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

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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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Introduction

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

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

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

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('cure'), it was concluded that NK cells and diverse ILC subsets may be redundant for most aspects of normal human immunity [3,8] that more recently has been extended to development and function of lymphoid tissues [9].

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

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

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Methods

Patient and control cohorts

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

Cell isolation and FACS analysis

Human peripheral blood mononuclear cells (PBMC) were isolated using density gradient centrifugation. For FACS analysis, cells were first stained with Flexible Viability Dye eFluor 506 (eBioscience) following by surface antibodies staining on ice. Fc receptors were blocked using IgG from human serum (MilliporeSigma). Samples were acquired with an LSRFortessa (BD) and analyzed by FlowJ10.7.1 (TreeStar). Bacterial species-specific antibody against microbiota were assessed as described previous [15].

Analysis of nasopharyngeal proteins

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

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

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

16S rRNA sequencing and analysis

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

Bacterial quantification by quantitative RT-PCR assays

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

Statistical analysis

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

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Results

This study analyzed a cohort of 21 SCID patients that have been successfully treated with HSCT at Necker Hospital since 1977. SCID patients with X-linked as well as autosomal etiologies were included, some patients required Ig replacement therapy (IgRT) by subcutaneous injection of IgG for specific clinical conditions (including recurrent respiratory tract infections [16-18]; Supplemental Table 1). Several patients have been previously reported [3,9]. The group 'GC/JAK3' were T-B+NK- SCID (IL2RG, JAK3) patients that received non-myeloablative HSCT, and the group 'SCID other' were T-B-NK+ or T-B+NK+ SCID (RAG1/2, ARTEMIS, MHC Class II, IL7RA) patients the majority of which received pre-transplant cytoreduction (Supplemental Table 1). All HSCT-treated SCID patients showed successful donor hematopoietic reconstitution and recovered T cell immunity. Once clinically stable, patients were discharged and followed up periodically in our outpatient clinic. The follow up period varied from 18 to 42 years.

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

While HSCT-treated SCID patients have stable T cell reconstitution with balanced CD4/CD8 ratios, naïve T cells and TRECs [1,3,19], an in-depth analysis of their differentiated T cell subsets has not been previously performed. ILC and NK cell reconstitution in SCID patients receiving myeloablative conditioning for HSCT has only been reported in 2 RAG-deficient patients [3]. As reciprocal interactions between ILCs and T cells have been documented [20-23], previously reported lack of NK and ILCs in the non-conditioned SCID recipients might be associated with perturbations in their T cell compartment. We paid particular attention to differentiated T cell subsets that can be identified by expression of specific chemokine receptors (see Methods; Supplemental Figure 1A, B for FACS gating) [24,25]. Non-conditioned GC/JAK3 patients receiving IgRT post-HSCT (Supplemental Table 1) were analyzed separately.

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

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and RAG-deficient patients [3] and further extending this observation to other SCID etiologies.

We next characterized naïve and differentiated CD4+ T cell subsets in this SCID cohort. Unsupervised clustering analysis allowed us to identify T cell subsets including naïve (CD45RA+), Treg (CD127loCD25+), Tfh (Treg-CD45RA-CXCR5+), Th1 (Treg-CD45RA-CXCR5-CCR6-CXCR3+CCR4-), Th2 (Treg-CD45RA-CXCR5-CCR6-CXCR3-CCR4+CRTH2+/-) and Th17 (Treg-CD45RA-CXCR5-CCR6+). We observed a significant reduction in clusters of CCR4+CRTH2+/- cells corresponding to the Th2 subset, as well as a reduction in naïve T cells (CD45RA+) in GC/JAK3 and GC/JAK3+IgRT patients compared to HC and SCID-other patients (Figures 1C, D). Other Th subsets (Th1, Th17, Tfh) were normally present in all HSCT SCID patients (Figure 1D, Supplemental Figure 2A, B).

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

The observations of a relative decrease in naïve CD4+ T cells with a compensatory increase in activated $CD8^+$ T cells following HSCT for SCID confirm earlier work [28], while our detailed assessment of differentiated T cell subsets allowed us to identify a selective deficiency in blood $CD4^+$ Th2 cells and CD8+ Tc2 cells in HSCT-treated GC/JAK3 patients. In all SCID etiologies, differentiation of other T helper subsets (Th1, Th17, Treg) appeared largely intact. These results suggest that non-conditioned HSCT-treated GC/JAK3 patients manifest a generalized and selective deficiency in innate and adaptive lymphocytes involved in Type-2 immunity.

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

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

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

Type-2 cytokine secretion is regulated by stromal-derived factors and promotes activation of hematopoietic (eosinophils, mast cells) as well as non-hematopoietic (goblet cells) targets.[29,30] We found that IL-33 (a major inducer of recruitment, activation and IL-5 and IL-13 production by type-2 lymphocytes [31-33]) was not reduced in nasopharyngeal samples (Figure 2D), whereas eosinophil cationic protein (ECP, marker for tissue eosinophilia [34,35]) was reduced in GC/JAK3 patients consistent with reduced IL-5 levels (Figure 2B, D). Nasopharyngeal IL-6 levels were not significantly elevated in any SCID patient although tended to be higher in GC/JAK3+IgRT patients (Figure 2D). When all data was clustered, the heatmap clearly distinguished the defective type-2 cytokine production (in particular, IL-5 and IL-13) in the context of non-conditioned HSCT for GC/JAK3 and irrespective of IgRT (Figure 2E).

Systemic and mucosal Ig subtypes in HSCT-treated SCID patients

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

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

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

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

We next analyzed the binding of different Ig isotypes to nasopharyngeal microbiota using a flow cytometer-based assay [12]. We found a significant decrease in the percentage of IgA-coated nasopharyngeal microbes as well as the density of IgA coating (IgA MFI, not shown) in GC/JAK3 patients compared to SCID-other patients and healthy controls (Figure 4A, B). Interestingly, GC/JAK3+IgRT patients showed a partial but significant increase in IgA-coated nasopharyngeal microbes (Figure 4B) consistent with the increase in nasopharyngeal IgA (Figure 3C). Whether the increase in IgA activity following IgRT results from an indirect effect following stimulation of type 2 cytokine production [39-41] remains unclear.

Compensatory IgG responses to gut commensal bacterial communities may operate in the absence of IgA-specific responses [42]. As other Ig isotypes and subclasses were normally present (IgD) or elevated (IgM, IgG) in GC/JAK3 patients (Figure 3B), we quantitated the fraction (%) and intensity (MFI) of nasopharyngeal microbes that were coated with IgG, IgA or IgD using a recently reported multiplexing

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

HSCT-treated GC/JAK3 patients have nasopharyngeal microbiota dysbiosis

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

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

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patients compared to healthy controls and a selective reduction in *Corynebacterium, Cutibacterium and Staphylococcus* genera in GC/JAK3 patients compared to other SCID patients and healthy controls (Figure 5D, E). These 'cornerstone' bacterial communities (particularly *Corynebacterium* and *Dolosigranulum* genera) can reduce carriage of several pathobionts (*Streptococcus pneumoniae*, *Haemophilus influenzae and Moraxella catarrhalis*) that may be present in normal healthy individuals.[49,50] Relative abundance of *Streptococcus, Haemophilus* and *Moraxella* were not significantly increased in HSCT SCID-other patients compared to healthy controls, although a higher abundance of *Streptococcus* and *Haemophilus* genera were detected in GC/JAK3 patients that were not treated with IgRT (Figure 5D, F). This was in part due to the increased abundance of *S. pneumoniae* (Figure 5G; Supplementary Figure 5D). Finally, a significant negative correlation of *S. pneumoniae* abundance with *Dolosigranulum* genus abundance could be detected in the nasopharynx (Figure 5H).

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

Role for type 2 immunity and nasal IgA in maintaining commensal microbiota diversity and protection against 'pathobiont' carriage

Secretory IgA is implicated in human nasopharyngeal microbial homeostasis [52] and may protect mucosal surfaces from pathogen invasion through agglutination [53,54].

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

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

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Discussion

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

As ILCs abundantly populate mucosal sites, we explored their potential immune roles by comparing HSCT-treated SCID patients that show variable innate lymphocyte reconstitution [3]. We observed clear differences in mucosal immune parameters in a subset of SCID patients with genetic defects in GC or JAK3 which were not conditioned prior to HSCT. We documented a generalized reduction in mucosal type 2 immunity and IgA production and an inability to maintain 'healthy' commensal bacterial communities consistent with a defect in nasopharyngeal mucosal barrier function. In contrast, other SCID etiologies (with or without myeloablative protocols prior to HSCT) showed normal type 2 cytokines and IgA production without nasal dysbiosis. Thus the underlying SCID etiology is apparently a major factor for the observed defects in mucosal immunity.

Local IgA production is a hallmark of mucosal immunity and is largely driven by specific immune responses to resident micro-organisms [57]. Cytokines promote IgG and IgE production [58]. In contrast, the key soluble factors that regulate switch to IgA are a matter of debate [57,59]. Previous studies have documented absence of serum IgA in about half of HSCT-treated GC/JAK3 patients [18,36-38]. Here we show that mucosal IgA is absent in all HSCT-GC/JAK3 patients examined, including those with normal circulating IgA. Interestingly, nasopharyngeal type 2 cytokines were most strongly perturbed in these patients, suggesting a causative link to mucosal IgA production. Type 2 cytokines can promote the survival and differentiation of tissue resident memory B cells and IgA secreting plasma cells [60-62,63,64] thereby increasing IgA production [30,65-68]. Along these lines, a recent report described a major role for

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

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

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

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

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

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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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Contributions

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

Competing interests

The authors declare no competing interests.

Data Sharing Statement

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

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

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Figure Legends

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

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

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

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

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

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

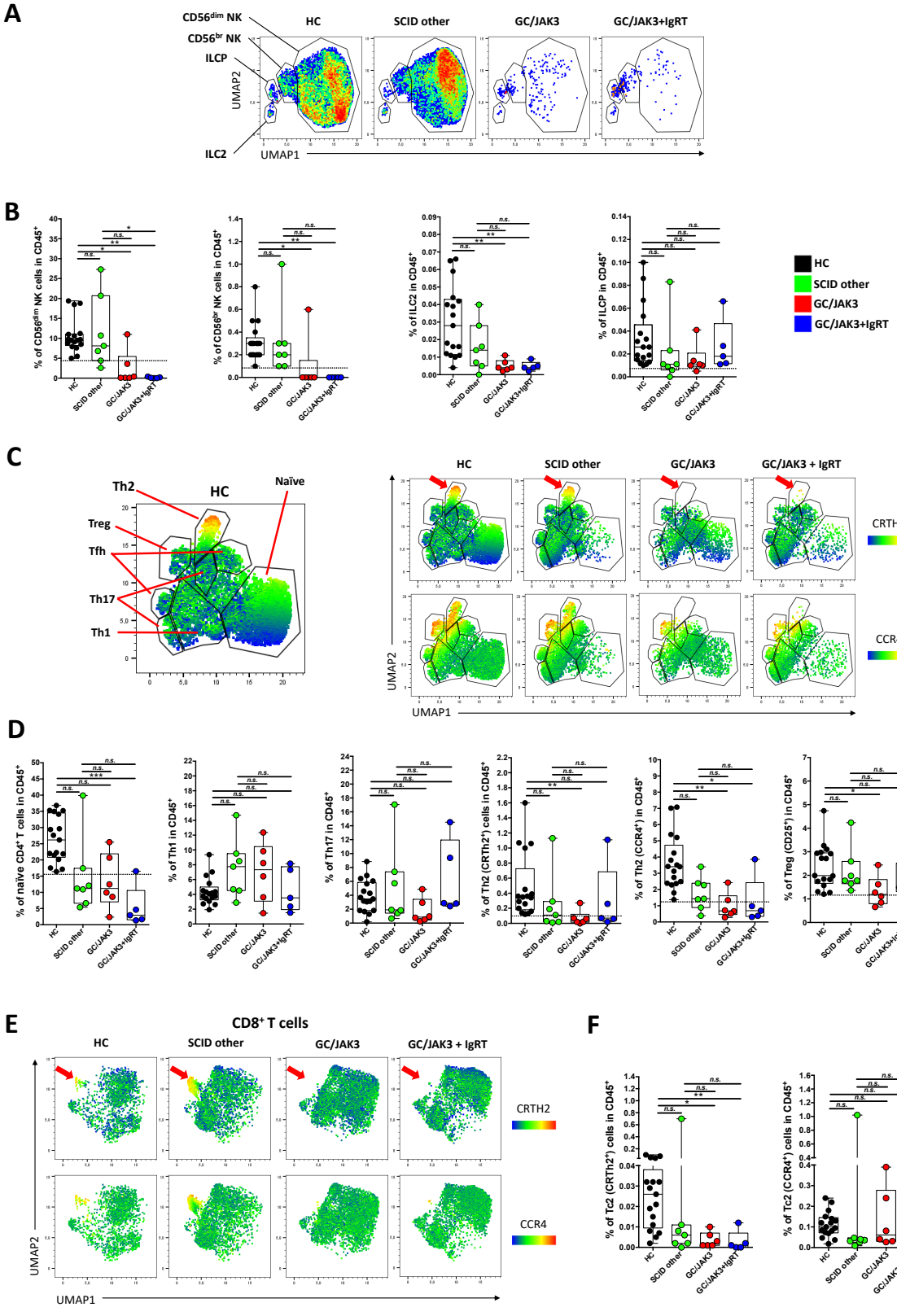
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indicated. **D,** Bar plot showing the mean of the microbiota Genus abundance (%) in healthy and HSCT-treated SCID patients. (**E**) and (**F**) Individual Genus abundance (%) plots in healthy and HSCT-treated SCID patients. **G,** Individual Streptococcus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

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

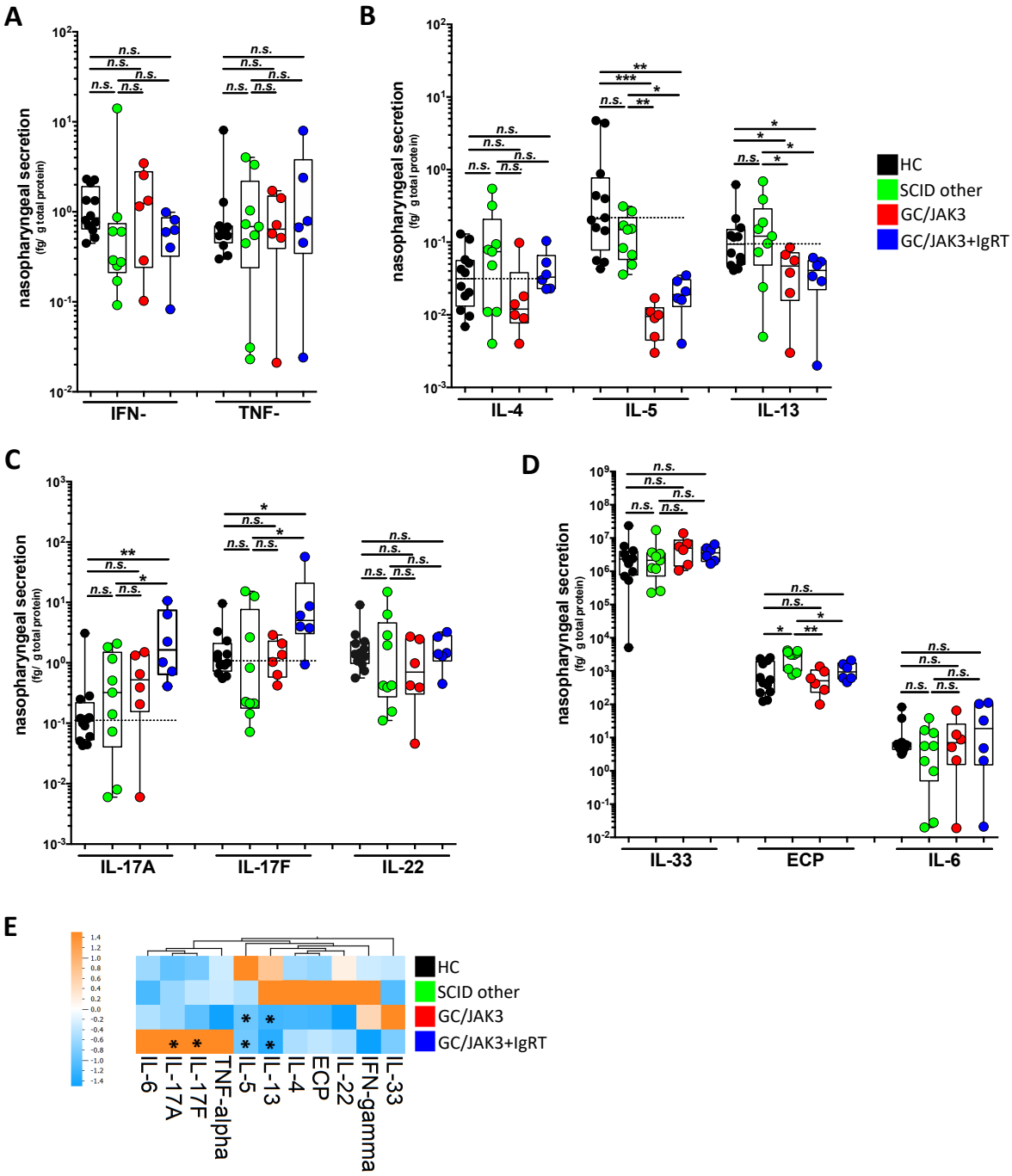
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Figure 1



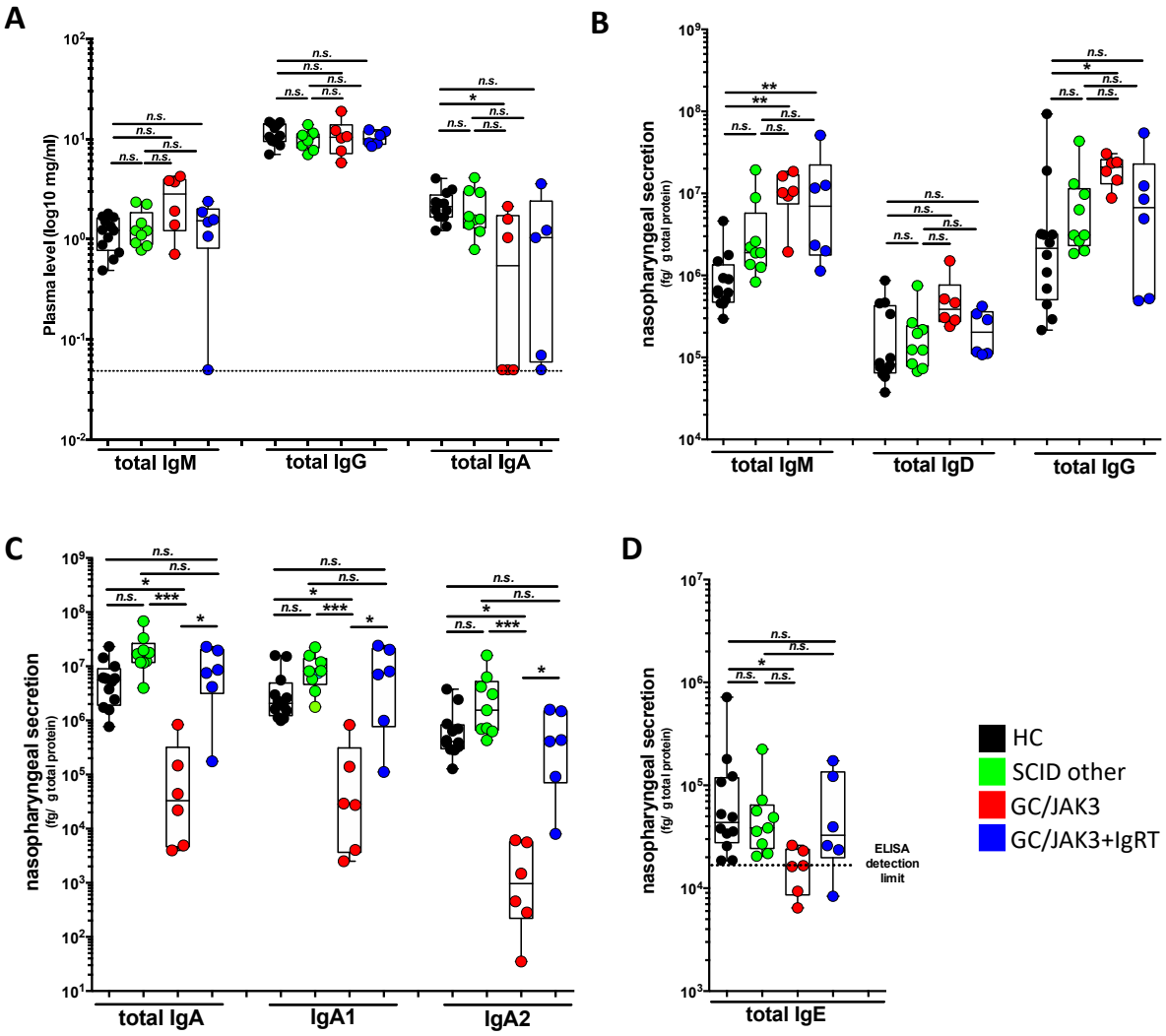
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Figure 2



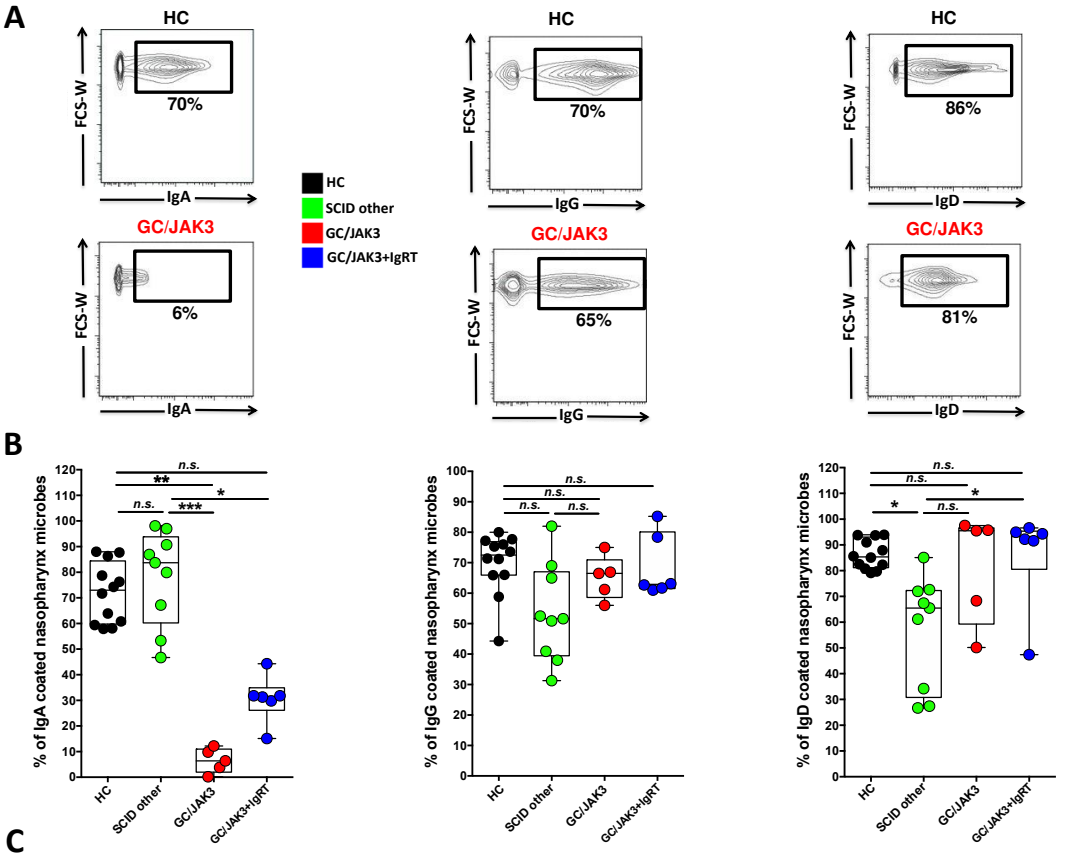
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Figure 3



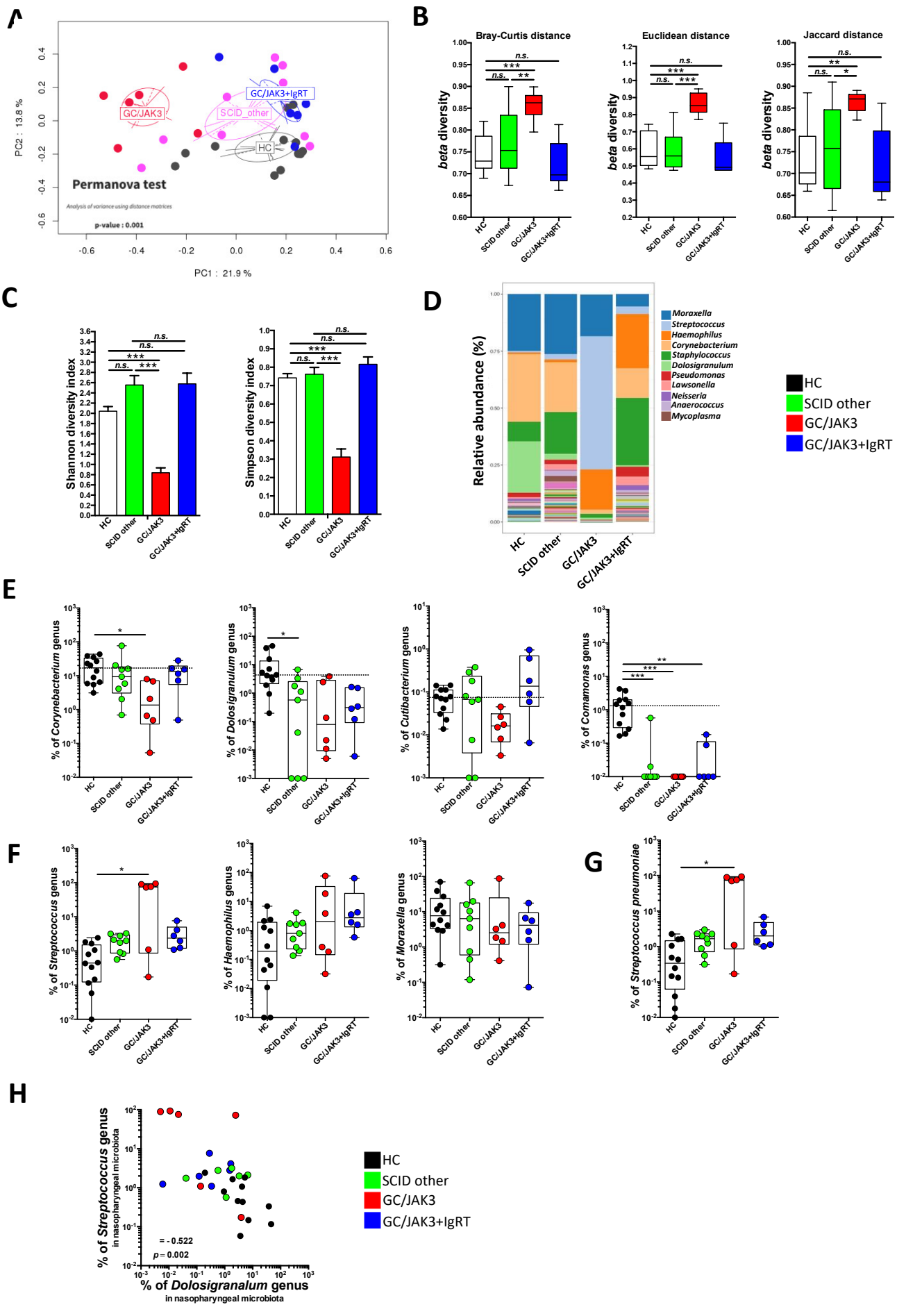
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Figure 4



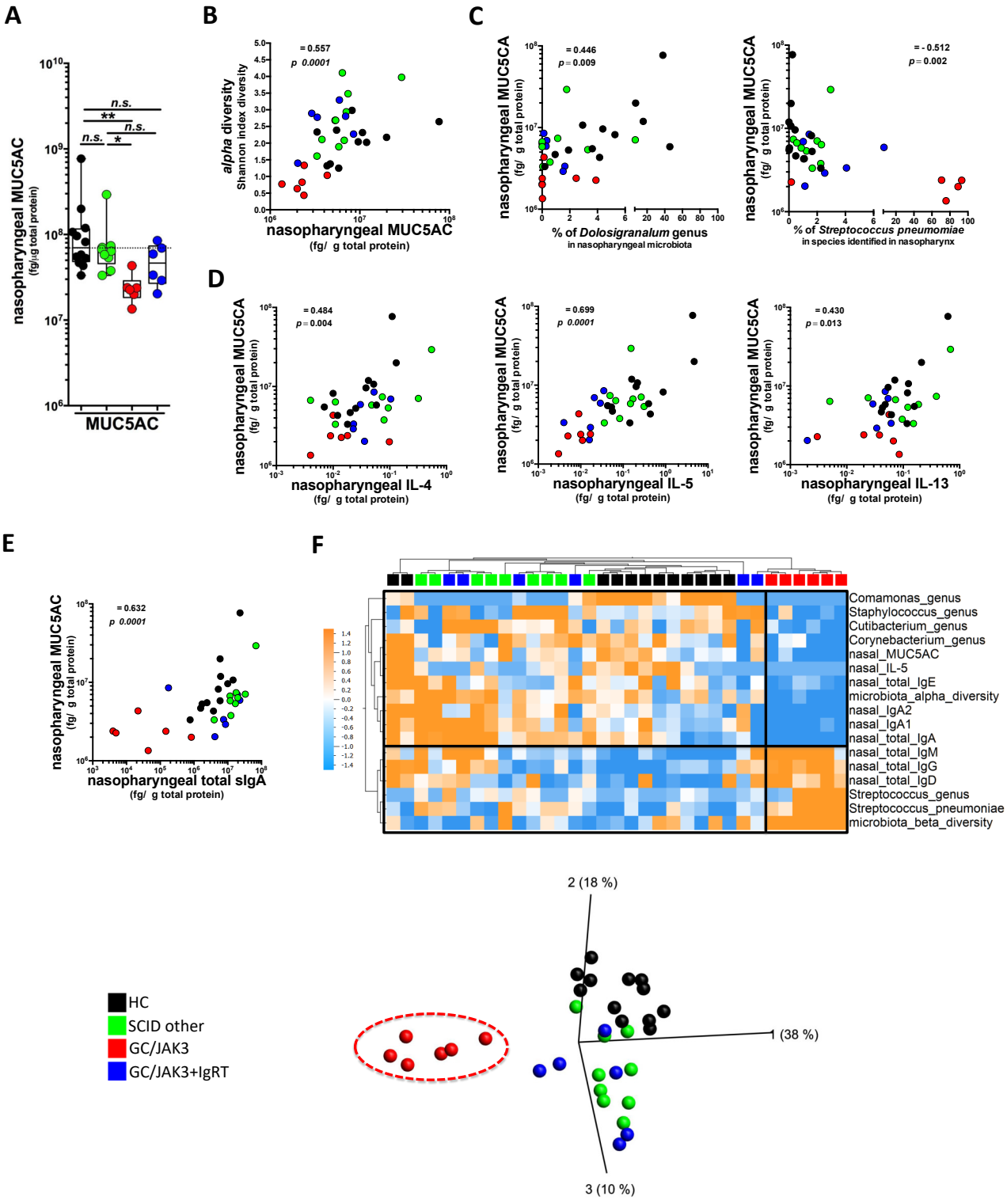
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Figure 5



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Figure 6



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