Supplementary Appendix

The Gut and Blood Microbiome in IgA Nephropathy and Healthy Controls

Table of contents:

Methods **2**

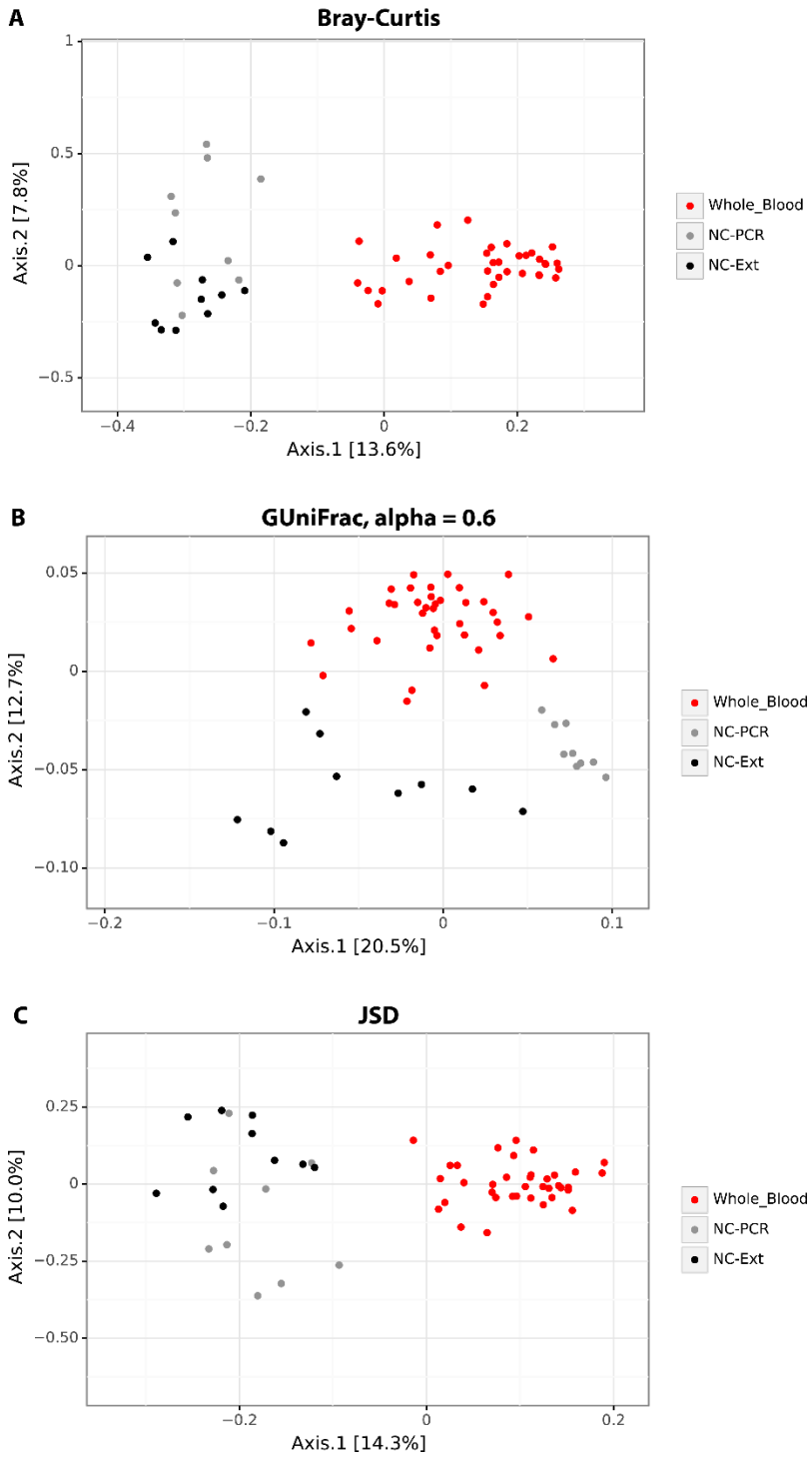
Supplementary figure 1..... **3**

Supplementary figure 2..... **4**

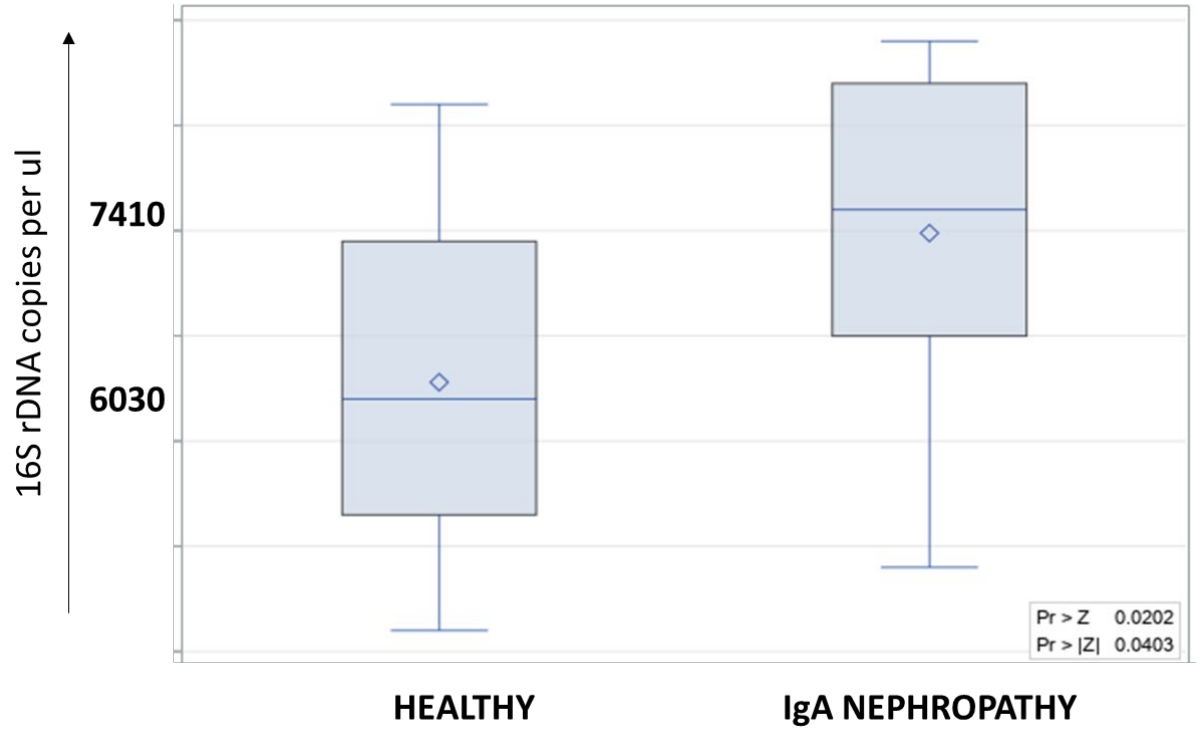
Methods:

16SrDNA amplification and measurement: Total 16S rDNA quantity in DNA extracted from blood samples was measured by qPCR in triplicates using 16S universal primers targeting the V3-V4 region of the bacterial 16S ribosomal gene and normalized using a plasmid-based standard scale20. The efficiency calculated from the standard curve was 91.75% (Normal 80-120%), and the R2 of the standard curve was 0.99 (Normal > 0.98). After successful extraction and amplification, 16S rDNA was measured as number of 16S copies per microliter of blood in triplicates and fell within the standard curve range.16S metagenomic sequencing taxonomic assignment and filters: The taxonomic assignment was performed against the Silva v132 database to determine community profiles.

The following specific filters were applied for this analysis to obtain the best results: a) The last 10 bases of reads R1 were removed; b) The last 40 bases of reads R2 were removed; c) Amplicons with a length of <350 or >500 nucleotides were removed; d) OTUs with abundance lower than 0.005% of the whole dataset abundance were removed. To increase the specificity of bacterial taxa truly different between the IgAN and healthy groups, we lowered the sensitivity by eliminating taxa having proportions <0.005% in more than half subjects in both groups and restricting statistical analysis to the genus level since approximately >70% of taxa at species level were either unknown or had multiple affiliations.



Supplementary figure 1: (A) Comparisons of beta diversities by ordination analysis of the 16S rRNA gene sequencing data using Bray-Curtis, (B) UniFrac with alpha parameters = 0.6; and (C) Jensen-Shannon divergence dissimilarity distances in the blood samples and negative controls (NC-PCR and NC-Ext). NC-PCR: molecular grade water added in an empty tube, amplified and sequenced at the same time as the DNA extracted from blood samples. NC-Ext: molecular grade water added in an empty tube, extracted, amplified and sequenced at the same time as the blood samples.



Supplementary Figure 2: Box and Whisker plot demonstrating significant differences in median 16S rDNA quantity between IgA Nephropathy and Healthy groups