s__Haemophilus parainfluenzae
s__Bifidobacterium longum
s__Lactobacillus delbrueckii
s__Lactococcus lactis
s__Streptococcus thermophilus
s__Streptococcus salivarius
s__Bacteroides stercoris
s__Streptococcus sanguinis
s__[Ruminococcus] gnavus
s__Bacteroides fragilis
s__Streptococcus mutans
s__Bacteroides thetaiotaomicron
s__Actinobacteria sp.
s__Veillonella parvula
s__Veillonella rogosae
s__Veillonella dispar
s__Veillonella atypica
s__Acidaminococcus intestini
s__Enorma massiliensis
s__Klebsiella pneumoniae
s__Butyrivibrio crossotus
Phylum
p__Actinobacteria
p__Bacteroidetes
p__Firmicutes
p__Proteobacteria
log(Relative Abundance)
−15 −10 −5 0 5
B
log2FoldChange
−5
−10
−15
−20
−25
s__Alistipes species incertae sedis
s__Klebsiella pneumoniae
Phylum
p__Bacteroidetes
p__Proteobacteria
C
log(Relative Abundance)
−2
−4
−6
−8
−10
ICP
D
log(Relative Abundance)
−3.0
−3.5
−4.0
−4.5
−5.0
ICP
Timepoint Pre−Dose Acute Late
E
Timepoint Pre−Dose Acute Late
log(Relative Abundance)
0.0
−2.5
−5.0
−7.5
−10.0
−12.5
s__Clostridiales species incertae sedis
s__Faecalibacterium prausnitzii
s__Ruminococcus bromii
s__Ruminococcaceae species incertae sedis
s__Prevotella copri
s__Bacteroides uniformis
s__Clostridium species incertae sedis
s__Bacteroides dorei/vulgatus
s__Lachnospiraceae species incertae sedis
s__[Eubacterium] rectale
s__Blautia obeum/wexlerae
s__Anaerostipes hadrus
s__Roseburia species incertae sedis
s__Firmicutes species incertae sedis
s__Faecalibacterium species incertae sedis
Species
F
log(Relative Abundance)
0.0
−2.5
−5.0
−7.5
−10.0
HC
ICP
PID
G
log(Relative Abundance)
−3
−6
−9
HC
ICP
PID
H
log(Relative Abundance)
0
−4
−8
−12
HC
ICP
PID
Timepoint Pre−Dose Acute Late

444

445 ***Figure 2.*** *Bacterial species demonstrate minimal change attributable to the COVID-19 vaccines.*

446 *(A) Differential abundance analysis using DESeq2 of relative abundance of the top 35 differential*
447 *species between samples taken at pre-dose (N = 29) and acutely (N = 69) after vaccination. (B) Log2*
448 *fold-change of the significant differential abundant species taken from the DESeq2 analysis. (C)*
449 *Relative abundance of Klebsiella pneumoniae* in ICP cohort samples. *(D) Relative abundance of*
450 *Butyrivibrio crossotus* in ICP cohort samples. (E) *Relative abundance of the top 15 abundant species*
451 *within the ICP cohort taken at each of the vaccine timepoints (N = 43 HC, 160 ICP, and 36 PID). Relative*
452 *abundance of various bacterial species correlated with immune-related diseases: Faecalibacterium*
453 *prausnitzii (F), Akkermansia muciniphila (G) and Escherichia coli (H) within patient samples taken at*
454 *each vaccine timepoint (N = 43 HC, 160 ICP, and 36 PID).*

455

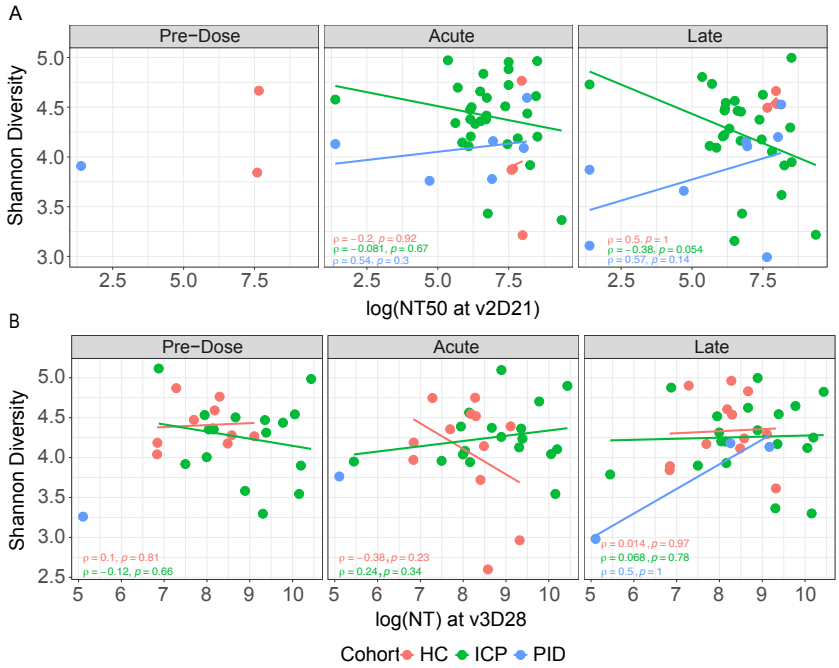
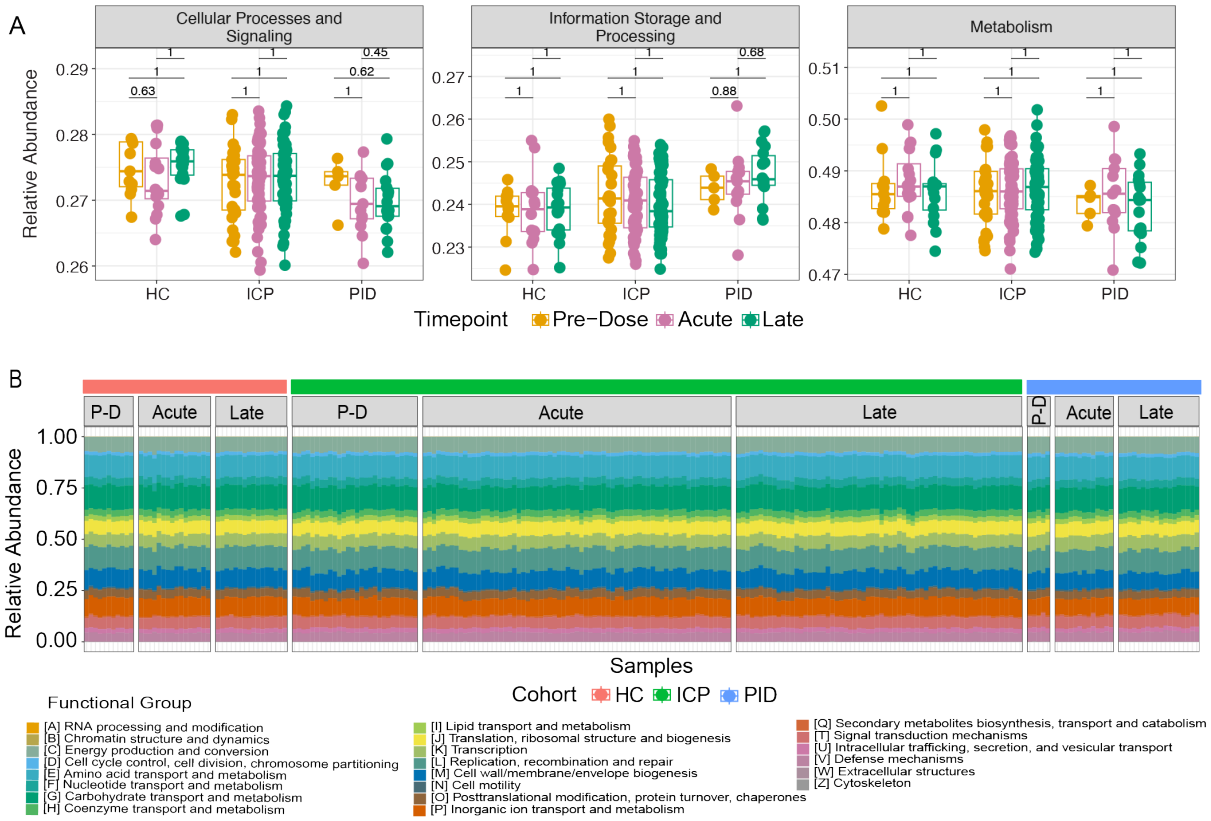


Figure 3. *Vaccine efficacy is not correlated with the gut microbiome diversity.*

Live-virus neutralisation (NT) capacity assessed against Shannon diversity of fecal samples, each point represents a different sample taken at one of the three vaccine timepoints. Colours represent cohorts, within healthy control (HC), immune-checkpoint therapy treated cancer patients (ICP) and patients with primary immunodeficiencies (PID). Correlated vaccine response through NT capacity of patient serum taken at second dose (N = 9 HC, 57 ICP, and 15 PID) (A) or third dose (N = 33 HC, 54 ICP, and 5 PID) (B). rho and p values from Spearman's Rank correlation testing displayed.


A
Cellular Processes and Signaling
Information Storage and Processing
Metabolism
Relative Abundance
HC
ICP
PID
Timepoint
Pre−Dose
Acute
Late
B
P-D
Acute
Late
Relative Abundance
Samples
Cohort
HC
ICP
PID
Functional Group
[A] RNA processing and modification
[B] Chromatin structure and dynamics
[C] Energy production and conversion
[D] Cell cycle control, cell division, chromosome partitioning
[E] Amino acid transport and metabolism
[F] Nucleotide transport and metabolism
[G] Carbohydrate transport and metabolism
[H] Coenzyme transport and metabolism
[I] Lipid transport and metabolism
[J] Translation, ribosomal structure and biogenesis
[K] Transcription
[L] Replication, recombination and repair
[M] Cell wall/membrane/envelope biogenesis
[N] Cell motility
[O] Posttranslational modification, protein turnover, chaperones
[P] Inorganic ion transport and metabolism
[Q] Secondary metabolites biosynthesis, transport and catabolism
[T] Signal transduction mechanisms
[U] Intracellular trafficking, secretion, and vesicular transport
[V] Defense mechanisms
[W] Extracellular structures
[Z] Cytoskeleton
C
[E] Amino acid transport and metabolism
[L] Replication, recombination and repair
[M] Cell wall/membrane/envelope biogenesis
Relative Abundance
HC
ICP
PID
Timepoint
Pre−Dose
Acute
Late

485 ***Figure 4.*** *Functional capacity of microbiome samples are not altered by the COVID-19 vaccines.*

486 *(A) The relative abundance of the highest functional annotation level using the EGGNOG database*
487 *within patient samples at different vaccine timepoints in each of our patient cohorts. (B) Functional*
488 *composition depicted by colours of each of the bars, from samples taken from each of the cohorts (HC,*
489 *ICP and PID), separated by the vaccine timepoints from which the sample was taken; PD = Pre-Dose,*
490 *Acute and Late. (C) Relative abundance of the 3 most abundant functional annotations in our patient*
491 *samples from within each of our patient cohorts and separated by the vaccine timepoint from which the*
492 *sample was taken. Statistical testing performed using Wilcoxon test and adjusted for multiple testing*
493 *using FDR (N = 43 HC, 160 ICP, and 36 PID).*

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