**SUPPLEMENTARY MATERIAL**

If not otherwise stated, all chemicals were from Sigma-Aldrich (Merck, Darmstadt, Germany).

## Preparation of fecal extracts

Details can be found in the supplementary material Frozen fecal samples were grinded into fine powder by cryomilling in liquid nitrogen using a fecal-specific milling program (SamplePrep 6875D, SPEX, Metuchen, USA) [1]. Pulverized samples were used for the preparation of protein extracts but also the extraction of DNA. Powdered fecal samples were resuspended in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, pH 8), 300 mg sample in 1.5 mL buffer and were subjected to bead-beating at 30 Hz for 2 x 10 min (Stainless Steel Beads 5 mm, Qiagen, Hilden, Germany); Mixer Mill MM400, Retsch, Haan, Germany). After overnight rotation at 4°C, samples were centrifuged at 14,770 x g for 45 min at 4°C. The resulting supernatant was used as a first fraction. The pellet was further extracted using lysis buffer with 4 M urea for protein stabilization and repeating the extraction steps [2]. This second fraction was combined with the first and used for analysis. A Bradford assay was used to determine the protein concentration of each fecal protein extract. 2 µL extract was diluted in 798 µL $H_2O$ and 200 µL assay dye was added (Protein assay dye 5000006, BIO-RAD, Winninglaan, Temse, Belgium). The quantification was performed according to the manufacturers' protocol, using a standard curve of known concentrations of bovine serum albumin (BSA), absorption was measured at 595 nm.

## Enzyme-linked immunosorbent assay (ELISA)

Also, fecal Calprotectin (fCal) was assessed as a measure for intestinal inflammation. Ground frozen fecal samples of peanut-allergic children and healthy age-matched controls were used according to the manufacturer's instructions provided in the kit (EK-CAL, Bühlmann Laboratories AG, Schönenbuch, Switzerland). The established cut-off for fCal is 50 µg/g, with levels higher than that indicating intestinal inflammation. In our cohort, fCal results did not discriminate between peanut-allergic children and controls. Healthy children had a mean fCal value of 75.8 µg/g (range 11.4-260.9 µg/g of fecal pellet), while peanut-allergic patients had mean value of 32.6 µg/g (range 0-185.6 µg/g). Indeed, it has been shown before that healthy children may have fCal levels over this cut-off level and that levels decrease with age [3,4].



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**Multiplex analysis for deep IgE-profiling**

Reactivity profiles of specific IgE (sIgE) antibodies in participant sera and fecal extracts were assessed using the ALEX2 macroarray with 298 allergens (MADX, Vienna, Austria) [5,6]. The assay was performed according to the manufacturer's protocol. Briefly, 100 μL of 1:5-diluted fecal extract or serum samples were used. The readout was done using array-specific software. Per patient, fecal sIgE values above the cut-off for positivity (0.3 $kU_A/L$) were added up to yield the sum of sIgE (e.g. Table 1). The mean sum of fecal sIgE was 31.2 kU/L (range 5.22-75.82 kU/L).



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