

SUPPLEMENTARY TEXT 5

VALIDATING THE ASSOCIATION OF THE DIET-RESPONSIVE TAXA WITHIN THE INTERVENTION AND CONTROL COHORTS AS WELL AS WITHIN INDIVIDUALS WITH VARYING ADHERENCE TO THE MED-DIET

There were 1224 microbiota datasets corresponding to 612 individuals having matched microbiome profiles for both the baseline and the follow-up time points. To further verify their association with the MedDiet adherence, we checked the variation of the relative abundances of these OTU-groups across an entire adherence landscape. For this we arranged the microbiota data (of the individuals) from the entire intervention study in increasing order of their adherence to the diet, and subsequently divided them into five equally sized overlapping windows (of increasing adherence scores; five overlapping windows of 204 samples with an overlap of 102 samples). Adopting such a window approach would illustrate the gradual transitions of specific changes across an entire adherence landscape (after eliminating variations caused due to specific samples). As expected, profiling the abundance variation of the two taxa groups across the windows identified a progressive increase of the DietPositive taxa (Kruskal Wallis H-test P-value $< 5e-4$) and a concomitant decrease of the DietNegative taxa (Kruskal Wallis H-test P-value $< 3.2e-7$) with increasing adherence to the Mediterranean diet (**Supplementary figure 8**). Performing this window-based analysis separately within the baseline and final time points also revealed the same pattern (**Supplementary figure 8**). We then checked whether the positive and negative associations of the DietPositive and DietNegative taxa in the intervention cohort were also reflected in the across time-point (final to baseline) changes in dietary adherence. For each of the diet-associated markers (i.e. the OTUs), we computed the log fold change in the gain/loss ratios (the number of individuals in whom an OTU is more abundant across the time-points divided by the number of individuals in whom it is decreased) in the intervention cohort with respect to the control cohort. We observed that for the DietPositive taxa, the intervention to control log fold difference of the gain/loss ratios were positive (indicating that the changes were more positive in the intervention cohort as compared to the controls) and significantly higher (Mann-Whitney U test P $< 1.3e-4$) than those obtained for the DietNegative taxa which were negative (indicating a decrease

across time-points in the intervention cohort as compared to the controls) (**figure 2c**). To further profile the changes in the abundance of the markers across individuals with varying degrees of changes in their adherence to the diet, we divided them into three equal tertiles, namely 'High Adherence', 'Medium Adherence' and 'Low Adherence' in decreasing order of their change in adherence across time-points. The abundance changes of the two groups of markers (DietPositive and DietNegative) were then profiled across the three groups separately. As expected, while the DietPositive OTUs had a significantly positive change in the High Adherence as compared to the Low Adherence individuals, an exactly opposite trend was observed for the DietNegative markers (**figure 2d**). These findings suggest that the associations of the specific taxa with diet are stable across cohorts as well as across the changes between time-points.