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Defects in mucosal immunity and nasopharyngeal dysbiosis in HSC transplanted SCID patients with IL2RG/JAK3 deficiency

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Abstract:

Both innate and adaptive lymphocytes have critical roles in mucosal defense that contain commensal microbial communities and protect against pathogen invasion. Here we characterize mucosal immunity in human severe combined immunodeficiency (SCID) patients receiving hematopoietic stem cell transplantation (HSCT) with or without myeloablation. We confirmed that pre-transplant conditioning impacted on innate (NK, ILC) and adaptive (B and T cells) lymphocyte reconstitution in these SCID patients and now demonstrate that this further extends to generation of Th2 and Tc2 cells. Using an integrated approach to assess nasopharyngeal immunity, we identify a local mucosal defect in type 2 cytokines, mucus production and a selective local IgA deficiency in HSCT-treated SCID patients with genetic defects in IL2RG/GC or JAK3. These patients have a reduction in IgA-coated nasopharyngeal bacteria and exhibit microbial dysbiosis with increased pathobiont carriage. Interestingly, IVIG replacement therapy can partially normalize nasopharyngeal Ig profiles and restore microbial communities in GC/JAK3 patients. Together, our results suggest a potential non-redundant role for type 2 immunity and/or of local IgA antibody production in the maintenance of nasopharyngeal microbial homeostasis and mucosal barrier function.

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Agreement to Share Publication-Related Data and Data Sharing Statement: Data Sharing Statement The raw 16S RNA sequence data for each patient were deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA772582. All other datasets generated during the current study are available upon reasonable request via email to the corresponding author.

Clinical trial registration information (if any):

1 **Defects in mucosal immunity and nasopharyngeal dysbiosis in HSC**
2 **transplanted SCID patients with IL2RG/JAK3 deficiency**

3
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26
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28
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- 32 - Pre-transplant conditioning impacts on innate (NK, ILC) and adaptive
33 lymphocyte reconstitution including the generation of Th2 and Tc2 cells
34 - GC/JAK3-deficient SCID receiving non-conditioned HSC grafts fail to develop
35 type 2 responses and have mucosal IgA deficiency with dysbiosis

36 **Abstract**

37

38 Both innate and adaptive lymphocytes have critical roles in mucosal defense that
39 contain commensal microbial communities and protect against pathogen invasion.
40 Here we characterize mucosal immunity in human severe combined
41 immunodeficiency (SCID) patients receiving hematopoietic stem cell transplantation
42 (HSCT) with or without myeloablation. We confirmed that pre-transplant conditioning
43 impacted on innate (NK, ILC) and adaptive (B and T cells) lymphocyte reconstitution
44 in these SCID patients and now demonstrate that this further extends to generation
45 of Th2 and Tc2 cells. Using an integrated approach to assess nasopharyngeal
46 immunity, we identify a local mucosal defect in type 2 cytokines, mucus production
47 and a selective local IgA deficiency in HSCT-treated SCID patients with genetic
48 defects in IL2RG/GC or JAK3. These patients have a reduction in IgA-coated
49 nasopharyngeal bacteria and exhibit microbial dysbiosis with increased pathobiont
50 carriage. Interestingly, IVIG replacement therapy can partially normalize
51 nasopharyngeal Ig profiles and restore microbial communities in GC/JAK3 patients.
52 Together, our results suggest a potential non-redundant role for type 2 immunity
53 and/or of local IgA antibody production in the maintenance of nasopharyngeal
54 microbial homeostasis and mucosal barrier function.

55

56 Introduction

57 Hematopoietic stem cell transplantation (HSCT) for severe combined immune
58 deficiency (SCID) represents a life-saving therapy for this heterogeneous group of
59 hematopoietic disorders ^{1,2}. HSCT generates a variable degree of hematolymphoid
60 reconstitution that depends on the pre-transplant conditioning regime (eg.
61 myeloablation) as well as the genetic defect being treated ^{2,3}. For example, T-B-
62 natural killer cells (NK)+ SCID resulting from defects in the antigen receptor
63 recombination pathway harbor immature lymphoid precursors in the thymus and
64 bone marrow. These patients may receive either myeloablation that can enhance
65 myeloid and lymphoid reconstitution following HSCT or reduced intensity conditioning
66 that may eliminate competitive but abnormal thymocyte precursor cells or NK cells. In
67 contrast, patients with T-B+NK- SCID (caused by mutations in the common γ chain
68 (γ c) gene *IL2RG* or the Janus kinase *JAK3*) lack lymphoid precursors and are
69 generally not cytoreduced prior to HSCT ³. As a result, lymphoid lineages engraft
70 rapidly but myeloid reconstitution is less robust.

71 Innate lymphoid cells (ILC) are tissue-resident lymphocytes, enriched at
72 mucosal barriers with roles in immune defense and tissue remodeling ^{4,5}. Diverse ILC
73 subsets (ILC1/2/3) produce a restricted range of cytokines that target hematopoietic
74 as well as non-hematopoietic (stromal, epithelial, endothelial...) cells. ⁶. Previous
75 studies have documented the developmental and functional parallels between ILCs
76 and T 'helper' (Th) cells ⁷. This homology suggests a potential functional redundancy
77 during immune responses but may also provide a means to synergistically promote
78 immune defense.

79 Recently it was shown that pre-transplant conditioning allows for better post-
80 HSCT immune reconstitution with higher frequencies of donor NK and ILC subsets in
81 myeloablated T-B-NK+ SCID and cancer patients compared with non-conditioned T-
82 B+NK- SCID recipients ³. The differential innate lymphoid cell engraftment in these
83 patients results from a combination of both absence of conditioning and inherent
84 genetic defects, providing a unique setting (ILC/NK+ versus ILC/NK- phenotypes) to
85 assess biological roles for ILC/NK cells in human immunity. Interestingly, both HSCT
86 groups showed robust donor T cell engraftment with restoration of cellular immunity
87 and recovery of immune competence. In a long-term follow-up of these two HSCT-
88 treated patient groups, no obvious differences in clinical course or disease
89 susceptibility were noted. As both HSCT groups showed similar clinical recoveries

90 ('cure'), it was concluded that NK cells and diverse ILC subsets may be redundant for
91 most aspects of normal human immunity^{3,8} that more recently has been extended to
92 development and function of lymphoid tissues⁹.

93 Diverse ILC subsets are enriched at mucosal surfaces, and in conjunction with
94 adaptive T and B cell responses, promote barrier defense and tissue regeneration
95 after infection and inflammation and regulate microbial communities that have a
96 symbiotic relationship with the host^{10,11}. The reciprocal interactions and coordinated
97 regulation of ILCs versus T helper cells for immune defense remains unclear. A
98 better characterization of mucosal immunity in ILC/NK+ versus ILC/NK- HSCT
99 patients may shed light on the specific and/or redundant roles of innate lymphocytes
100 at barrier surfaces for protection from disease.

101 Here we further analyzed a large cohort of HSCT-treated SCID patients to
102 assess impact of hematopoietic reconstitution on mucosal barrier function. We
103 document a selective deficiency in type 2 immunity, strong decreases in
104 nasopharyngeal IgA and nasal microbial dysbiosis in IL2RG/JAK3-deficient patients
105 receiving non-conditioned HSCT.

106 **Methods**

107

108 **Patient and control cohorts**

109 Healthy donors were recruited originally as part of the Milieu Intérieur cohort
110 (<https://www.milieuinterieur.fr/en/>; Supplementary Materials and Methods). HSCT-
111 treated SCID patients were followed at Hôpital Necker-Enfants Malades (French
112 National Reference Center for Primary Immunodeficiencies). Pathogenic mutations
113 were identified in all cases (Supplementary Table 1). Written informed consent was
114 obtained from all patients and/or parents. Nasopharyngeal swabs were obtained
115 concurrently with blood samples during routine visits (no evidence of ongoing
116 infection, autoimmunity or allergy; no antibiotic use) and were processed as
117 described^{12,13}.

118

119 **Cell isolation and FACS analysis**

120 Human peripheral blood mononuclear cells (PBMC) were isolated using density
121 gradient centrifugation. For FACS analysis, cells were first stained with Flexible
122 Viability Dye eFluor 506 (eBioscience) following by surface antibodies staining on ice.
123 Fc receptors were blocked using IgG from human serum (MilliporeSigma). Samples
124 were acquired with an LSRFortessa (BD) and analyzed by FlowJ10.7.1 (TreeStar).
125 Bacterial species-specific antibody against microbiota were assessed as described
126 previous¹⁵.

127

128 **Analysis of nasopharyngeal proteins**

129 Total IgA, IgM, IgG1, IgG2, IgG3 and IgG4 were determined using the Bio-Plex Pro
130 Human Isotyping Assay Panel (Biorad, Hercules, CA, USA). Data were acquired on a
131 Bio-Plex 200 System (Bio-Rad) and analyzed with Bio-Plex Manager v5 (Bio-Rad).
132 IgA1 and IgA2 subclass were measured by Simoa (Quanterix). Total IgD were
133 determined using a ELISA kit (MBS564048, Mybiosource, San Diego). Total IgE
134 were determined using a ELISA kit (88-50610, Invitrogen, Massachusetts). Data
135 were collected with the Multiskan Spectrum (Thermo Fisher Scientific).

136

137 Cytokines were quantified by Simoa® Cytokine 3-Plex B, Discovery or Advantage
138 Kits (Quanterix) except IFN- γ and IL-17F that used Quanterix Homebrew assays.
139 Nasopharyngeal mucin levels were analyzed using a MUC5AC ELISA Kit (NBP2-

140 76703, Novus Biologicals, diluted 1/50). Eosinophil cationic protein (ECP) were
141 determined for swabs medium using an ELISA kit (MBS2602477, Mybiosource, San
142 Diego, USA, diluted 1/2). Total protein content of the supernatants by the Bradford
143 method ¹⁴.

144

145 **16S rRNA sequencing and analysis**

146 16S rRNA sequencing and sequence processing and statistical analysis was
147 described previous ^{12,13}. A total of 2.974.329 reads (90.131 reads on average per
148 sample) was obtained. Raw sequence data have been deposited in the NCBI
149 Sequence Read Archive (SRA) under accession number (PRJNA772582).

150

151 **Bacterial quantification by quantitative RT-PCR assays**

152 Bacterial qPCR used universal 16S rRNA primers to measure total bacteria (16S_F:
153 5'-ATTACCGCGGCTGCTGG-3' and 16S_R: 5'-ATTACCGCGGCTGCTGG-
154 3') and *Streptococcus pneumoniae* (*LytA* gene, F: 5'- ACGCAATCTAGCAGATGAAGC-
155 3' and R: 5'- TGTTTGGTTGGTTATTCGTGC -3').

156

157 **Statistical analysis**

158 Statistics were performed using GraphPad Prism (San Diego, USA). P values were
159 determined by a Kruskal-Wallis test, followed by Dunn's post-test for multiple group
160 comparisons with median reported; *P < 0.05; **P < 0.01; ***P < 0.001. Correlations
161 between the different assays were calculated using Spearman test. Heatmaps were
162 generated with Qlucore OMICS explore Version 3.5(26). Correlation matrices were
163 built using the Spearman correlation and computed using R (v4.0.3).

164

165 **Results**

166 This study analyzed a cohort of 21 SCID patients that have been successfully
167 treated with HSCT at Necker Hospital since 1977. SCID patients with X-linked as well
168 as autosomal etiologies were included, some patients required Ig replacement
169 therapy (IgRT) by subcutaneous injection of IgG for specific clinical conditions
170 (including recurrent respiratory tract infections¹⁶⁻¹⁸; Supplemental Table 1). Several
171 patients have been previously reported^{3,9}. The group 'GC/JAK3' were T-B+NK- SCID
172 (IL2RG, JAK3) patients that received non-myeloablative HSCT, and the group 'SCID
173 other' were T-B-NK+ or T-B+NK+ SCID (RAG1/2, ARTEMIS, MHC Class II, IL7RA)
174 patients the majority of which received pre-transplant cytoreduction (Supplemental
175 Table 1). All HSCT-treated SCID patients showed successful donor hematopoietic
176 reconstitution and recovered T cell immunity. Once clinically stable, patients were
177 discharged and followed up periodically in our outpatient clinic. The follow up period
178 varied from 18 to 42 years.

179

180 *HSCT-treated GC/JAK3 patients have reduced circulating type 2 lymphocytes*

181 While HSCT-treated SCID patients have stable T cell reconstitution with
182 balanced CD4/CD8 ratios, naïve T cells and TRECs^{1,3,19}, an in-depth analysis of
183 their differentiated T cell subsets has not been previously performed. ILC and NK cell
184 reconstitution in SCID patients receiving myeloablative conditioning for HSCT has
185 only been reported in 2 RAG-deficient patients³. As reciprocal interactions between
186 ILCs and T cells have been documented²⁰⁻²³, previously reported lack of NK and
187 ILCs in the non-conditioned SCID recipients might be associated with perturbations
188 in their T cell compartment. We paid particular attention to differentiated T cell
189 subsets that can be identified by expression of specific chemokine receptors (see
190 Methods; Supplemental Figure 1A, B for FACS gating)^{24,25}. Non-conditioned
191 GC/JAK3 patients receiving IgRT post-HSCT (Supplemental Table 1) were analyzed
192 separately.

193 Circulating ILCs include CD56+ natural killer (NK) cells (CD56+++CD16- and
194 CD56+CD16+ cells) and CD127+ ILC2 (CRTH2+) and ILCP (ILC precursors,
195 CRTH2-CD117+ CD45RA+NKp44-)²⁶. Using unsupervised clustering analysis, we
196 observed a significant reduction in frequencies of NK cells and ILC2 but not ILCP in
197 GC/JAK3 and GC/JAK3+IgRT patients compared with HC and SCID-other patients
198 (Figures 1A, B) confirming previous reports in HSCT-treated IL2RG/JAK3-deficient

199 and RAG-deficient patients³ and further extending this observation to other SCID
200 etiologies.

201 We next characterized naïve and differentiated CD4⁺ T cell subsets in this
202 SCID cohort. Unsupervised clustering analysis allowed us to identify T cell subsets
203 including naïve (CD45RA⁺), Treg (CD127^{lo}CD25⁺), Tfh (Treg-CD45RA-CXCR5⁺),
204 Th1 (Treg-CD45RA-CXCR5-CCR6-CXCR3+CCR4⁻), Th2 (Treg-CD45RA-CXCR5-
205 CCR6-CXCR3-CCR4+CRTH2^{+/-}) and Th17 (Treg-CD45RA-CXCR5-CCR6⁺). We
206 observed a significant reduction in clusters of CCR4+CRTH2^{+/-} cells corresponding
207 to the Th2 subset, as well as a reduction in naïve T cells (CD45RA⁺) in GC/JAK3
208 and GC/JAK3+IgRT patients compared to HC and SCID-other patients (Figures 1C,
209 D). Other Th subsets (Th1, Th17, Tfh) were normally present in all HSCT SCID
210 patients (Figure 1D, Supplemental Figure 2A, B).

211 A similar analysis was performed on CD8⁺ T cells. The unsupervised
212 clustering analysis showed a significant decrease in the CCR4+CRTH2⁺ cluster
213 corresponding to Tc2 subset in CD8⁺ T cells in GC/JAK3 and GC/JAK3+IgRT
214 patients (Figure 1E, F) whereas other CD8⁺ T cell subsets were similar in SCID-other
215 patients as compared to HC (Supplemental Figure 3).

216 The observations of a relative decrease in naïve CD4⁺ T cells with a
217 compensatory increase in activated CD8⁺ T cells following HSCT for SCID confirm
218 earlier work²⁸, while our detailed assessment of differentiated T cell subsets allowed
219 us to identify a selective deficiency in blood CD4⁺ Th2 cells and CD8⁺ Tc2 cells in
220 HSCT-treated GC/JAK3 patients. In all SCID etiologies, differentiation of other T
221 helper subsets (Th1, Th17, Treg) appeared largely intact. These results suggest that
222 non-conditioned HSCT-treated GC/JAK3 patients manifest a generalized and
223 selective deficiency in innate and adaptive lymphocytes involved in Type-2 immunity.

224

225 *HSCT-treated GC/JAK3 patients have reduced nasopharyngeal type 2 cytokines*

226 We next assessed mucosal immune responses in HSCT-treated SCID
227 patients. Nasopharyngeal swabs were obtained concurrently with blood samples and
228 were processed as described^{12,13} to yield nucleic acids as well as a soluble fraction
229 that harbored cytokines, antibodies, anti-microbial peptides and various metabolites.
230 As differentiated Th cells and ILCs maintain mucosal homeostasis^{4,5}, we used digital
231 ELISA (Simoa) to quantitate cytokines associated with Th1/ILC1, Th2/ILC2 and
232 Th17/ILC3 responses in these nasopharyngeal samples.

233 We found no significant differences in the levels of type-1 cytokines (IFN- γ ,
234 TNF- α) in normal controls compared to HSCT-treated GC/JAK3 or SCID-other
235 patients (Figure 2A). In contrast, levels of type-2 cytokines IL-5 and IL-13 were
236 clearly decreased in non-conditioned GC/JAK3 patients whereas IL-4 levels were
237 within healthy ranges (Figure 2B). While the deficiency in nasopharyngeal IL-13
238 persisted in GC/JAK3 patients receiving IVIG, IL-4 and IL-5 levels were somewhat
239 higher in these patients compared to non-conditioned GC/JAK3 patients that did not
240 receive IgRT (Figure 2B). Concerning inflammatory type-3 cytokines (IL-17A, IL-17F,
241 IL-22), nasopharyngeal mucosal levels were not different from controls except in
242 GC/JAK3 patients being treated with IgRT (Figure 2C). Whether these increases in
243 nasopharyngeal cytokines result from IgRT treatment or are related to other
244 mechanisms that underlie the need to treat (eg. infections) with IgRT is unclear.

245 Type-2 cytokine secretion is regulated by stromal-derived factors and
246 promotes activation of hematopoietic (eosinophils, mast cells) as well as non-
247 hematopoietic (goblet cells) targets.^{29,30} We found that IL-33 (a major inducer of
248 recruitment, activation and IL-5 and IL-13 production by type-2 lymphocytes³¹⁻³³)
249 was not reduced in nasopharyngeal samples (Figure 2D), whereas eosinophil
250 cationic protein (ECP, marker for tissue eosinophilia^{34,35}) was reduced in GC/JAK3
251 patients consistent with reduced IL-5 levels (Figure 2B, D). Nasopharyngeal IL-6
252 levels were not significantly elevated in any SCID patient although tended to be
253 higher in GC/JAK3+IgRT patients (Figure 2D). When all data was clustered, the
254 heatmap clearly distinguished the defective type-2 cytokine production (in particular,
255 IL-5 and IL-13) in the context of non-conditioned HSCT for GC/JAK3 and irrespective
256 of IgRT (Figure 2E).

257

258 *Systemic and mucosal Ig subtypes in HSCT-treated SCID patients*

259 Previous studies have analyzed the impact of pre-HCST conditioning on
260 systemic antibody levels following hematopoietic reconstitution for SCID^{18,36}. In
261 particular, busulfan administration is correlated with higher donor chimerism,
262 especially in the B cell compartment³⁶. We found that non-conditioned GC/JAK3
263 patients demonstrated somewhat elevated total serum IgM, but all six patients
264 studied had normal total serum IgG levels (Figure 3A), confirming that GC/JAK3-
265 deficient B cells can produce switched IgGs in the presence of normal T cells¹⁸. In
266 contrast, serum IgA was undetectable (selective IgA deficiency, SIgAD) in half of

267 HSCT-treated GC/JAK3 patients, mirroring previous reports^{37,38}. SCID-other patients
268 harbored normal Ig subtype distributions and levels (Figure 3A). Interestingly, need
269 for IgRT in GC/JAK3 patients did not clearly correlate with any selective serum Ig
270 deficiency (Figure 3A) but was dictated by the clinical context (history of recurrent
271 respiratory tract infections).

272 We next assessed mucosal Igs in HSCT-treated SCID patients by measuring
273 Ig isotypes and subclasses in paired nasopharyngeal samples. We found that total
274 nasopharyngeal IgM and IgG was elevated in GC/JAK3 patients compared to
275 controls but less so compared to SCID-other patients (Figure 3B) which appeared
276 related to higher levels of IgG3 (Supplemental Figure 4A). In contrast, all GC/JAK3
277 patients showed a strong reduction in total nasopharyngeal IgA that concomitantly
278 involved both IgA1 and IgA2 (Figure 3C, Supplemental Figure 4B) and a significant
279 reduction in total nasopharyngeal IgE (Figure 3D). Interestingly, total nasopharyngeal
280 IgD levels were significantly increased in GC/JAK3 patients. Lastly, GC/JAK3+IgRT
281 patients 'normalized' nasopharyngeal Ig distributions with increased IgA and IgE
282 (Figures 3C, D), although the mechanistic basis remains unclear. Together, our
283 results demonstrate distinct profiles of systemic and local mucosal IgA responses in
284 HSCT-treated SCID patients.

285

286 *IgG and IgD may provide mucosal protection in the context of IgA deficiency*

287 We next analyzed the binding of different Ig isotypes to nasopharyngeal
288 microbiota using a flow cytometer-based assay¹². We found a significant decrease in
289 the percentage of IgA-coated nasopharyngeal microbes as well as the density of IgA
290 coating (IgA MFI, not shown) in GC/JAK3 patients compared to SCID-other patients
291 and healthy controls (Figure 4A, B). Interestingly, GC/JAK3+IgRT patients showed a
292 partial but significant increase in IgA-coated nasopharyngeal microbes (Figure 4B)
293 consistent with the increase in nasopharyngeal IgA (Figure 3C). Whether the
294 increase in IgA activity following IgRT results from an indirect effect following
295 stimulation of type 2 cytokine production³⁹⁻⁴¹ remains unclear.

296 Compensatory IgG responses to gut commensal bacterial communities may
297 operate in the absence of IgA-specific responses⁴². As other Ig isotypes and
298 subclasses were normally present (IgD) or elevated (IgM, IgG) in GC/JAK3 patients
299 (Figure 3B), we quantitated the fraction (%) and intensity (MFI) of nasopharyngeal
300 microbes that were coated with IgG, IgA or IgD using a recently reported multiplexing

301 technique ¹². The majority of nasopharyngeal microbes in healthy individuals are
302 coated with IgA in combination with IgD and to a lesser extent with IgG (Figure 4C)
303 ¹². SCID-other patients showed a similar pattern of nasopharyngeal microbe coating,
304 while nasopharyngeal microbes in GC/JAK3 patients were more abundantly coated
305 with IgG and IgD alone (Figure 4C). Finally, we observed an increase in IgA/IgG-
306 double coated nasopharyngeal microbes in GC/JAK3 patients receiving IgRT (Figure
307 4C), consistent with the increased nasopharyngeal IgA in these individuals. These
308 results are consistent with the notion that IgG ⁴² and IgD ⁴³⁻⁴⁵ may provide a layer of
309 mucosal protection during IgA deficiency.

310

311 *HSCT-treated GC/JAK3 patients have nasopharyngeal microbiota dysbiosis*

312 Secretory IgA (sIgA) produced locally at mucosal sites plays an essential role
313 in host defense ¹² and shapes commensal microbiota composition and activity in
314 each individual ⁴⁶. To characterize local nasopharyngeal microbial communities, we
315 performed 16S ribosomal RNA (rRNA) gene sequencing from nasopharyngeal
316 samples, calculated bacterial beta diversity and subjected sequenced OTU to
317 Principal Coordinates Analysis (PCoA). We found that nasopharyngeal samples
318 derived from HSCT-treated GC/JAK3 patients without IVIG replacement therapy
319 clustered distinctively from the healthy controls and SCID-other patient samples
320 (Figure 5A) and had elevated beta diversity based on Bray-Curtis, Euclidian and
321 Jaccard distance matrices (Figure 5B). Accordingly, Shannon and Simpson diversity
322 indices were reduced in these GC/JAK3 patients (Figure 5C). We further applied a
323 non-metric multidimensional scaling (NMDS) using Bray-Curtis distances and found a
324 similar distinctive clustering of GC/JAK3 patients not treated with IVIG
325 (Supplementary Figure 5A). In contrast, SCID-other patients with more complete
326 hematopoietic reconstitution showed nasopharyngeal microbial communities that
327 were more similar to healthy controls (Figures 5A-C, Supplementary Figure 5B).
328 Total bacterial load was also increased in GC/JAK3 patients not treated with IgRT
329 compared to healthy controls and other SCID patients (Supplementary Figure 5C).

330 Nasopharyngeal microbial communities in these different SCID patients were
331 further characterized by annotation of the 16S rRNA datasets. It has been reported
332 that the nasopharyngeal microbiota of healthy individuals is enriched in commensal
333 bacteria including *Corynebacterium* and *Dolosigranulum* genera ^{47,48}. We found a
334 general reduction in *Dolosigranulum* and *Comamonas* genera in HSCT SCID

335 patients compared to healthy controls and a selective reduction in *Corynebacterium*,
336 *Cutibacterium* and *Staphylococcus* genera in GC/JAK3 patients compared to other
337 SCID patients and healthy controls (Figure 5D, E). These ‘cornerstone’ bacterial
338 communities (particularly *Corynebacterium* and *Dolosigranulum* genera) can reduce
339 carriage of several pathobionts (*Streptococcus pneumoniae*, *Haemophilus influenzae*
340 and *Moraxella catarrhalis*) that may be present in normal healthy individuals.^{49,50}
341 Relative abundance of *Streptococcus*, *Haemophilus* and *Moraxella* were not
342 significantly increased in HSCT SCID-other patients compared to healthy controls,
343 although a higher abundance of *Streptococcus* and *Haemophilus* genera were
344 detected in GC/JAK3 patients that were not treated with IgRT (Figure 5D, F). This
345 was in part due to the increased abundance of *S. pneumoniae* (Figure 5G;
346 Supplementary Figure 5D). Finally, a significant negative correlation of *S.*
347 *pneumoniae* abundance with *Dolosigranulum* genus abundance could be detected in
348 the nasopharynx (Figure 5H).

349 IgRT is an established treatment to combat infections in HSCT-treated SCID
350 patients for which *S. pneumoniae* is a leading cause of disease.⁵¹ Nasopharyngeal
351 samples from GC/JAK3 patients receiving IgRT clustered closer to SCID-other
352 patients and healthy controls (Figure 5A). Moreover, IgRT in GC/JAK3 patients
353 ‘normalized’ microbiota diversity (beta Shannon and Simpson diversity indices) with
354 higher representation of *Corynebacterium* and reduced levels of *Streptococcus*
355 (Figure 5B-D). Moreover, we could confirm a decrease in *S. pneumoniae* in
356 GC/JAK3+IgRT patients (Figure 5G). Intriguingly, IgA level under IgRT correlated
357 with microbial α -diversity indicating the intricate interplay between immune selection
358 and maintenance of complex commensal communities (Supplementary Figure 5E).
359 Taken together, these data suggest that HSCT-treated GC/JAK3 patients without
360 IgRT can present persistent nasopharyngeal microbial dysbiosis with expansion of
361 bacteria associated with increased morbidity and mortality risk⁵¹. As IgRT appears to
362 ameliorate the nasopharyngeal dysbiosis, one may consider potential use of IgRT in
363 all GC/JAK3 patients to pre-empt or correct this abnormality.

364

365 *Role for type 2 immunity and nasal IgA in maintaining commensal microbiota*
366 *diversity and protection against ‘pathobiont’ carriage*

367 Secretory IgA is implicated in human nasopharyngeal microbial homeostasis⁵²
368 and may protect mucosal surfaces from pathogen invasion through agglutination^{53,54}.

369 Still, other 'non-specific' barriers, including a dense mucus layer, restrict commensal
370 and pathobiont colonization at mucosal sites ⁵⁵. While total protein levels in
371 nasopharyngeal samples from SCID patients and healthy controls were not
372 significantly different (Supplemental Figure 3B), we found that GC/JAK3 patients
373 (treated or not with IgRT) had markedly reduced MUC5AC levels compared to SCID-
374 Other and healthy controls (Figure 6A). MUC5AC levels were positively correlated
375 with α -diversity, *Dolosigranulum* and *Corynebacterium* genera abundance and
376 strongly negatively correlated with *S. pneumoniae* abundance (Figures 6B, C). These
377 results suggest that nasopharyngeal mucus plays a role in homeostasis of local
378 commensal microorganisms and is selectively reduced following HSCT for GC/JAK3.

379 We next looked for possible correlations in the cytokine dataset that could
380 associate with reduced MUC5AC levels in these SCID patients. The reduction in
381 nasopharyngeal IgA and type 2 cytokine levels in GC/JAK3 patients (Figures 2, 3)
382 paralleled the observed reductions in MUC5AC (Figure 6D, E), suggesting a possible
383 link. Type 2 cytokines are known to regulate barrier immunity by promoting mucus
384 production ^{29,30} but other factors stimulate epithelial cell renewal and differentiation,
385 including IL-22 ⁵⁶. IL-22 levels were correlated with MUC5AC levels (Supplemental
386 Figures 6C; see Supplemental Figures 6D for all 2-parameter correlations). Together,
387 these results identify critical soluble factors that coordinate local mucosal immune
388 defense in the nasopharynx via mucus production. Finally, a supervised analysis of
389 nasopharyngeal factors that were significantly different in GC/JAK3 patients reiterate
390 the key parameters that integrate the unique mucosal immune profile of these
391 individuals (Figure 6F), including reduced type 2 cytokines, reduced MUC5AC levels,
392 reduced IgA and microbial dysbiosis.

393

394 Discussion

395 In this report, we assess local mucosal immunity in the human nasopharynx in
396 a well-characterized cohort of SCID patients treated with curative HSCT. Previous
397 studies on systemic immune reconstitution after BMT have highlighted differences in
398 homeostasis of peripheral pools of innate and adaptive lymphocytes^{1,3,18,19}. Pre-
399 transplant conditioning regimes may allow for differential engraftment of donor
400 hematopoietic precursor cells and downstream myeloid and innate lymphocyte pools,
401 the latter including natural killer (NK) and innate lymphoid cells (ILCs)³. The impact
402 of differences in 'innate reconstitution' on overall systemic immune responses
403 appeared limited given the similar clinical profiles of these HSCT-treated SCID
404 patients³. These studies raised questions concerning the specific versus redundant
405 functions of innate lymphocytes in human immunity.

406 As ILCs abundantly populate mucosal sites, we explored their potential
407 immune roles by comparing HSCT-treated SCID patients that show variable innate
408 lymphocyte reconstitution³. We observed clear differences in mucosal immune
409 parameters in a subset of SCID patients with genetic defects in GC or JAK3 which
410 were not conditioned prior to HSCT. We documented a generalized reduction in
411 mucosal type 2 immunity and IgA production and an inability to maintain 'healthy'
412 commensal bacterial communities consistent with a defect in nasopharyngeal
413 mucosal barrier function. In contrast, other SCID etiologies (with or without
414 myeloablative protocols prior to HSCT) showed normal type 2 cytokines and IgA
415 production without nasal dysbiosis. Thus the underlying SCID etiology is apparently a
416 major factor for the observed defects in mucosal immunity.

417 Local IgA production is a hallmark of mucosal immunity and is largely driven
418 by specific immune responses to resident micro-organisms⁵⁷. Cytokines promote
419 IgG and IgE production⁵⁸. In contrast, the key soluble factors that regulate switch to
420 IgA are a matter of debate^{57,59}. Previous studies have documented absence of
421 serum IgA in about half of HSCT-treated GC/JAK3 patients^{18,36-38}. Here we show
422 that mucosal IgA is absent in all HSCT-GC/JAK3 patients examined, including those
423 with normal circulating IgA. Interestingly, nasopharyngeal type 2 cytokines were most
424 strongly perturbed in these patients, suggesting a causative link to mucosal IgA
425 production. Type 2 cytokines can promote the survival and differentiation of tissue
426 resident memory B cells and IgA secreting plasma cells^{60-62,63,64} thereby increasing
427 IgA production^{30,65-68}. Along these lines, a recent report described a major role for

428 ILC2-derived IL-5 in promoting local mucosal IgA production in mice ⁶⁹. Whether
429 locally generated IL-5 (by ILC2 or Th2 cells) regulates mucosal IgA production in
430 humans remains unclear.

431 An alternative explanation for the observed IgA deficiencies may result from
432 defective Ig switch in residual GC/JAK3-deficient host B cells, the latter not being
433 fully replaced by donor HSCT after non-ablative conditioning ³⁶. The inability of
434 GC/JAK3-deficient B cells to respond to any γ_c -dependent cytokine might also reduce
435 IgA switch mechanisms and result in selective IgA deficiency. Still, nasopharyngeal
436 IgG subclasses were not decreased in HSCT-treated GC/JAK3 patients, and serum
437 IgG levels were normal consistent with the ability of GC/JAK3-deficient host B cells to
438 switch Ig isotypes ¹⁸. However, these patients have an intrinsic B cell deficiency
439 (defective response to IL-2, IL-9 and in part to IL-4) that can affect antibody
440 production. γ_c -dependent signals in epithelial cells ⁷⁰ may also play a role. These
441 observations suggest a contribution of impaired γ_c -dependent signaling pathways to
442 the defective mucosal IgA production in these patients.

443 Th2 differentiation is considered as a 'default' pathway which can be
444 subverted to alternative Th fates by environmental signals ^{71,72}. Still, Th2
445 differentiation requires reinforcing signals through STAT6 (IL-4, IL-13) to upregulate
446 GATA3 expression and seal Th2 cell fate ⁷³. The defect in generation of Th2 cells in
447 HSCT-treated GC/JAK3 patients may result from the absence of these STAT6-
448 dependent signals, perhaps delivered by innate lymphocytes (NKT cells, ILC2). In
449 addition, ILC2 can prime tissue Th2 responses via DC recruitment ²⁰⁻²³.

450 The mucus layer that lines mucosal surfaces provides a physical barrier to
451 commensal micro-organisms as well as pathogens and segregates 'niches' harboring
452 complex microbial biofilms ⁷⁴. Secreted mucins (MUC5AC) are produced by goblet
453 cells in the nasopharyngeal mucosa and tracheobronchial surface epithelium of the
454 lower respiratory tract that acts as a "scaffold" to present and organize secreted
455 proteins such as sIgA, antimicrobial peptides (AMPs) and cytokines ⁷⁴. Type 2
456 cytokines (including IL-13) activate goblet cells to produce mucus ^{29,30}. Whether the
457 loss of MUC5AC in GC/JAK3 patients secondary to reduced IL-13 predisposes these
458 individuals to microbial dysbiosis will require further study.

459 Finally, nasopharyngeal IgA deficiency in HSCT-treated GC/JAK3 patients is
460 associated with local microbial dysbiosis that may have been present prior to HSCT.

461 It is well established that mucosal IgA plays a major role in regulating bacterial
462 communities in the gut ^{46,75} and the reduction in nasopharyngeal IgA observed in
463 GC/JAK3 patients is correlated with loss of microbial diversity and frequently
464 accompanied by increased pathobiont carriage. While other Ig subclasses are
465 present (and even elevated) in the nasopharynx in these patients and are able to
466 coat bacteria, IgA remains a non-redundant immune factor required for microbial
467 mucosal homeostasis.

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476

477

478 **Contributions**

479 P.G. conducted experiments, analyzed the data and prepared the manuscript.
480 J.M.D., T.E., V.B., C.P., A.L., A.C., B.C. performed experiments. C.L. processed
481 samples. D.D. provided logistical support and access to samples from normal
482 individuals via the Milieu Intérieur Consortium. B.N. and A.F. were responsible for
483 patient care, collected samples, designed experiments and prepared the manuscript.
484 J.P.D. designed experiments, analyzed the data, obtained funding, supervised
485 research and prepared the manuscript.

486

487 **Competing interests**

488 The authors declare no competing interests.

489

490 **Data Sharing Statement**

491 The raw 16S RNA sequence data were deposited in the NCBI Sequence Read
492 Archive (SRA) under accession number PRJNA772582. All other datasets generated

493 during the current study are available upon reasonable request via email to the
494 corresponding author.

495

496

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707

708 **Figure Legends**

709

710 **Figure 1. FACS analysis of peripheral blood mononuclear cells (PBMCs)**
 711 **reveal a defect in differentiation of type-2 lymphocytes in HSCT-treated**
 712 **GC/JAK3 patients. A,** Unsupervised Uniform Manifold Approximation and
 713 Projection (UMAP)²⁷ of single live CD45+Lin-CD3-CD4-CD7+ cells was applied for
 714 CD56, CD16, CD94, NKp46, CD94, CD127, CD161, CD25, CD117 and CRTH2
 715 fluorescence parameters. **B,** Supervised analysis of circulating innate lymphoid cells
 716 (ILCs) including two CD56+ subsets of natural killer (NK) cells and two CD127+
 717 subsets denoted as ILC2 (CRTh2+) and ILCP (ILC precursors, CRTh2-
 718 CD117+CD45RA+NKp44-) (manually gating strategy on Supplemental Figure 1A). **C,**
 719 UMAP analysis on CD4+ T cells including CXCR3, CCR4, CRTH2, CCR6, CXCR5,
 720 CD25, CD127 and CD45RA fluorescence parameters (Supplemental Figure 2A). **D,**
 721 Supervised analysis of circulating T cell populations. The different subsets were
 722 identified as follow: Naïve (CD45RA+), Th1 (Treg-CD45RA-CXCR5-CCR6-
 723 CXCR3+CCR4-), Th17 (Treg-CD45RA-CXCR5-CCR6+), Th2 (Treg-CD45RA-
 724 CXCR5-CCR6-CXCR3-CCR4+CRTH2+/-) and Treg (CD127loCD25+) (manually
 725 gating strategy on Supplemental Figure 1B). **E,** UMAP analysis on CD8+ Tc2 subset
 726 including CCR4 and CRTH2 fluorescence parameters (Supplemental Figure 3). **F,**
 727 Supervised analysis of circulating CD8+ Tc2 subset defined as CD45RA-CD25-
 728 CD94-CD56-CXCR5-CCR6-CXCR3-CCR4+CRTH2+/- . In **(B)**, **(D)** and **(F)**, box plots
 729 with median ± minimum to maximum. P values were determined with the Kruskal
 730 Wallis test followed by with Dunn's post test for multiple group comparisons; *P <
 731 0.05, **P < 0.005, ***P < 0.001.

732

733 **Figure 2. HSCT-treated GC/JAK3 patients have a generalized defect in**
 734 **nasopharyngeal type 2 immunity. A,** Nasopharyngeal levels of type 1 cytokines
 735 (IFN- γ and TNF- α). **B,** Nasopharyngeal levels of type 2 cytokines (IL-4, IL-5 and IL-
 736 13). **C,** Nasopharyngeal levels of type 3 cytokines (IL-17A, IL-17F and IL-22). **D,**
 737 Nasopharyngeal levels of IL-33, IL-6 and Eosinophil cationic protein (ECP)/RNase 3.
 738 **E,** Heatmap representation of the mean of the concentration of all nasopharyngeal
 739 cytokines in healthy and HSCT-treated SCID patients. In **(A)**, **(B)**, **(C)**, and **(D)**, box
 740 plots with median ± minimum to maximum. P values were determined with the

741 Kruskal Wallis test followed by with Dunn's post test for multiple group comparisons;
 742 *P < 0.05, **P < 0.005, ***P < 0.001.

743

744 **Figure 3. HSCT-treated GC/JAK3 patients without Igs replacement therapy**
 745 **have decrease in nasopharyngeal secretory IgA (SIgA).** **A,** Serum Igs
 746 concentration in healthy and HSCT-treated SCID patients. **B,** Nasopharyngeal
 747 concentrations of total IgM, IgD and IgG in healthy and HSCT-treated SCID patients.
 748 **C,** Nasopharyngeal concentrations of total IgA and IgA1 and IgA2 in healthy and
 749 HSCT-treated SCID patients. **D,** Nasopharyngeal concentrations of total IgE in
 750 healthy and HSCT-treated SCID patients. In **(A), (B), (C),** and **(D),** box plots with
 751 median \pm minimum to maximum. P values were determined with the Kruskal Wallis
 752 test followed by with Dunn's post test for multiple group comparisons; *P < 0.05, **P
 753 < 0.005, ***P < 0.001.

754

755 **Figure 4. HSCT-treated GC/JAK3 patients without IVIG replacement therapy**
 756 **have decreased IgA-coating of nasopharyngeal bacteria.** **A,** Representative dot
 757 plots of IgA, IgG and IgD binding to nasopharyngeal microbiota in healthy and HSCT-
 758 treated SCID patients. **B,** % of IgA/IgG/IgD coated nasopharynx microbes in healthy
 759 and HSCT-treated SCID patients. **C,** Double IgA/IgG or IgA/IgD coated nasopharynx
 760 microbes in healthy and HSCT-treated SCID patients. In **(A), (B), (C),** box plots with
 761 median \pm minimum to maximum. P values were determined with the Kruskal Wallis
 762 test followed by with Dunn's post test for multiple group comparisons; *P < 0.05, **P
 763 < 0.005, ***P < 0.001.

764

765 **Figure 5. HSCT-treated GC/JAK3 patients without IVIG replacement therapy**
 766 **have nasopharyngeal microbiota dysbiosis.** **A,** Unsupervised principal coordinate
 767 analysis (PCoA) of 16S RNA sequencing Operational Taxonomic Units (OTU) of the
 768 healthy controls and HSCT-treated SCID patients along the first two principal
 769 coordinate (PC) axes, based on Bray-Curtis distances. The respective PERMANOVA
 770 test showing that nasopharynx microbiota from SCIDX/JAK3 patients is significantly
 771 different from healthy controls. **B,** The beta (β) diversity, calculated using the Bray-
 772 Curtis, Jaccard and Euclidean distances, among subjects by group. **C,** The alpha (α)
 773 diversity, calculated using the Shannon index and Simpson index in healthy and
 774 HSCT-treated SCID patients. Mean and standard error of mean (SEM) values are

775 indicated. **D**, Bar plot showing the mean of the microbiota Genus abundance (%) in
 776 healthy and HSCT-treated SCID patients. **(E)** and **(F)** Individual Genus abundance
 777 (%) plots in healthy and HSCT-treated SCID patients. **G**, Individual *Streptococcus*
 778 *pneumoniae* (%) plot in healthy and HSCT-treated SCID patients. **H**, show individual
 779 correlation plot between *Streptococcus* Genus abundance (%) and *Dolosigranalum*
 780 Genus abundance (%). In **(B)**, **(E)**, **(F)** and **(G)** box plots with median \pm minimum to
 781 maximum. P values were determined with the Kruskal Wallis test followed by with
 782 Dunn's post test for multiple group comparisons; *P < 0.05, **P < 0.005, ***P <
 783 0.001. In **(H)**, σ represents Spearman coefficient and p the p value.

784

785 **Figure 6. Defective type 2 immunity underlies nasopharyngeal dysbiosis in**
 786 **HSCT-treated GC/JAK3 patients. A**, Nasopharyngeal MUC5AC concentration in
 787 healthy and HSCT-treated SCID patients. **B**, Individual correlation plot between
 788 nasopharyngeal MUC5AC concentration and α -diversity. **C**, Individual correlation plot
 789 between nasopharyngeal MUC5AC concentration and *Dolosigranalum* genus
 790 abundance (%) or *Streptococcus pneumoniae* abundance (%). **D**, Individual
 791 correlation plot between nasopharyngeal MUC5AC concentration and
 792 nasopharyngeal IL-4, IL-5, and IL-13 concentration. **E**, Individual correlation plot
 793 between nasopharyngeal MUC5AC concentration and nasopharyngeal secretory IgA
 794 (SIgA). **F**, Heatmap representation of statistically different (P<0.05) nasopharyngeal
 795 features between GC/JAK3 patients and the other patients and the 3D PcoA
 796 representation. In **(A)** box plots with median \pm minimum to maximum. P values were
 797 determined with the Kruskal Wallis test followed by with Dunn's post test for multiple
 798 group comparisons; *P < 0.05, **P < 0.005. In **(B)**, **(C)**, **(D)** and **(E)** σ represents
 799 Spearman coefficient and p the p value.

Figure 1

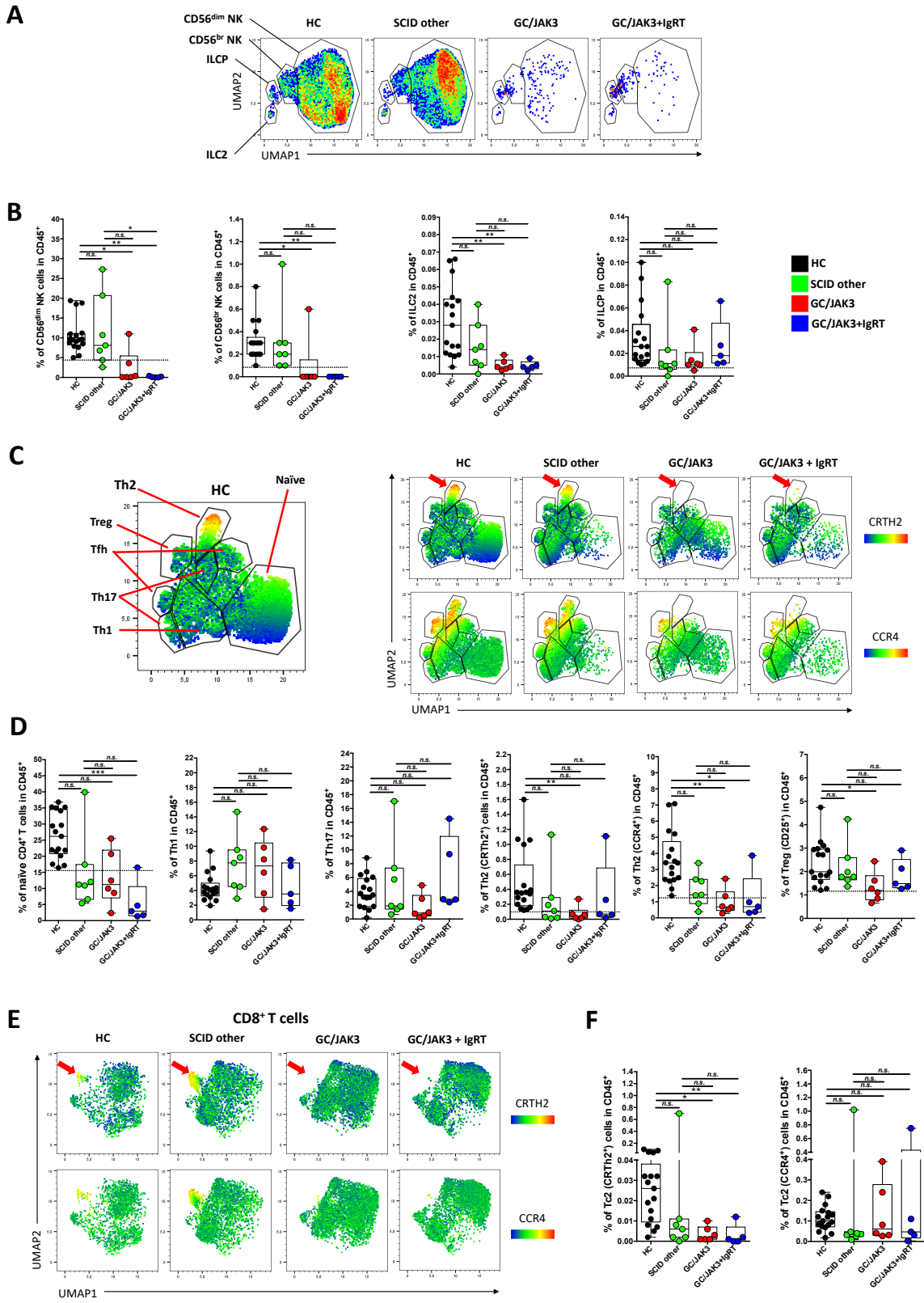


Figure 2

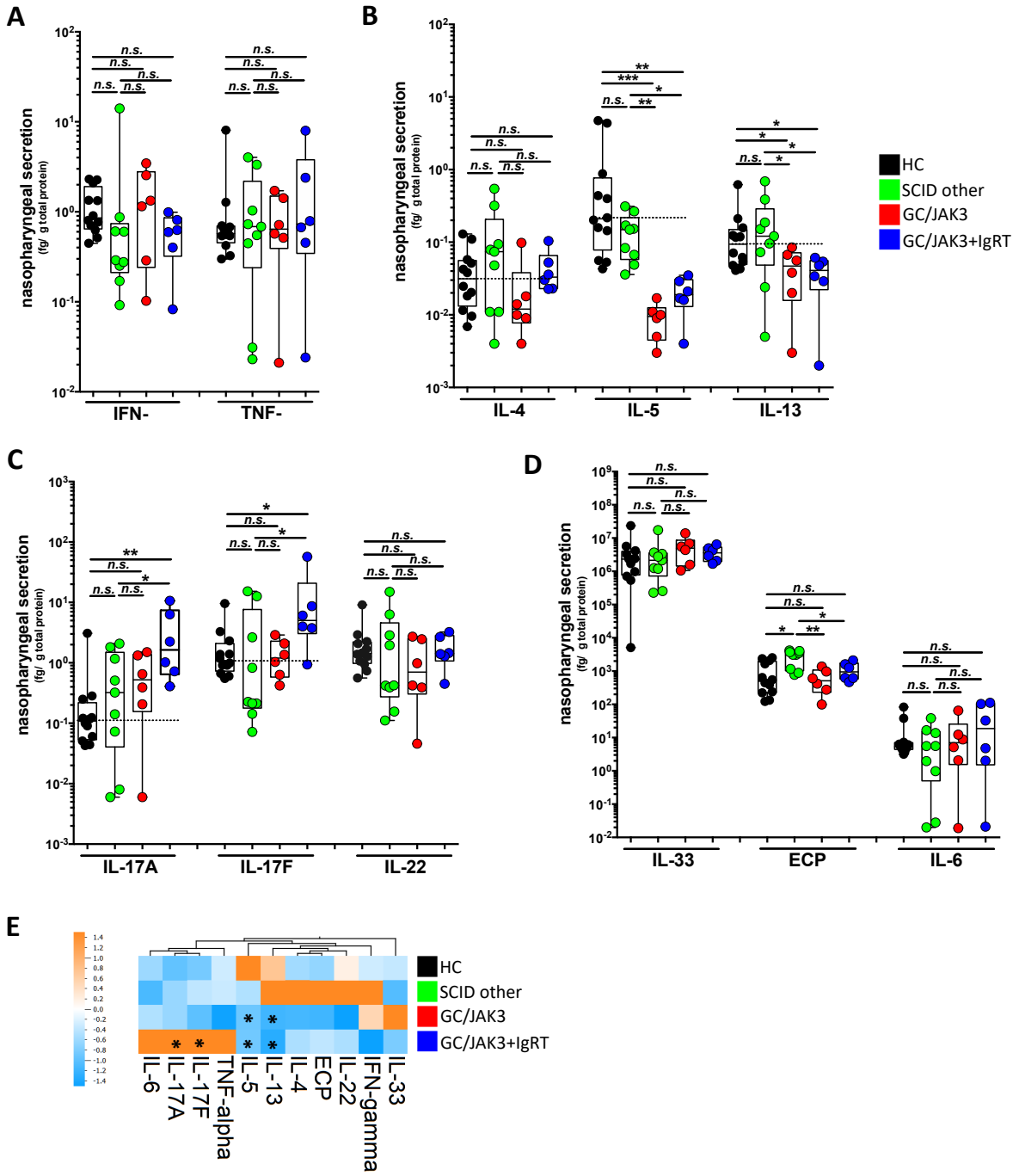


Figure 3

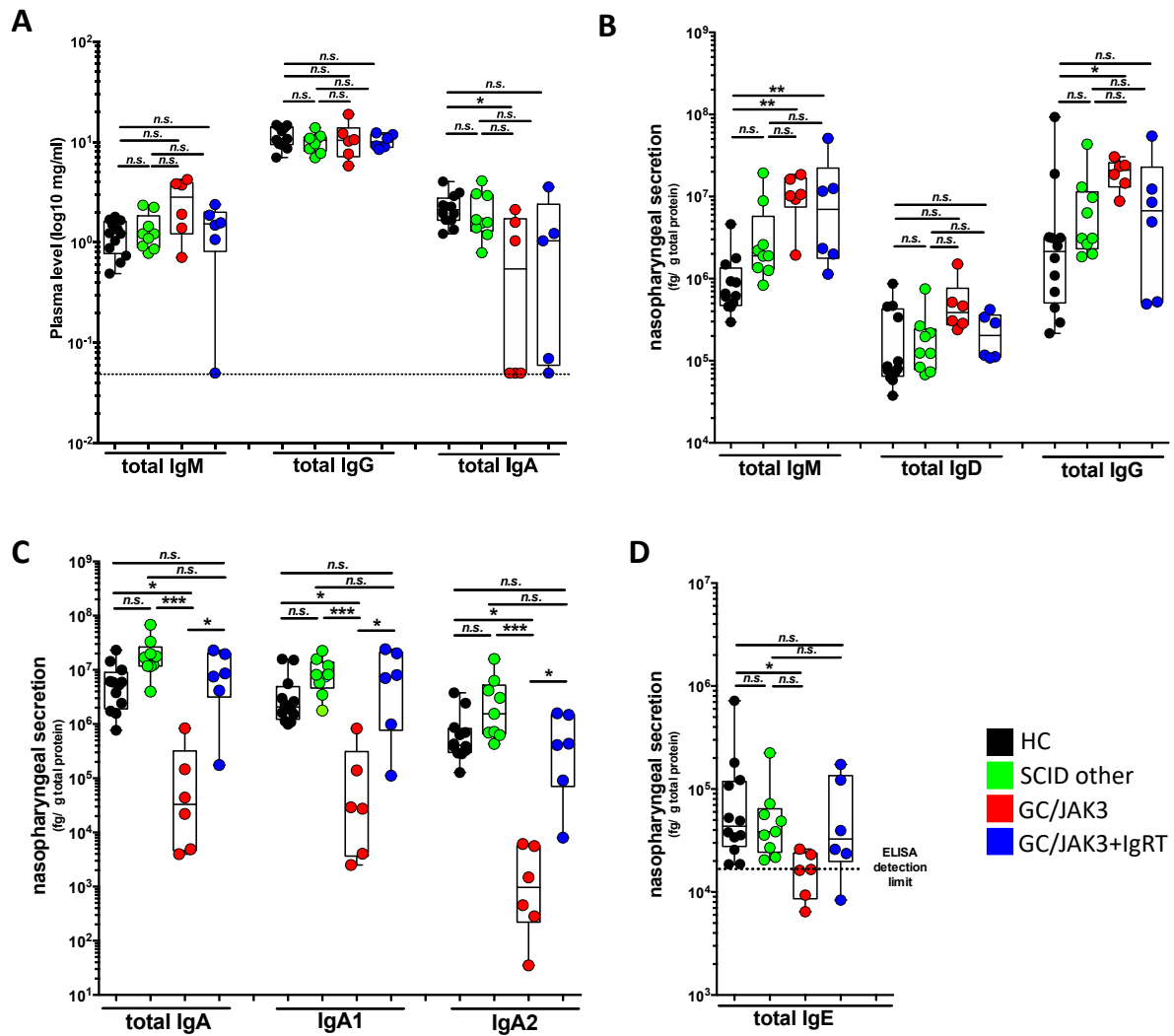


Figure 4

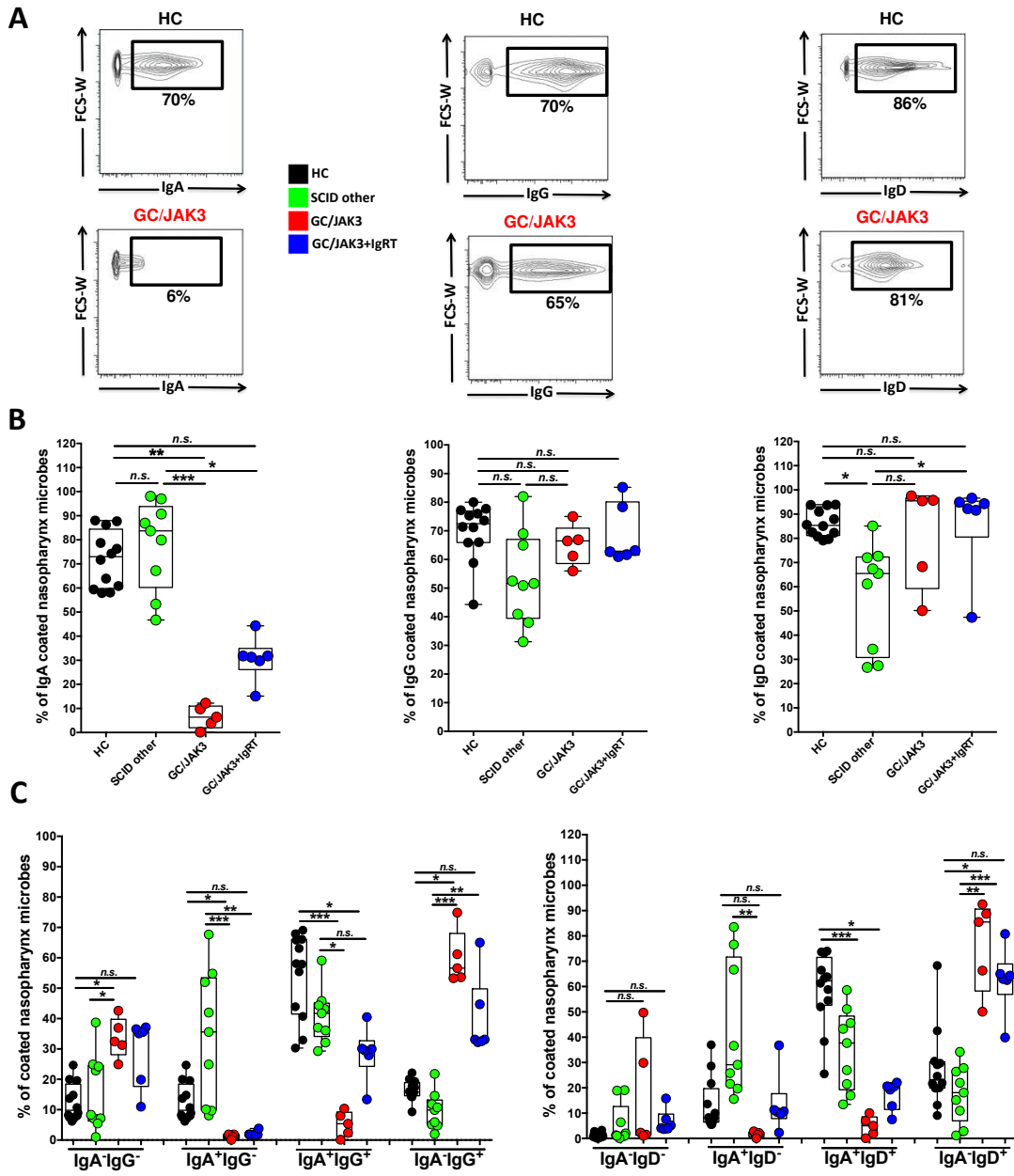


Figure 5

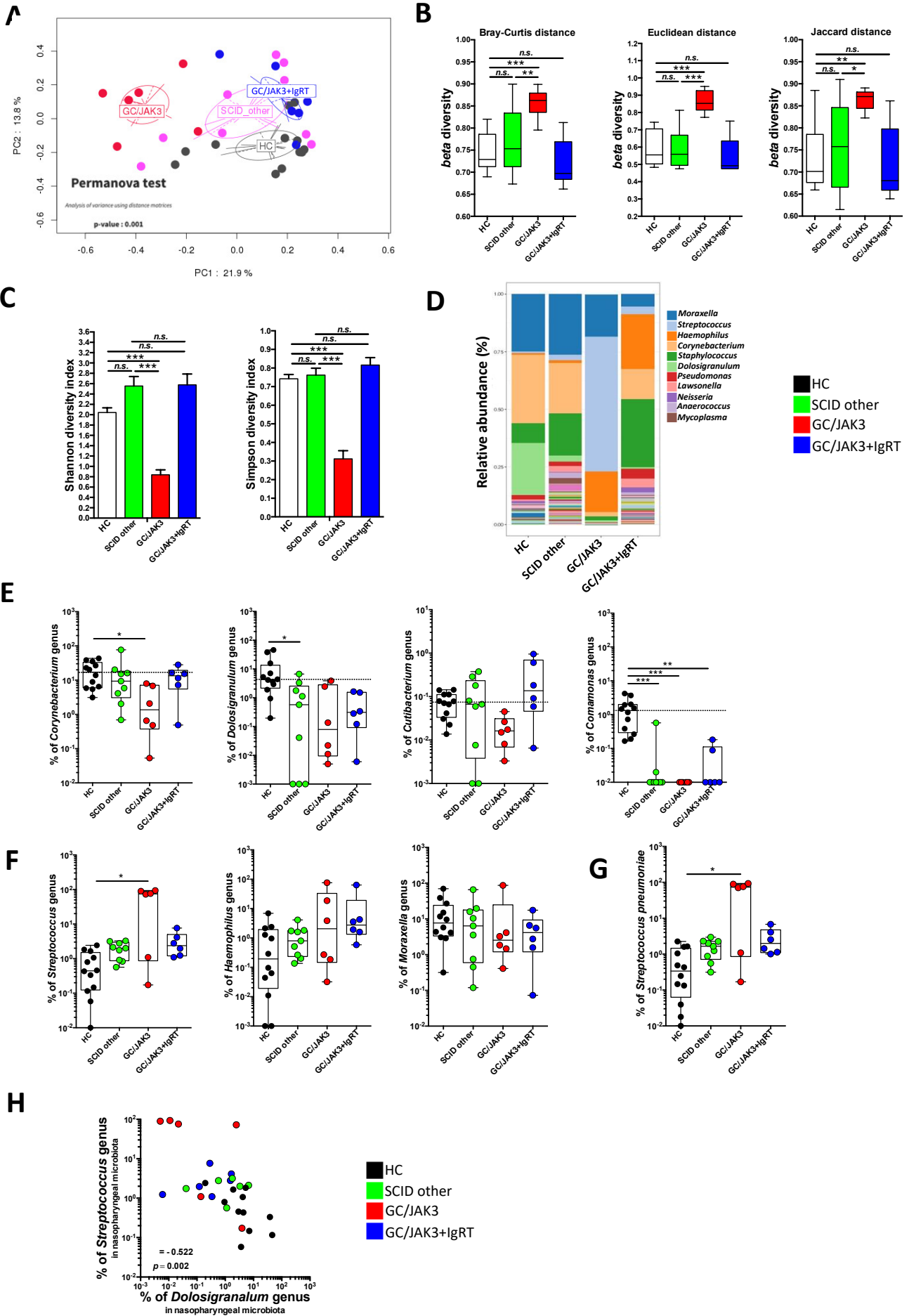


Figure 6

