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. 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 immunosuppressive therapy. We find that the gut microbiome, assessed using shotgun metagenomics, remained stable post-vaccination irrespective of diverse immune status, vaccine response, and microbial composition spanned by the cohort. The stability is evident at all tested levels including phylum, species, and functional capacity. Our results show 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.

Introduction

As of 9th July 2023, there have been reported almost 800 million cases of COVID-19 infection and approaching 7 million related deaths globally[1]. To reduce this severity, vaccines were deployed with the aim of promoting anti-SARS-CoV-2 immunity, with almost 13.5 billion vaccine doses administered globally, 150million of which administered in the United Kingdom[1].

The SARS-CoV-2 mRNA and viral vector vaccines induce a strong immune response through the promotion of spike mRNA translation in host cells, resulting in the expression of spike protein on their cell surface. The spike protein is recognised by either antigen-presenting cells (APCs), which recognise spike antigens and are activated, inducing the migration to draining lymph nodes where these cells can present spike antigens to T- and B-cells resulting in antibody production, or by B-cells themselves with the cognate receptor for the spike protein, ultimately both resulting in antibody production[2]. Although sequestration of the spike mRNA by antigen-presenting cells aims to limit the spread into systemic circulation, SARS-CoV-2 spike protein can also be found in blood plasma[3], potentially leading to inflammation at different sites of the body other than the site of vaccination.

Although numerous studies have analysed the gut microbiome during COVID-19 infection, since the initiation of the vaccination programme against COVID-19, comparably few studies have addressed the impact of the vaccination on the gut microbiome[5]. The previous work has addressed the link between the gut microbiome and vaccine immunogenicity[6,7,8]. Primarily, baseline abundances of species before the first vaccine dose have been correlated with a defined end point of vaccine efficacy, typically a vaccine-related readout, such as virus neutralisation or spike-specific antibody titres.

Yet, an open question is how the gut microbiome is affected by COVID-19 vaccination in the first few days following vaccination, as well as elucidating whether any immediate changes in the gut microbiome are maintained or resolved over time. Addressing this knowledge-gap could help understanding the extent and the nature of reciprocal links between the gut microbiome and systemic immunity in the context of vaccination. We therefore sought to analyse the gut microbiome of patients receiving doses of the COVID-19 mRNA vaccine to decipher whether there were any notable, characteristic changes in the gut microbiome in either healthy or immunocompromised individuals [Table 1]; the gut microbiomes in these cohorts are often characterised by distinct compositions when compared to healthy controls.

The immunocompromised patients recruited were either cancer patients receiving immune checkpoint modulating therapies (ICP) or patients with primary immunodeficiencies (PID). These cohorts thus consist of patients with impaired immunity resulting from either therapeutic intervention for the ICP cohort, or patients with inborn errors of immunity with monogenic defects in immunoregulatory genes for the PID cohort. This therefore also presented us with the unique opportunity to elucidate whether the COVD-19 vaccines alter the gut microbiome in the absence of complete, functional immunity and subsequent impairments of the regulation of the gut microbiome.

Methods

77 ***Patient recruitment and ethics***

78 Patients were enrolled and placed into one of 3 cohorts, healthy controls (HCs), cancer
79 receiving either monotherapy or combination therapy of immune checkpoint inhibitors (ICP),
80 or PID patients with defined mutations in key immunoregulatory genes (CTLA4, NFKB1,
81 CD40L) as well as patients with clinical presentation aligning to that of defined PID, including
82 antibody deficiency, termed as undefined PID [Table 1]. The research was conducted in
83 accordance with the principles of Good Clinical Practice and following approved protocols of
84 the NIHR National Bioresource. Samples were collected with the written informed consent of
85 all study participants under the NIHR National BioResource - Research Tissue Bank (NBR-
86 RTB) ethics (REC:17/EE/0025) or under the Barts and the London Immunology Registry
87 (REC:361 11/LO/1689). The patients and healthy controls were consented under the East of
88 England Cambridge South national research ethics committee (REC) reference 13/EE/0325
89 or Barts and the London Immunology Registry (REC: 11/LO/1689).

90 ***Patient sampling***

91 Peripheral blood and fecal samples for were collected longitudinally over the course of 3 doses
92 of the vaccines against COVID-19 (sample and patient coverage varied across doses).
93 Peripheral blood mononuclear cells (PBMCs) were extracted from blood samples using
94 density gradient centrifugation, stored temporarily at -80°C, before being transferred to long-
95 term storage in liquid nitrogen. Serum was isolated from peripheral blood via centrifugation
96 and stored at -80°C until required. Fecal samples were collected at 3 timepoints around each
97 vaccine dose: pre-dose, acute (day 2 or 3 after vaccination) or late (day 16–28 after
98 vaccination). Fecal matter was collected in OMNIgene•GUT kits (DNA Genotek, Canada)
99 whereby samples are stored in a stabilizing, inactivating solution. Samples were transported
100 to the lab and homogenized upon arrival before being stored at -80°C until required.

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

102 Serum samples were thawed, heat-inactivated at 56°C for 30 mins and measured for the
103 dilution of serum that reduces viral activity by 50% (NT_{50}); the method for which has been
104 previously described[9]. For anti-SARS-CoV-2 specific IgG antibodies, we utilised a previously
105 described method[10,11], in which Luminex bead sets are covalently coupled to the recombinant
106 SARS-CoV-2 proteins nucleocapsid protein (NCP), spike (S) and receptor-binding domain
107 (RBD) to quantify antibody levels.

108 ***Fecal DNA extraction and sequencing***

109 DNA was extracted from fecal samples using QIAamp® PowerFecal® Pro DNA kits (Qiagen).
110 Samples were thawed and approximately 250mg of fecal sample was lysed via bead beating.
111 According to the kit protocol, the sample was then cleaned of non-DNA organic and inorganic

material, then washed using ethanol. DNA is eluted into 10mM Tris and quantified using the Qubit™ 1X dsDNA High Sensitivity (HS) Assay Kit (Thermo Fisher Scientific, UK) using the Qubit™ fluorometer (Thermo Fisher Scientific, UK). DNA at a concentration of 10mg/uL was sent for sequencing. Shotgun metagenomic sequencing was performed using Illumina NextSeq 2000 sequencing technology using paired end reads of 150bp in length.

Shotgun metagenomic analysis

Raw sequencing data was pre-processed with PRINSEQ++[12] in paired read mode, quality trimming to a minimal PHRED score of 30 in a window of 15 bases and removing reads of less than 75 bp length after trimming. Additionally, host contamination was removed by mapping against the HG38 human database using Bowtie2[13] v2.4.5 and removing any mapped reads from the dataset. Raw, trimmed and filtered reads were checked for quality using FastQC[14]. From the remaining read pairs, taxonomic profiling was determined using mOTUs3[15] profiler, whilst for functional profiling, the remaining read pairs after filtering were assembled using metaSPAdeS[16] with a k-mer size of 55. The resulting scaffolds were filtered for at least 200 bp length and weighted by their average coverage of the filtered reads to enable quantitative analysis. The remaining scaffolds were aligned to the EGGNOG database[17] (downloaded on 2022/04/08) using DIAMOND version 2.0.13. Microbiome analysis was performed in R using phyloseq[18] and vegan[19] packages. Differential abundance analysis was performed using DESeq2[20].

Statistical analysis

Wilcoxon tests with multiple testing correction using the false discovery rate were deployed throughout. Correlation was determined using Spearman rank.

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 our three patient cohorts (43 HC, 160 ICP and 36 PID samples) 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].

145 Samples taken at different vaccine timepoints from within each cohort were not significantly
146 different [Figure 1B], however we noted significant diversity differences between the cohorts
147 [Supplementary figure 1A]. This indicates that despite the distinct microbial diversity between
148 the patient cohorts, the COVID-19 vaccine is not affecting the diversity of the gut microbiome.

149 We next used principal component analysis to reduce the dimensionality of our microbiome
150 composition data (starting with a very high dimension dataset including the abundance of all
151 detected operational taxonomic units (OTUs)) [Fig1C]. The first 5 principal components that
152 were responsible for the 2.9%, 2.4%, 2.1%, 2.0% and 1.9% of variation in the data
153 respectively, were further analysed by using linear models with multiple input variables from
154 our available metadata. The principal components describing the largest variation components
155 in the dataset representing shifts in microbiome composition, potentially reflecting to the
156 parallel abundance changes of multiple bacterial species between the samples. In our linear
157 models we asked if either the cohort and/or the vaccination timepoint association of the
158 samples have effect(s) on the value of the principal component. We found that the cohort
159 grouping has significant effect on PC1, PC2, PC3 and PC5 while none of these major principal
160 components were dependent on the timepoints in which the samples were taken. This
161 suggests that the variance we see in our samples is not a signature of the COVID-19 vaccines,
162 rather those of the unique cohorts presenting with different microbiome compositions.

163 Next, we asked whether the COVID-19 vaccines induce any changes in the phylum-level
164 composition of the gut microbiome, we observed the relative abundance of taxa at a phylum
165 level across all samples [Fig1D]. There were no significant differences observed when
166 comparing samples at the three vaccine timepoints within each of our three cohorts in the top
167 6 most prevalent phyla [Fig1E], this is despite significant differences in these phyla between
168 cohorts [Supplementary figure 1B]. This demonstrates that the COVID-19 vaccines do not
169 alter the composition of the gut microbiome irrespective of the unique compositions found in
170 our cohort samples.

171

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

173 We next sought to analyse differentially abundant microbial species between vaccine
174 timepoints using DESEq2. All cohorts were analysed independently for the abundance
175 changes in samples taken at each vaccine timepoint with the top 35 differentially abundant
176 species presented in a representative heatmap of log2 fold-change in abundance. The ICP
177 cohort demonstrates that when assessing the change in species abundance between samples
178 taken pre-dose and acutely, unsupervised clustering does not demonstrate clustering based
179 on the top 35 bacterial species [Fig2A]. Two species that were significantly increased in acute

180 samples compared to pre-dose samples [Fig2B], *Klebsiella pneumoniae* and *Butyrivibrio*
181 *crossotus* found in 11 and 5 samples [p = 1.01e-24 and 7.63e-12 respectively]. The former is
182 only representative in a quarter of the cohort, only melanoma patients, and within those has
183 an average relative abundance of 0.7% [Figure 2C]; the latter in 2 renal cancer patients,
184 representing on average 3% of the relative abundance. This indicates that we see minimal
185 changes in the relative abundance of bacterial species within the patient samples considering
186 over 2600 species are represented across all our patient samples, and that the changes we
187 do see are attributable to species that are not represented by the entire cohort and that
188 represent a fraction of the microbial niche. Similar findings were seen for our other two cohorts,
189 HC and PID [Supplementary figure 2], indicating that on a species level there is no unified,
190 biologically relevant change in abundance of microbial species induced by the COVID-19
191 vaccines.

192 As the differential abundance analysis tool DESEq2 considers the change in abundance of all
193 species irrespective of their relative abundance within each sample, we were curious whether
194 there were any noticeable changes, falling below DESEq2 threshold, in the abundance of the
195 top 15 most abundant species found within each patient cohort that could be attributed to the
196 vaccine timepoints. There was no significant difference in any of the 15 species in samples
197 taken at any of the three vaccine timepoints [Figure 2D]. This indicates that we see no effect
198 of the vaccine on the species occupying the highest proportion of the abundant microbial
199 species. Thus, on a species level there is no unified, biologically relevant change in abundance
200 of microbial species induced by the COVID-19 vaccines.

201 There was considerable concern both at the time of the vaccine programme, and to this day,
202 on the safety of COVID-19 vaccines. Given that, we sought to observe if there was any
203 differential outgrowth of species that have been associated to various immune-related
204 diseases, including gastric cancer and autoimmunity, as well as metabolic and neurological
205 diseases. When observing the abundance of *Faecalibacterium prausnitzii* found to be reduced
206 in both gastric cancers, autoimmunity, and Crohn's disease[21], we found no significant
207 abundance changes at the vaccine timepoints in our cohorts [Fig2E]. We also found no
208 presence of *Helicobacter pylori*, which is strongly associated with the initiation and
209 development of gastric cancers[22]. *Akkermansia muciniphila,* found to be increased in
210 obesity[23], was not altered [Fig2F]. In studies of Alzheimer's, *Escherichia coli* has been
211 demonstrated to promote neurodegeneration[24], in our samples we did not see significant
212 difference induced by the vaccine in any of our cohorts [Fig2G]. This supports that the COVID-
213 19 vaccines does not promote the change in abundance of microbes that are negatively
214 associated with various immune-related diseases from within our patient cohorts.

215 ***The magnitude of the response to the BNT162b2 vaccine is not correlated with microbiome***
216 ***diversity***

217 Within the current literature, a few studies have reported changes in the gut microbiome that
218 correlate with vaccine efficacy[5,6,7], so we sought to determine whether the gut microbiome
219 composition was influenced by the magnitude of the BNT162b2 vaccine response. We
220 performed a gold-tier assessment of the vaccine response using a live-virus neutralisation
221 assay to assess whether patients with great vaccine efficacy had any correlation with the
222 microbial diversity. When taking the neutralising capacity (NT50) of patient serum at both
223 second dose [Fig3A] and third dose [Fig3B], we asked whether the Shannon diversity at
224 different vaccine timepoints, correlated with neutralisation. We did not see any correlation of
225 diversity with vaccine efficacy in any of our patient cohorts; the same is true for the quantity of
226 anti-spike IgG antibodies [Supplementary figure 3]. This indicates that the magnitude of the
227 immune response was not correlated with the gut microbiome diversity in our patient cohorts.

228 ***The microbiome gene functional annotations were not affected by COVID-19 vaccines***

229 Having investigated the composition and relative abundance of the microbial species that
230 constitute the gut microbiome, we next sought to investigate whether the functional capacity
231 of the microbial species was altered by the COVID-19 vaccines. Using the EGGNOG
232 database, we assigned functional annotations to the sequenced metagenomes. The highest
233 level of functional annotation depicts three functional groups, cellular processes and
234 signalling, information storage and processing, and metabolism. In these, we did not see any
235 significant differences between the vaccine timepoints within our cohorts [Fig4A]; similarly to
236 taxonomic data presented earlier, when combining samples from within the same cohorts,
237 there are significant changes.

238 We next observed the abundance of the 22 defined functional groups in the next functional
239 annotation level down in the separate vaccine timepoints within each patient cohort [Fig4B].
240 Representative graphs of the most abundant functional annotations within each of the highest
241 three level functional levels remained unchanged after vaccination within our patient cohorts
242 [Fig4C], the same is true for the remaining 19.

243 At the lowest functional annotation level, we interrogated the abundance of cluster of
244 orthologous genes (COGs) at separate vaccine timepoints within our cohorts. Remarkably,
245 only two COGs were significantly different as a result of the COVID-19 vaccines, in HC
246 samples COG2243 Precorrin-2 c20-methyltransferase between acute and late vaccine
247 samples (padj value = 0.04 [FDR]), and COG4750 cytidylyltransferase choline kinase between
248 pre-dose and late samples in ICP patients (padj value = 0.03 [FDR]); not a single COG was
249 found to be significantly altered in PID patient samples as a result of the vaccine. This

250 demonstrates that the functional annotations of the gut microbiome are not practically
251 unaltered by the administration of the COVID-19 vaccines.

252 **Discussion**

253 To our knowledge, this study is the first to assess the gut microbiome composition in response
254 to the COVID-19 vaccines across multiple doses and at multiple timepoints after each vaccine
255 dose, with samples take pre-dose, acutely and late following vaccination. The study is also
256 the first to assess the effect of the COVID-19 vaccines on the gut microbiome in cancer
257 patients and the rare population of individuals born with inborn errors of immunity. As sampling
258 across the cohorts varied throughout, we opted to combine the three vaccine doses and
259 assess vaccine timepoints or samples from within each cohort. This allowed us to better
260 observe the influence of the COVID-19 vaccines in these contexts.

261 The relative abundance of microbes within the gut microbiome has more recently been
262 assessed with vaccine immunogenicity including that of vaccines against SARS-CoV-2 virus.
263 The first reported study of the gut microbiome in COVID-19 vaccinated patients correlated
264 vaccine immunogenicity of the inactivated virus, CoronaVac, and the messenger RNA vaccine
265 encoding spike protein, BNT162b2 vaccine, with the baseline abundance of gut
266 *Bifidobacterium adolescentis* and *Roseburia faecis* respectively[5]. Another study investigated
267 the variability of the COVID-19 vaccine response to the gut microbiome by correlating RNAseq
268 data with microbial abundance using 16S amplicon sequencing[7]. They identified several
269 differentially abundant taxa between high- and low-antibody responders and high- and low-T-
270 cell responders. In the context of immunocompromised cohorts, a previous study assessed
271 patients with inflammatory bowel disease[6] well known to be characterised by gut microbiome
272 dysbiosis[25], who were receiving anti-TNF immunomodulators. This study did not demonstrate
273 changes in diversity in above geometric mean vaccine responders but found *Bilophia*
274 abundance correlated to an improved response. They also assessed the fecal metabolome
275 showing that various metabolites were associated with a better vaccine response; several
276 metabolites were also associated with a reduced response. What these studies have in
277 common is associating microbiome composition to vaccine immunogenicity, however these
278 studies have not addressed temporal changes that can be seen in the gut microbiome,
279 whereas our longitudinal analysis affords us the opportunity to do so.

280 In our patient cohorts, we did not find any significant effect on the diversity of the gut
281 microbiome following COVID-19 vaccination despite considerable differences between the
282 cohorts. In line with the studies investigating the gut microbiome of PID patients[26], we also
283 observed decreased diversity in our cohort compared to HC samples. These patients have
284 been demonstrated to exhibit increased gut permeability with higher rates of bacterial

285 translocation[27], perhaps indicative that there is bidirectional permeability of systemic immunity
286 affecting the gut microbiome. With regards to vaccination, PID patients are reported to respond
287 poorly due to their antibody deficiencies; while able to mount some form of protective antibody
288 responses, it is often not durable [28,29,30]. Further, in live vaccines there is evidence of induction
289 of severe disease[26]. In our study, we find no influence on microbiome variation after
290 vaccination in our patient samples.

291 In melanoma patients, various bacteria species from the *Actinobacteria* and *Firmicutes* phylum
292 have been associated with better responses to immune checkpoint blockade therapies[27,28,29]
293 An *Akkermansia muciniphilia* signature was also found in renal cancer patients responding
294 better to immune checkpoint inhibitors (ICIs)[35,36]. While cancer progression is reported to be
295 linked to gut microbiome composition and its derived metabolites, these associations vary
296 between cancer types[37]. We saw wide compositional variation within our ICP cohort samples,
297 perhaps due to the wide range of disease presentation and treatment included in our patients.
298 Our study cannot address the influence of the non-immune modulating drugs taken by the ICP
299 patients. The patients who undergo both chemotherapy and immunotherapy will typically be
300 administered a multitude of immunomodulators to aid their prevention of disease and infection.
301 We are not able to rule out the influence of these drugs at this time, known to affect the gut
302 microbiome[38]. Cancer patients' response to vaccination depend significantly on cancer type,
303 for example antibody-related immune responses in solid cancers are better than in
304 haematological cancers[39], perhaps indicative of their gut microbiome compositional
305 differences[40]. It is therefore notable that in such a varied cohort of individuals, we did not
306 observe any effect of the COVID-19 vaccination, showing stability of the gut microbiome
307 irrespective of pre-dose composition.

308 While we did not observe any changes also in the functional capacity at the metagenomic
309 level, further experimental validation using a metabolomics approach may be useful. Bacterial-
310 derived short-chain fatty acids (SCFAs), tryptophan and bile acid (BAs) metabolites have the
311 capacity to activate or suppress the immune system[41]. For example, SCFAs have been shown
312 to reduce APC activation[42], whilst BA metabolites prevent APC maturation[43] and induce B-cell
313 apoptosis[44] and prevent antibody production[45], both essential immune cell types within the
314 promotion of a vaccine response. It would therefore be of importance to correlate the predicted
315 functional annotations of the bacterial species found within our patient samples, with the
316 metabolites found with the patients' fecal samples to draw a more definitive conclusion on
317 whether the COVID-19 vaccines induced a biologically relevant changes in the gut
318 microbiome.

319 Considering limitations of our study, sampling from the PID cohort was limited reflecting the
320 rarity of the individuals within the general population. Despite this, we were able to recruit

321 patients from characterised by monogenic defects in both intrinsic and extrinsic B-cell
322 aetiologies, thus representing multiple facets within the rare PID population. We could not
323 account for known confounding factors like diet due to the lack of the metadata; however, this
324 is likely to be less critical since we observe considerable stability over an extended duration.
325 Further, we were unable to sample all patients at all vaccine timepoints across all vaccine
326 doses. Nevertheless, our findings still bare relevance as we assess patient cohorts individually
327 and where possible the paired sample data analysis aligns with the overall findings.

328 Although the global vaccination efforts have controlled the spread of the SARS-CoV-2 virus,
329 there were still reported to be 2.6 million new cases within the past month[1], highlighting that
330 management of disease through vaccination is still relevant for public health. Our findings
331 provide evidence that that the gut microbiome remains stable post-vaccination suggesting
332 minimal if any impact on microbiome-mediated processes.

333

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338

339 **Competing interests**

340 The authors declare no competing interests.

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437

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

Healthy Controls (HC)	
Participants	***Age***
6F 9M	28–59

Immune checkpoint treated cancer patients (ICP)			
Participants	***Age***	***Disease***	***Treatment***
9F 26M	39–86	11 Metastatic Melanoma 10 Adjuvant Melanoma 5 Melanoma controls 6 Metastic Renal 3 Renal controls	3 Nivolumab 13 Pembrolizumab 10 Ipilimumab + Nivolumab 1 Ipulumumab + Pembrolizumab

Primary Immunodeficient patients (PID)			
Participants	***Age***	***In-born error of immunity***	***Treatment***
4F 5M	19–61	1 CD40L 2 CTLA4 4 NFKB1 2 Undiagnosed	5 IvIg 3 Antibiotics

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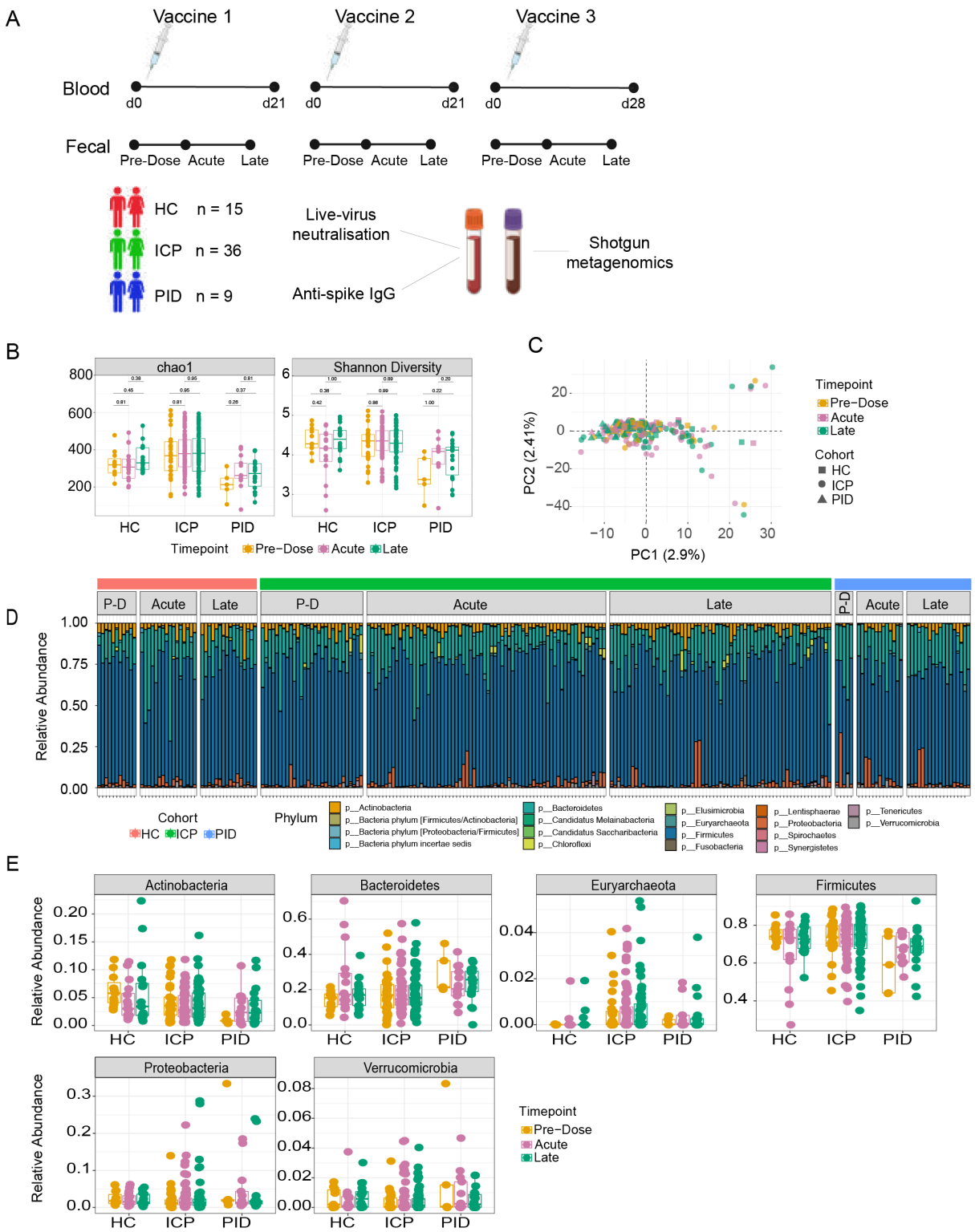
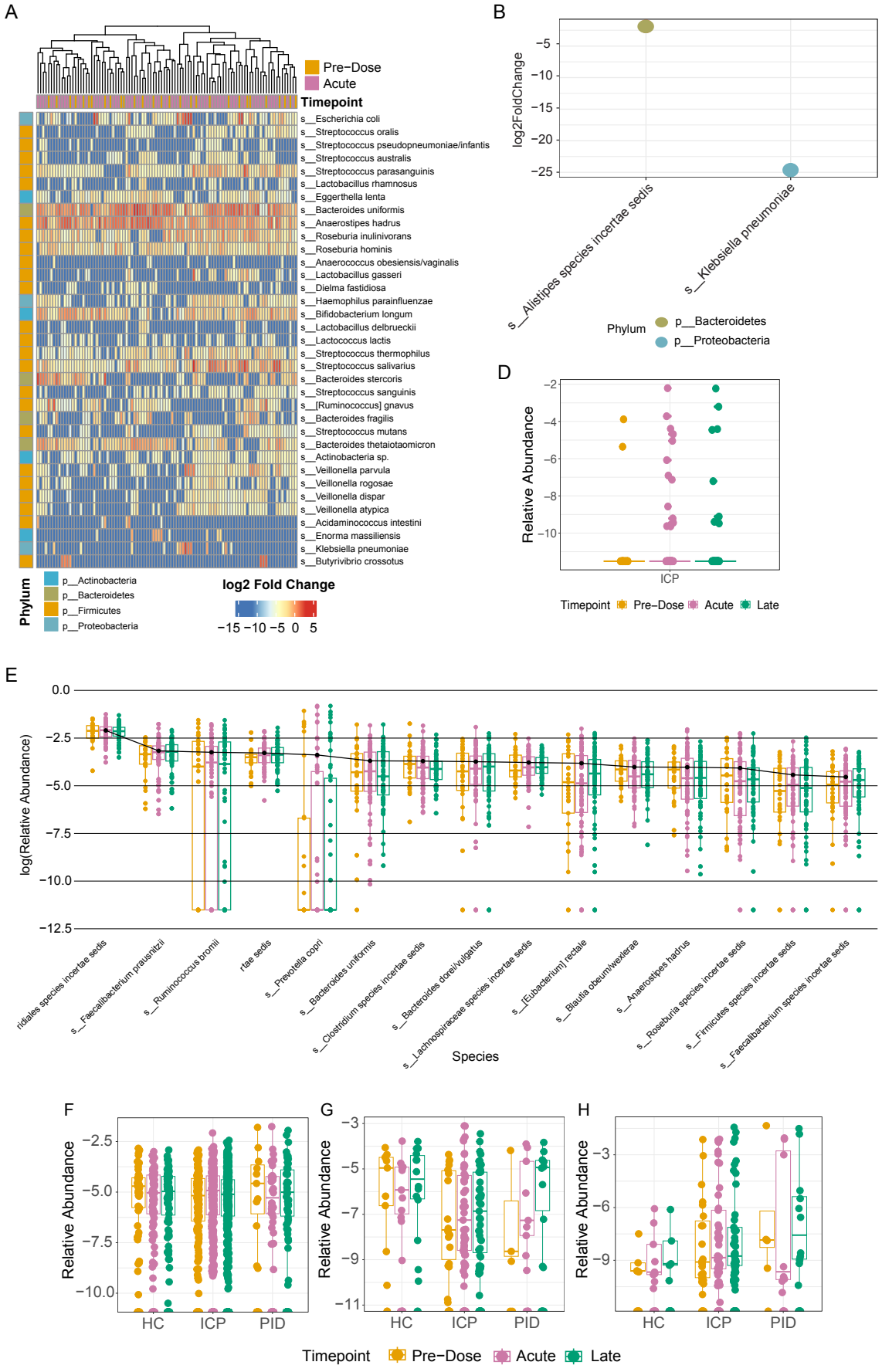


Figure 1. *(A) Study design. 60 patients were recruited for longitudinal analysis of the effect of the vaccines against COVID-19. Samples were assigned to one of three patient cohorts, healthy control (HC), immune-checkpoint therapy treated cancer patients (ICP), or patients with primary immunodeficiencies (PID). Blood samples were analysed for their live-virus neutralisation capacity and quantifying the amount of anti-spike IgG antibodies, whilst fecal sample were analysed with shotgun metagenomics for taxonomic and functional annotations. (B) Diversity measures of chao1 and Shannon*

assessed in fecal samples taken from different vaccine timepoints, from within healthy control (HC), immune-checkpoint therapy treated cancer patients (ICP) and patients with primary immunodeficiencies (PID). Statistical testing performed using Wilcoxon test and adjusted for multiple testing using FDR correction. (C) Principal component (PC) analysis at the operational taxonomic unit level. Each dot represents a unique sample from within each cohort (shapes) taken at unique timepoints following vaccination (colours). (D) Phylum composition depicted by colours of each of the bars, from samples taken from each of the cohorts (HC, ICP and PID), separated by the vaccine timepoints from which the sample was taken; PD = Pre-Dose, Acute and Late. (E) Relative abundance of the 6 most prevalent phyla in patient samples from within each of the cohorts and separated by the vaccine timepoint from which the sample was taken. Statistical testing performed using Wilcoxon test and adjusted for multiple testing using FDR correction.


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
log2 Fold Change
−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
D
Relative Abundance
−2
−4
−6
−8
−10
ICP
Timepoint
Pre−Dose
Acute
Late
E
log(Relative Abundance)
0.0
−2.5
−5.0
−7.5
−10.0
−12.5
ridiales species incertae sedis
s__Faecalibacterium prausnitzii
s__Ruminococcus bromii
rtae 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
Relative Abundance
−2.5
−5.0
−7.5
−10.0
HC
ICP
PID
G
Relative Abundance
−3
−5
−7
−9
−11
HC
ICP
PID
H
Relative Abundance
−3
−6
−9
HC
ICP
PID
Timepoint
Pre−Dose
Acute
Late

464 ***Figure 2.*** *Representative analysis of the ICP cohort demonstrates minimal change attributable to the*
465 *COVID-19 vaccines. (A) Differential abundance analysis using DESeq2 of the log2 fold-change of the*
466 *top 35 differential species between samples taken at pre-dose and acutely after vaccination. (B) Log2*
467 *fold-change of the significant differential abundant species taken from the DESeq2 analysis. (C)*
468 *Relative abundance of Klebsiella pneumoniae* in ICP cohort samples. *(D) Relative abundance of the*
469 *top 15 abundant species within the ICP cohort taken at each of the vaccine timepoints. Relative*
470 *abundance of various bacterial species correlated with immune-related diseases: Faecalibacterium*
471 *prausnitzii (E), Akkermansia muciniphila (F) and Escherichia coli (G) within patient samples taken at*
472 *each vaccine timepoint.*

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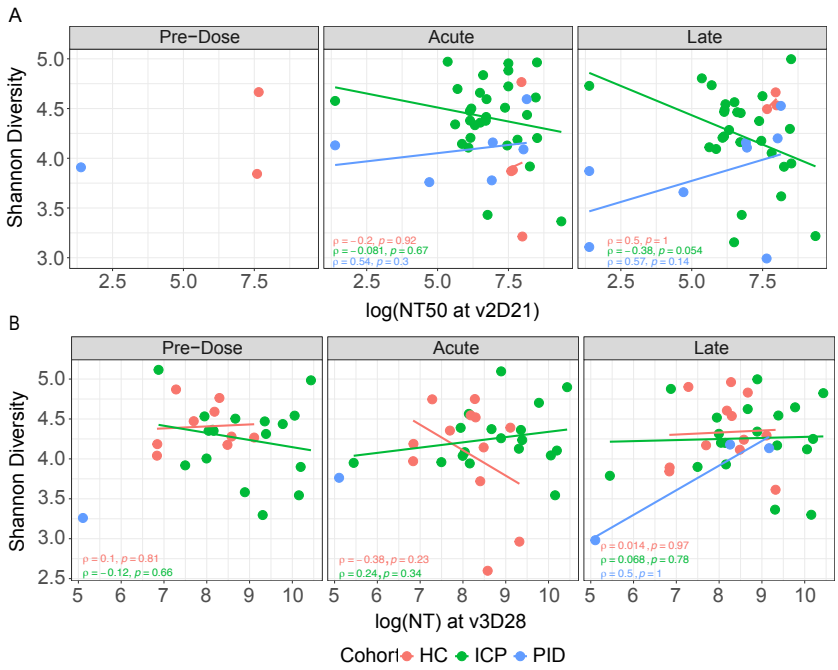


Figure 3. *Vaccine efficacy is not correlated with the gut microbiome composition. Live-virus neutralisation (NT) capacity assessed against Shannon diversity of fecal samples, each point represents a different sample taken at one of the three vaccine timepoints. Colours represent cohorts, within healthy 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 (A) or third dose (B). rho and p values from Spearman correlation testing displayed.*

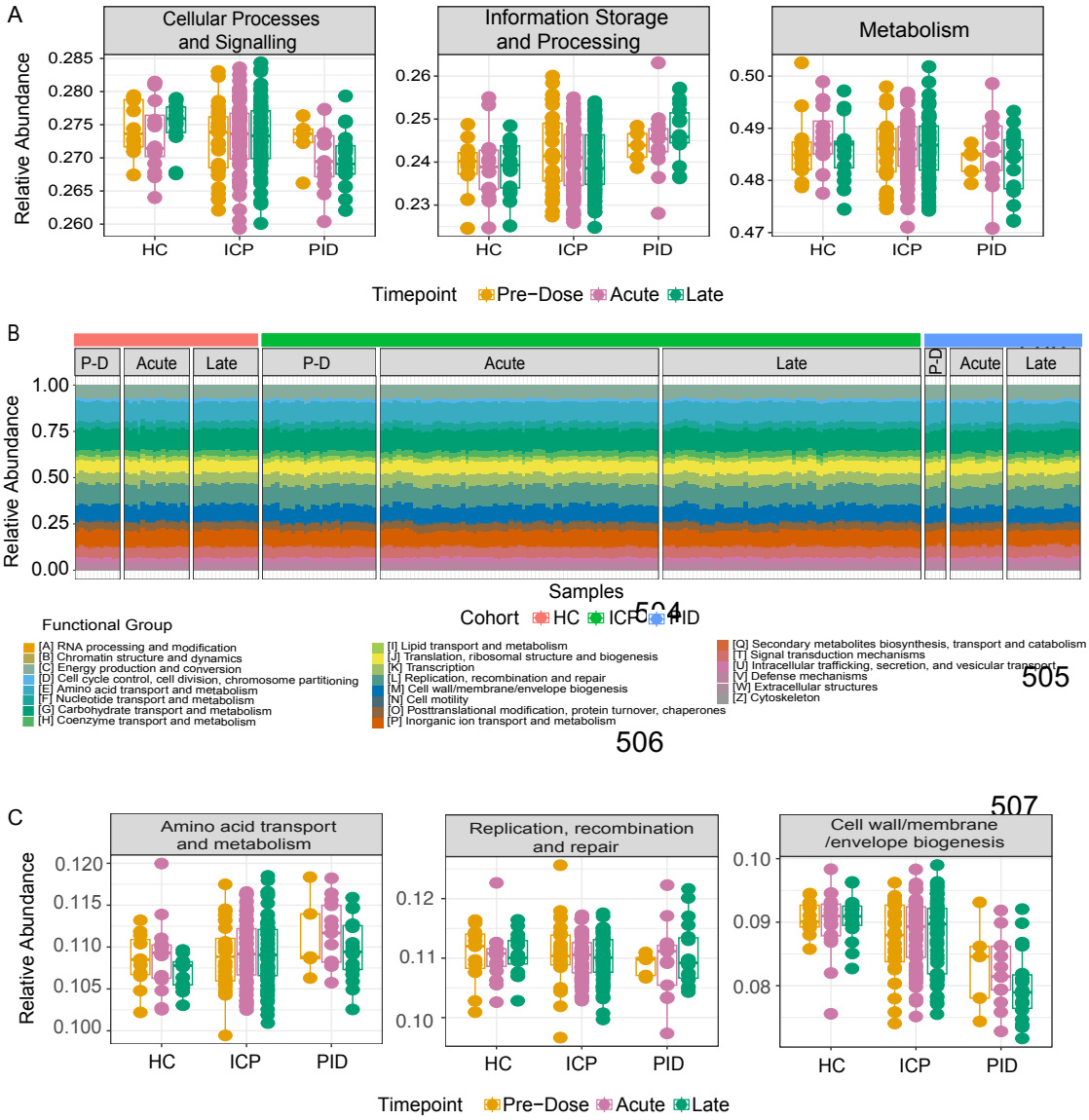
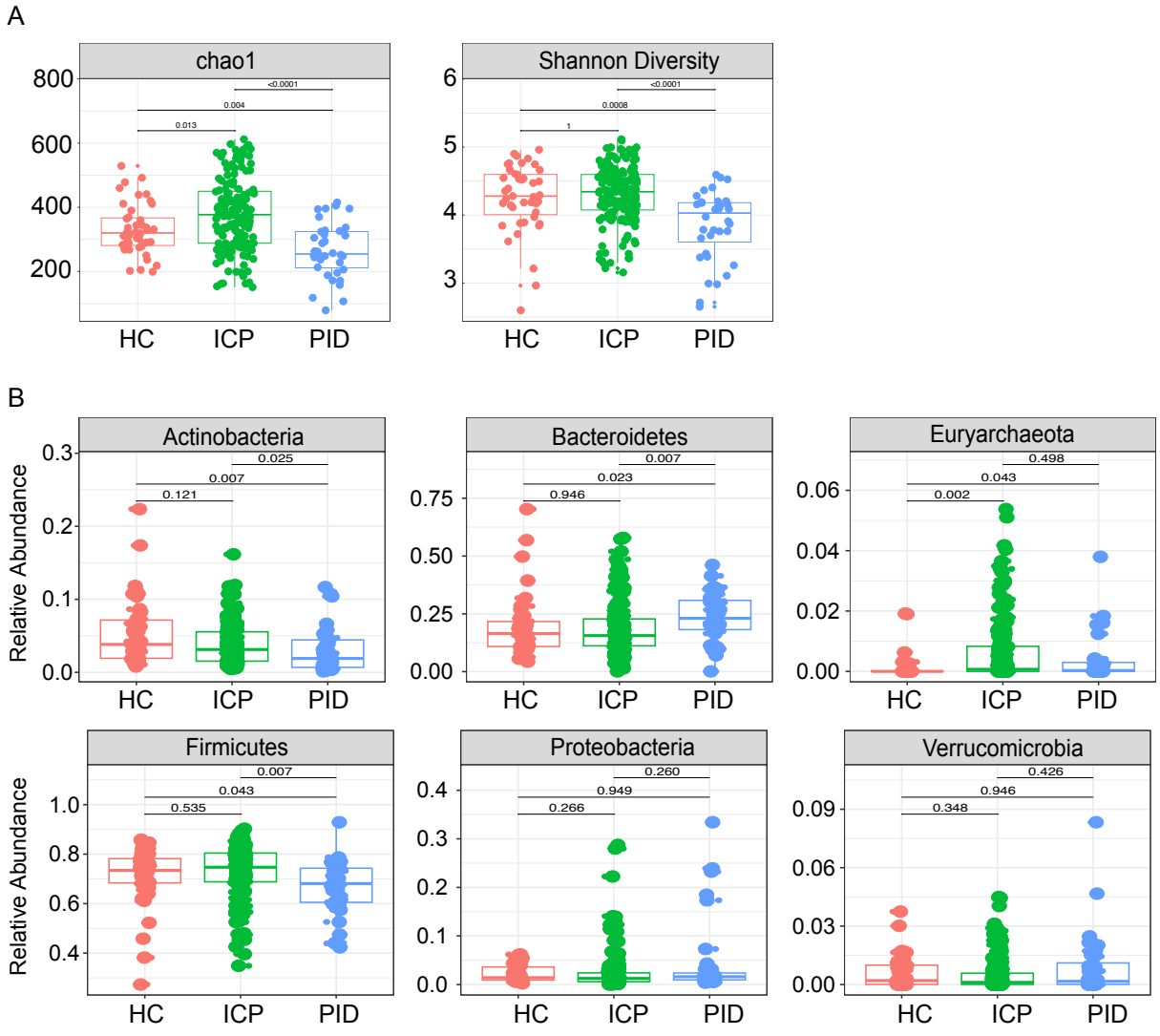
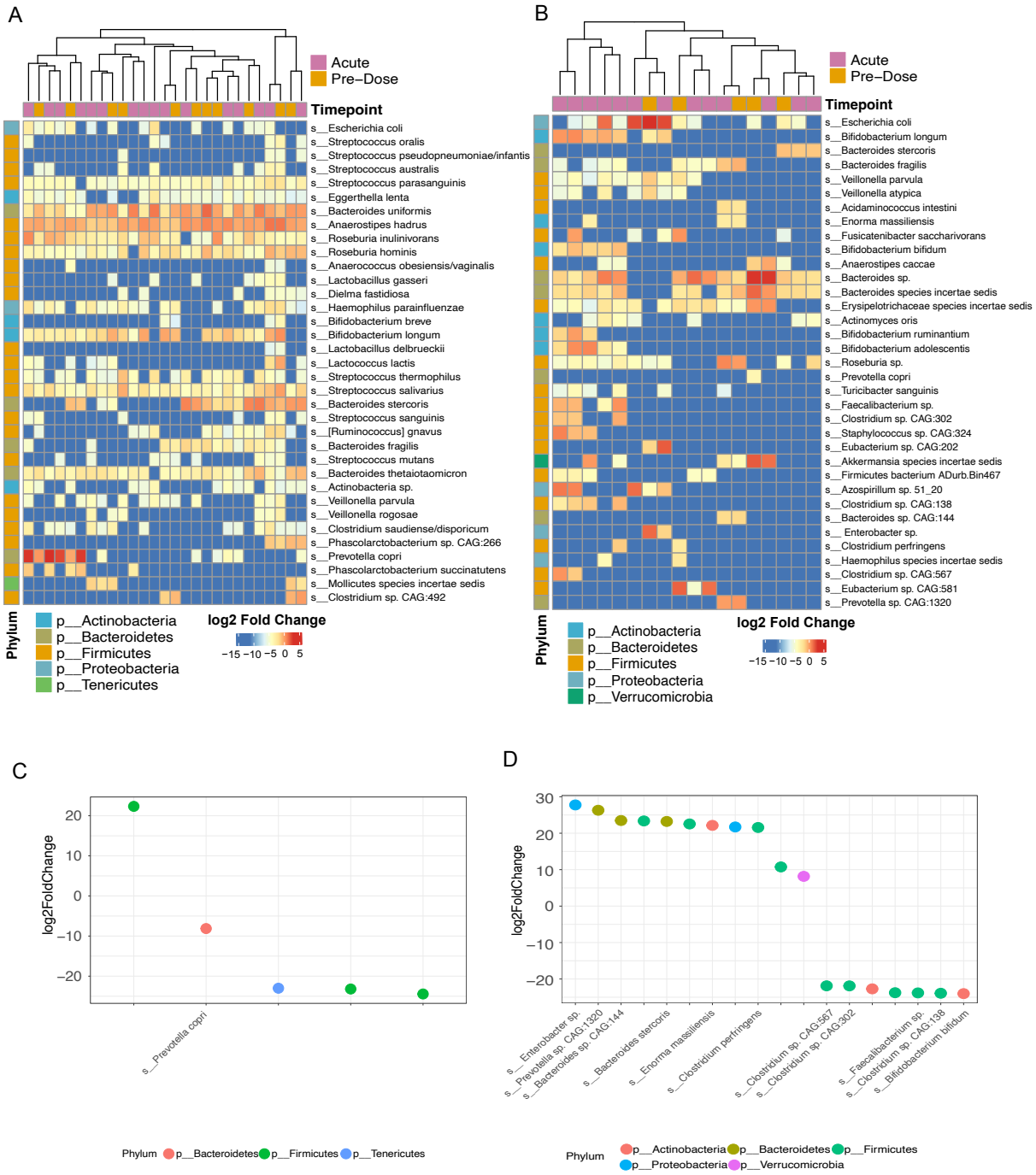


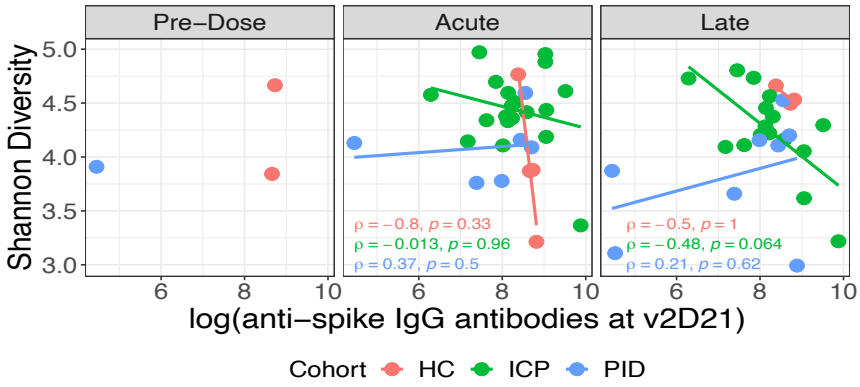
Figure 4. *Functional annotations of metagenomics samples using EGGNOG database are not altered by the COVID-19 vaccines. (A) The relative abundance of the highest functional annotation level within patient samples at different vaccine timepoints in each of our patient cohorts. (B) Functional composition depicted by colours of each of the bars, from samples taken from each of the cohorts (HC, ICP and PID), separated by the vaccine timepoints from which the sample was taken; PD = Pre-Dose, Acute and Late. (C) Relative abundance of the 3 most abundant functional annotations in our patient samples from within each of our patient cohorts and separated by the vaccine timepoint from which the sample was taken. Statistical testing performed using Wilcoxon test and adjusted for multiple testing using FDR.*



Supplementary Figure 1. Gut microbiome compositional differences are evident between patient cohorts. (A) Alpha diversity measures of chao1 and Shannon diversity in samples from different patient cohorts, *healthy control (HC), immune-checkpoint therapy treated cancer patients (ICP), or patients with primary immunodeficiencies (PID).* Statistical testing performed using Wilcoxon test and adjusted for multiple testing using FDR. (B) Relative abundance of the 6 most prevalent phyla in our patient samples from within each of our patient cohorts and separated by the vaccine timepoint from which the sample was taken. Statistical testing performed using Wilcoxon test and adjusted for multiple testing using FDR.



Supplementary Figure 2. Differential abundance analysis of HC and PID cohort samples taken at pre-dose and acutely after COVID-19 vaccination. (A) DESeq analysis of HC cohort and PID cohort (B), along with corresponding log2foldchange analysis of significantly different bacterial species between pre-dose and acute samples in HC samples (C) and PID samples (D), colours represent different phyla.



Supplementary Figure 3. Second dose *anti-spike IgG antibody levels 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. rho and p values from Spearman correlation testing displayed.*