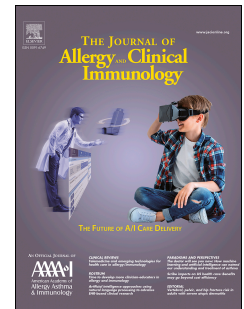


# Journal Pre-proof

Peanut diversity and specific activity are the dominant IgE characteristics for effector cell activation in children

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PII: S0091-6749(21)00358-4

DOI: <https://doi.org/10.1016/j.jaci.2021.02.029>

Reference: YMAI 15006

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 27 August 2020

Revised Date: 4 February 2021

Accepted Date: 18 February 2021

Please cite this article as: Hemmings O, Niazi U, Kwok M, James LK, Lack G, Santos AF, Peanut diversity and specific activity are the dominant IgE characteristics for effector cell activation in children, *Journal of Allergy and Clinical Immunology* (2021), doi: <https://doi.org/10.1016/j.jaci.2021.02.029>.

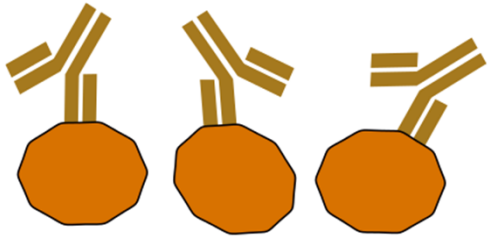
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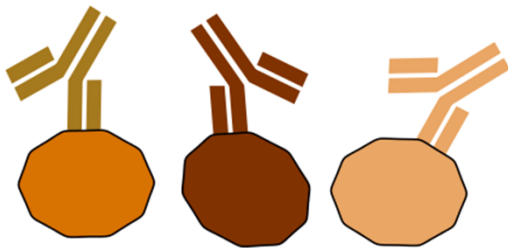
# Peanut diversity and specific activity are the dominant IgE characteristics for effector cell activation in children

## Peanut IgE Specificity



**Specificity** = levels of specific IgE in serum by ImmunoCAP (KU/L)

## Peanut IgE Diversity



**Diversity** = Shannon's diversity index of serum IgE directed to peanut allergen components using ImmunoCAP ISAC



n= 100 children

- 50 peanut allergic
- 40 peanut sensitised tolerant
- 10 non-allergic non sensitised

## Mast cell / Basophil activation

$\beta=0.225$

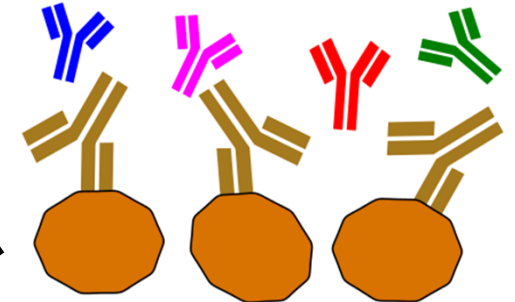
$\beta=0.447$

$\beta=0.440$

$\beta=-0.118$

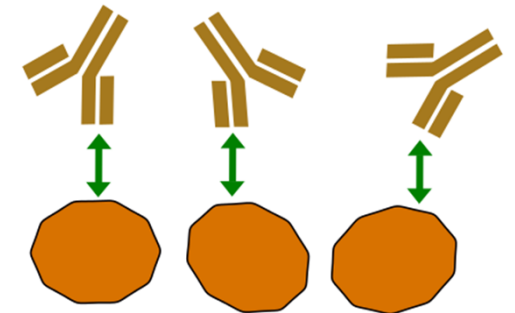
$\beta$ =standardized  $\beta$  coefficient of contribution of each IgE characteristic

## Peanut IgE Specific Activity



**Specific Activity** = ratio of specific IgE to total IgE in serum (%)

## Peanut IgE Avidity



**Avidity** = change in serum IgE binding by chaotropic agent SCN on ImmunoCAP (%)

**Peanut diversity and specific activity are the dominant IgE characteristics for effector cell activation in children**

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**Funding:** This work was supported by the Medical Research Council (MRC Clinician Scientist Fellowship MR/M008517/1, MRC Centenary Early Career Award and MRC Clinical Research Training Fellowship G0902018 awarded to A.F. Santos), Asthma UK (AUK-BC-2015-01) and by the UK National Institute for Health Research comprehensive Biomedical Research Centre award to Guy's and St Thomas' National Health Service (NHS) Foundation Trust, in partnership with the King's College London and King's College Hospital NHS Foundation Trust.

**Conflicts of Interest (COI):** Dr. Santos reports grants and personal fees from Medical Research Council (MR/M008517/1); grants from Asthma UK and the NIHR through the Biomedical Research Centre (BRC) award to Guy's and St Thomas' NHS Foundation Trust, during the conduct of the study; grants from Immune Tolerance Network/National Institute of Allergy and Infectious Diseases (NIAID, NIH), grants from Asthma UK; personal fees from Thermo Scientific, Nutricia, Infomed, Novartis, Allergy Therapeutics, Buhlmann, as well as research support from Buhlmann and Thermo Fisher Scientific through a collaboration agreement with King's College London. The other authors have nothing to disclose.

**Word count:** 3492

**Tables:** 3

**Figures:** 3

**Online repository:** 6 tables and 4 figures

**Abstract:**

**Background:** IgE mediates allergic reactions to peanut; however, peanut-specific IgE (sIgE) levels do not always equate to clinical peanut allergy. Qualitative differences between sIgE of peanut sensitised but tolerant (PS) and peanut allergic (PA) individuals may be important.

**Objective:** To assess the influence of IgE characteristics on effector cell activation in peanut allergy.

**Methods:** A cohort of 100 children was studied. The levels of IgE to peanut and peanut components were measured. Specific activity (SA) was estimated as the ratio of allergen-sIgE to total IgE. Avidity was measured by ImmunoCAP with sodium thiocyanate. IgE diversity was calculated based on ImmunoCAP-ISAC (Immuno Solid-phase Allergen Chip) assays for 112 allergens or for 6 peanut allergens. Whole blood basophils and mast cell line Laboratory of Allergic Diseases 2 (LAD2) sensitized with patients' plasma were stimulated with peanut or controls and assessed by flow cytometry.

**Results:** SA to peanut ( $p<0.001$ ), Ara h 1 ( $p=0.004$ ), Ara h 2 ( $p<0.001$ ), Ara h 3 ( $p=0.02$ ) and Ara h 6 ( $p<0.001$ ) and the avidity of peanut-sIgE ( $P<0.001$ ) was higher in PA than in PS individuals. Diversity for peanut allergens was greater in PA individuals ( $p<0.001$ ). All IgE characteristics were correlated with basophil and mast cell activation. Peanut SA ( $R=0.447$ ) and PD ( $R=0.440$ ) had the highest standardised  $\beta$ -coefficients in combined multivariable regression models (0.447 and 0.440, respectively).

**Conclusions:** IgE specificity, SA, avidity and peanut diversity was statistically different in PA and PS individuals. IgE Peanut SA and PD had the greatest influence on effector cell activation and could be employed clinically.

Abstract word count: 247

**Key messages:**

- IgE specificity, specific activity, avidity and diversity are significantly different between peanut allergic and peanut sensitized tolerant individuals and are positively correlated with effector cell activation.

- Multivariable models indicate that IgE diversity to peanut allergens and peanut-IgE specific activity have the greatest influence on effector cell activation.

#### **Capsule Summary (36 words):**

The functional characteristics of IgE can discriminate between peanut allergic and tolerant individuals and can modulate effector cell activation. Peanut diversity and specific activity (proportion of allergen-specific IgE) have the greatest effect on the allergic response.

**Key words:** Avidity, diversity, specificity, specific activity, specific IgE, mast cell activation test, peanut allergy, CD63

#### **Abbreviations:**

AIT – Allergen-specific immunotherapy

AUC - Area Under the Curve

BSA – Bovine serum albumin

LAD2 - cell line named after the NIH's Laboratory of Allergic Diseases

(ImmunoCAP) ISAC - Immuno Solid-phase Allergen Chip

MAT - Mast Cell Activation Test

NA – Non sensitized Non-Allergic

OFC - Oral Food Challenge

PA - Peanut Allergic

PD – Peanut Diversity

PS - Peanut Sensitized but Tolerant

ROC - Receiver Operating Characteristic

RPMI - Roswell Park Memorial Institute media

SA – Specific Activity

sIgE - Specific IgE

- 108 SPT - Skin Prick Test
- 109 tIgE - Total IgE
- 110 WD - Whole diversity

## 111 Introduction:

112 Allergen-specific IgE (sIgE) detection as a diagnostic tool reports many false-positives<sup>1, 2</sup>. For  
 113 some foods, including peanut, sIgE testing has improved dramatically over recent years  
 114 through incorporation of allergen components<sup>3, 4</sup>. Alongside component testing, *in vitro*  
 115 effector cell assays, such as the basophil activation test (BAT), have shown better diagnostic  
 116 accuracy and discrimination between allergic and sensitised but tolerant individuals<sup>5</sup>. A  
 117 possible explanation for the superiority of BAT in reflecting individuals' food allergic  
 118 phenotype is that it considers all characteristics of sIgE, rather than only levels, as well as  
 119 possible blocking antibodies and intrinsic basophil sensitivity<sup>6, 7</sup>. The mast cell activation test  
 120 (MAT) is also more specific than sIgE alone<sup>8</sup> and has the advantage of not requiring fresh  
 121 blood like the BAT. As the mast cells are homogenous and propagated from a single human  
 122 mast cell<sup>9</sup>, sensitized with plasma from different subjects and washed prior to allergen  
 123 stimulation, the MAT is well suited as an *in vitro* model to study the functional characteristics  
 124 of IgE.

125 Examples of intrinsic functional characteristics of IgE likely to influence effector cell  
 126 degranulation in response to allergen are: specificity, specific activity, diversity and affinity<sup>6</sup>.  
 127 IgE specificity is known to correlate with allergic status, illustrated by the dominance of Ara h  
 128 2-sIgE as a diagnostic marker in peanut allergy<sup>3, 4, 10</sup>. Conversely, levels of total IgE (tIgE) alone  
 129 are considered useless as standalone in the diagnosis of specific allergies; however, the  
 130 proportion of specific IgE to total IgE, known as IgE specific activity (SA), which reflects the  
 131 proportion of IgE bound to effector cells that is specific for a given allergen, may be more  
 132 informative<sup>11-13</sup>. An individual's reactivity to allergen is likely affected by the proportion of  
 133 non-sIgE and its occupation of FcεRI on the surface of effector cells<sup>12, 14</sup>. Perhaps, an  
 134 increased diversity in IgE repertoire is likely to dampen an allergen-specific response through  
 135 the reduced chance of crosslinking on the cell surface<sup>13</sup>. Conversely, IgE diversity to different  
 136 epitopes within one allergen has shown to increase basophil sensitivity to allergen and can  
 137 possibly increase basophil reactivity<sup>15-17</sup>. Both IgE diversity and basophil reactivity have been  
 138 associated with severity of peanut allergy. IgE affinity for allergen is likely to influence  
 139 effective IgE crosslinking able to cause effector cell activation<sup>18</sup>. For polyclonal IgE responses  
 140 to multivalent allergens, avidity, which refers to the collective effect of a combination of  
 141 antigen-antibody interactions, reflects more accurately the strength with which IgE binds to  
 142 allergen than affinity, which refers to molecular interactions between IgE and its epitopes<sup>19</sup>.

In this study, we aimed to determine the relative importance of IgE characteristics through the assessment of their diagnostic accuracy, influence on basophil and mast cell degranulation and interplay with each other.

## **Methods:**

### **Study population:**

Peanut-allergic (PA), peanut-sensitized but tolerant (PS), and non-sensitized non allergic (NA) individuals were evaluated as previously described<sup>5</sup>. Peanut allergy was determined by positive response to oral food challenge (OFC), except for patients who had convincing clinical history of systemic allergic reactions within 1 year of sample collection, together with a skin prick test (SPT) wheal size  $\geq 8$  mm and/or peanut-sIgE  $\geq 15$  KU<sub>A</sub>/L<sup>20</sup>. Peanut tolerance was defined by a negative OFC or the ability to ingest  $\geq 4$ g of peanut protein, twice weekly, without demonstrating an allergic response, as assessed by a validated peanut consumption questionnaire<sup>21</sup>. Peanut sensitization was determined by SPT  $\geq 1$ mm and/or a peanut-sIgE  $\geq 0.1$  KU<sub>A</sub>/L. Children who were clinically unwell, had significant chronic illness, or were unwilling to participate were not included. One hundred patients were selected consecutively in chronological order of recruitment based on availability of plasma samples with sufficient volume. The study was approved by the South East London Research Ethics Committee 2, and written informed consent was obtained from parents of all children.

### **Allergen-specific IgE measurements:**

ImmunoCAP ISAC (Immuno Solid-phase Allergen Chip) (Thermo Fisher, Uppsala, Sweden) was carried out according to manufacturer's instructions. IgE binding to each allergen array was recorded using a LuxScan 10K scanner (Core Life Sciences, Irvine, California) and data analyzed using the Phadia Microarray Image Analysis (MIA) Software.

### **IgE specific activity determination**

IgE specific activity was determined by calculating the ratio of allergen-sIgE to total IgE levels, measured using the ImmunoCAP assays as detailed above, and expressed as percentage, using the following formula:

$$\frac{\text{Specific IgE to peanut/Ara h 1/2/3/6/8/9}}{\text{Total IgE}} \times 100$$

### Avidity measurements

Avidity assays were performed to estimate the cumulative affinities of peanut sIgE in plasma of both PA (n=36) and PS (n=24) patients. The assay was developed based on avidity ELISA's using thiocyanate solutions to interfere with antibody:antigen interactions<sup>22, 23</sup>. To determine optimal concentration of sodium thiocyanate (NaSCN), plasma samples from 3 individuals, one PA (AS103) with peanut sIgE levels closer to top of detection limit (100 kUA/L), one PA individual (AS163) with peanut sIgE levels towards the lower detection limit of the assay, however with enough IgE to allow accurate inhibition (~20kUA/L), and a PS individual (AS183) with similar peanut IgE levels (~20kUA/L). We assayed plasma from each individual, in a 1:1 ratio, with NaSCN from 0-8M concentrations. 2M NaSCN was selected as the optimal concentration, as the lowest concentration to cause measurable reduction in IgE binding, across both PA and PS individuals, see figure E3. For avidity assays across the whole cohort, samples were selected based on volume and peanut-sIgE level  $\geq 1\text{kUA/L}$ . This minimum detection limit ( $\geq 1\text{kUA/L}$ ) was based upon linearity experiments, in which serial dilutions spanning the detection limit of the whole assay were measured. For avidity estimations, patient plasma was incubated for 20 minutes at room temperature, with either PBS (negative control) or 2M NaSCN. Pre-incubated samples were then assayed for peanut-sIgE using ImmunoCAP. Avidity results were reported as '100 - %binding decrease' following NaSCN incubation and calculated using the formula below:

$$100 - \frac{(\text{sIgE level w/NaSCN})}{\text{sIgE level w/PBS}} \times 100$$

### Diversity measurements:

The diversity of an individual's IgE repertoire was generated from ImmunoCAP ISAC assays. Shannon's diversity index<sup>24</sup>, was employed to transform the data as follows:

$$H = - \sum_{i=1}^s p_i \ln p_i$$

where  $H$  = diversity index and is the reported output;  $i = (1, \dots S)$ ,  $S$  = total number of allergens measured,  $P_i$ =the proportion (relative abundance) of allergen,  $i$ , in total amount of allergen specific IgE levels.

#### Basophil and mast cell activation tests:

Whole blood BAT and MAT were performed as previously described<sup>5, 8, 25</sup>, with BAT performed on 54 individuals (PA=23, PS=31) and MAT performed on 100 individuals (PA=50, PS=40, NA=10). For the BAT, heparinized whole blood was stimulated for 30 minutes at 37°C with peanut extract diluted in RPMI media resulting in serial 10-fold dilutions from 10 µg/mL to 0.1 ng/mL. Anti-IgE (1 µg/mL) and fMLP (1 µM) were used as positive controls and RPMI alone as the negative control. For the MAT, LAD2 cells<sup>26</sup> were cultured in rIL-4 5 days prior to overnight sensitization with patient plasma, as previously described. Serial dilutions of peanut extract (0.1-1000 ng/mL) or controls (Anti-IgE 1 µg/ml, ionomycin 1 µg/mL or 0.04% BSA RPMI) were added to pre-sensitized cells and incubated for one hour at 37°C. Basophils were identified as SSClow / CD203c+ / CD123+ / HLA-DR- and mast cells as FSC/viable cells. For both BAT and MAT, surface expression of CD63 was measured by flow cytometry using FACS Canto II with FACSDiva software (BD Biosciences) and data was analyzed using FlowJo software version 7.6.1 (TreeStar).

#### Statistical analyses:

Comparisons of groups were done using Mann-Whitney U test and Kruskal-Wallis P tests. The diagnostic performance of all measurements was examined against allergic status to peanut using receiver-operating characteristic (ROC)-curve analyses. Associations between individuals' characteristics and mast cell activation have been analysed using spearman's rank correlation, with mast cell activation distributed in a non-normal fashion. Linear regression models were generated for each characteristic of IgE investigated in this study, with their regression ( $\beta$ ) coefficients from univariable analyses reported in table 2. Multivariable linear regression models were designed including peanut sIgE levels, peanut SA, peanut avidity, peanut diversity and whole diversity, in a number of combinations as outlined in table 3. For standardized beta ( $\beta$ ) coefficients, input data was standardized by generating Z scores, using the following formula, prior to performing linear regression analyses:

$$Z = \frac{x - \mu}{\sigma}$$

$\sigma$

Where,  $Z$ = standardized data point,  $x$ = observed data point,  $\mu$ =mean of sample and  $\sigma$ =standard deviation of the sample. Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, San Diego, CA), SPSS 25.0 (IBM SPSS Statistics, New York) and MedCalc 19.4.1 (MedCalc Software, Ostend, Belgium).

## **Results:**

### **Study population**

One hundred children (50 PA, 40 PS, 10 NA), aged 5 months to 17 years, 69% male, were studied. PA individuals were significantly older than PS individuals ( $p=0.003$ ). All demographic, clinical and serological features of the study population are shown in **Table 1**.

### **Peanut-specific IgE is strongly correlated with mast cell activation**

We previously reported, sIgE levels to peanut ( $p<0.01$ ), Ara h 2 ( $p<0.0001$ ), Ara h 3 ( $p=0.046$ ) and Ara h 6 ( $p<0.0001$ ) were significantly higher in PA than in PS individuals, with Ara h 2 and Ara h 6 showing the greatest diagnostic utility<sup>10</sup>. The other components tested did not discriminate between PA and PS individuals (**Figure E4**).

Here we assessed to what degree levels of sIgE correlated with peanut-induced mast cell activation. Peanut-sIgE was strongly correlated with peanut-induced mast cell activation reporting an R value of 0.787 ( $p<0.0001$ ), with Ara h 2 reporting 0.666 ( $p<0.0001$ ), Ara h 6 0.658 ( $p<0.0001$ ) and Ara h 1 0.621 ( $p<0.0001$ ) - **Figure 3A, figure E1, Table 2**. A Full characteristic correlation matrix, with corresponding P values has been reported in Tables E4a/b.

### **IgE specific activity improves the discrimination between peanut allergic and peanut sensitized tolerant**

IgE SA to peanut ( $p<0.0001$ ), Ara h 1 ( $p=0.0038$ ), Ara h 2 ( $p<0.0001$ ), Ara h 3 ( $p=0.02$ ) and Ara h 6 ( $p<0.0001$ ) were higher in PA than PS individuals (**Figure 1A, Table 2**). In ROC analyses, peanut and allergen components showed a larger area under the ROC curve for SA than sIgE alone – for instance, Ara h 2 SA had an AUC of 0.933, followed by Ara h 6 (0.930), whole peanut (0.862), Ara h 3 (0.738) and Ara h 1 (0.716), (**Figure 2A, Table E2**), with peanut SA

showing a significantly higher AUC ( $p=0.0038$ ) than peanut sIgE, in the same population (Table E6). SA correlated with mast cell activation across both PA and PS individuals ( $n=90$ ), with peanut SA reporting the highest R value of 0.659 ( $p<0.0001$ ), followed by Ara h 2 (0.515,  $p<0.0001$ ) and Ara h 6 (0.513,  $p<0.0001$ )- Figure 3B, figure E2; Table 2.

#### **Avidity of IgE for peanut is higher in allergic patients and correlates with mast cell activation**

PA individuals ( $n=36$ ) showed a significantly higher avidity score (mean  $\pm$  standard deviation= $41.82\pm13.00$  %inhibition of IgE binding), than PS ( $n=24$ ) individuals (mean  $\pm$  standard deviation = $27.41\pm10.95$ ) ( $p<0.0001$ , Mann-Whitney U test); however, there was an overlap between the groups (Figure 1B). The avidity ROC analyses exhibited an AUC of 0.795 (Figure 2B, Table E2/E3). There was a very weak, but insignificant, correlation between the avidity of peanut-sIgE and mast cell activation in response to peanut ( $R=0.236$ ;  $p=0.069$  Figure 3C).

#### **IgE diversity for peanut allergens strongly correlates with mast cell activation**

To measure IgE diversity, we transformed the ISAC data using Shannon's index to create two variables: whole diversity (WD), incorporating all 112 allergens, and peanut diversity (PD), considering the peanut allergens Ara h 1, 2, 3, 6, 8 and 9. Diversity within the peanut allergens was significantly greater ( $p<0.0001$ ) in PA (mean=0.89) than PS (mean=0.16) individuals; however, WD did not discriminate ( $p=0.495$ ) between PA (mean=1.96) and PS (mean=1.81) individuals (Figures 1C/D). ROC analyses were performed to see how these variables were distinct in allergic versus non-allergic subjects (Figure 2C, Table E2). The AUC was 0.898 for PD and 0.628 for WD. PD also reported greater correlation with mast cell activation ( $R=0.649$ ,  $p<0.0001$ ) compared to WD ( $R=0.494$ ,  $p<0.0001$ ) - Figures 3D/E.

#### **Peanut IgE diversity and specific activity are the major determinants of mast cell and basophil activation**

Having studied each characteristic of IgE separately, we assessed their relative importance and influence on effector cell activation. We generated logistic regression models incorporating the most important IgE characteristics based on their ability to discriminate between the PA and PS populations, their correlation with LAD2 cell activation and significant ( $p\text{-value}<0.2$ ) standardised  $\beta$ -coefficients in simple logistic regression (Table 3).

Four multivariable mechanistic logistic regression models were designed, incorporating three characteristics dissimilar in nature and acquisition, to reduce the probability of collinearity. Model 1 incorporated peanut-sIgE, PD and avidity, generating standardised  $\beta$ -coefficients of 0.225 ( $p=0.066$ ), 0.534 ( $p<0.0001$ ) and -0.092 ( $p=0.436$ ), respectively. This suggested peanut IgE diversity to peanut allergens was the most influential characteristic of IgE, in mast cell activation. This effect was mirrored when assessing basophil activation, see supplementary model s5, albeit with a greater relative influence reported by peanut diversity. In model 2, peanut-sIgE was replaced with peanut-SA, and the influence that PD had on effector cell activation was lower compared to model 1 but still statistically significant. Peanut SA, PD and avidity displayed coefficients of 0.447 ( $p<0.0001$ ), 0.440 ( $p<0.0001$ ) and 0.118 ( $p=0.2580$ ) respectively, with both PD and peanut-SA reporting significant impact on effector cell activation.

Models 3 and 4 mirrored models 1 and 2; however, WD was incorporated to replace PD. In model 3, peanut-sIgE was the only characteristic to influence effector cell activation with any significance, exhibiting a standardised  $\beta$ -coefficient of 0.472 ( $p<0.0001$ ). A similar effect was seen in model 4 with peanut-SA replacing peanut-sIgE. Similar trends were seen considering basophil activation in models S6 and S7. Peanut-SA greater influenced activation, reporting a standardised  $\beta$ -coefficient of 0.668 ( $p<0.0001$ ). Models 3 and 4 showed the positive impact peanut avidity measurements may have in the prediction of effector cell activation, albeit with poor significance. Supplementary models were designed to directly compare the influence of similar characteristics (**Table E5**). Of note, supplementary model S2, containing only peanut-sIgE and SA, reports SA with a greater standardised  $\beta$ -coefficient and significance.

Given that the biology of primary basophils can differ from LAD2 cells, namely in terms of IgE receptor density and intrinsic cellular sensitivity, which can vary between patients, we assessed the relative importance of the same IgE characteristics on basophil activation. Univariable and multivariable logistic regression models were fitted using the available whole blood BAT results in the same patient population ( $n=54$ ) - **Table 1**<sup>5</sup>. Both peanut-SA and PD reported the highest standardised  $\beta$ -coefficients across both univariable, 0.762( $p<0.0001$ ) and 0.843 ( $p<0.0001$ ), respectively, and multiple multivariable regression models, such as supplementary model 4, reporting standardised  $\beta$ -coefficients of 0.335 and 0.690 respectively (**Tables E7 and E8**).

## Discussion:

Levels of allergen-sIgE do not always reflect clinical reactivity to peanut, which raises the hypothesis that, beyond levels, the qualitative characteristics of sIgE may influence effector cell response to peanut allergens. In this study, we tested samples from a well-characterized cohort of 100 children being assessed for peanut allergy on the BAT/MAT to peanut, which reflects *in vitro* the clinical reactivity to peanut<sup>8</sup>, and quantified specific activity, diversity and avidity of IgE for peanut allergens in each subject. PA children had higher proportion of allergen s-IgE, higher diversity and higher avidity of IgE for peanut allergens. These variables, as well as peanut and peanut component specific IgE correlated with effector cell activation to a greater or lesser extent. Using multivariable logistic regression models, the IgE characteristics that appeared to be most determinant of effector cell activation following peanut stimulation were SA and PD, suggesting that the IgE repertoire bound to receptors on the surface of mast cells is key in influencing degranulation. The greater proportion of allergen sIgE and the broader array of peanut allergens that IgE recognises, the greater the mast cell activation in response to peanut and, consequently the greater the likelihood and severity of peanut allergy<sup>8</sup>. To our knowledge, this is the first study assessing the functional characteristics of polyclonal-sIgE in a patient population, as well as the first study to quantify avidity using ImmunoCAP and to introduce the concept of diversity as a quantitative variable, which showed unprecedented influence on effector cell activation.

Quantifying allergen-sIgE has shown diagnostic utility in that elevated levels are associated with a higher likelihood of food allergy<sup>27</sup>. Component-sIgE and epitope-sIgE detection added further resolution in peanut allergy diagnosis<sup>3, 4, 28-30</sup>. We recently showed the importance of IgE specificity to peanut 2S albumins, Ara h 2 and Ara h 6, with Ara h 2 being the most dominant IgE binding allergen and inducer of effector cell activation<sup>10</sup>. Unsurprisingly, in this study, we made similar observations. However, because whole peanut extract was employed as the stimulant in the MAT and whole peanut extract represents better the peanut ingested by patients, peanut-sIgE levels, rather than sIgE to peanut components, were used to represent specificity across multivariable models. The peanut components included Ara h 8 and also Ara h 9, which are less likely to cause systemic reactions, but we opted to include them in the analyses, as, from a mechanistic perspective, low affinity interactions can contribute to effector cell degranulation<sup>31</sup>.

We used two in vitro systems to test the functionality of IgE and use as endpoints for the univariate and multivariate models: the BAT and the MAT. The use of the two cell types is an important validation of our findings. We used the LAD2 cells as the mast cells in the MAT<sup>32</sup>. The uniformity of using human mast cell line removes the heterogeneity that others have seen when using primary mast cells<sup>33</sup>, and allows us an excellent functional effector cell platform for exploring isolated IgE characteristics, with the least possible 'noise' from mast cell intrinsic features<sup>33</sup>. Moreover, the MAT assay isolates receptor bound antibodies, through initial wash steps, removing unbound antibodies from assay. This ablates the effect of blocking antibodies in the periphery, such as allergen specific IgG4, and encourages the assessment of receptor bound IgE<sup>25</sup>.

We report that peanut-sIgE significantly influences both basophil and mast cell activation, as exemplified in the univariable logistic regression models and multivariable models 1, 3 and S6. Impressively, SA improved discrimination between PA and PS individuals and had greater influence on both basophil and mast cell activation. It can be observed for some components, such as Ara h 2, that SA in the NA group reports higher medians than the PS group. This is due to the difference in total IgE levels between the two groups, in which total IgE is typically within the normal range in the NA group, and largely elevated in the PS group due to their atopic nature. The importance of SA has been highlighted by Christensen *et al*, showing that higher relative concentrations of allergen-sIgE to non-allergen sIgE increased both basophil sensitivity and maximum basophil degranulation<sup>6</sup>. Clinically, IgE SA has been shown as a strong predictor in the outcome of OFC's<sup>11</sup> and the efficacy of anti-IgE treatment in allergic individuals<sup>34</sup>. However, other studies have failed to demonstrate improved diagnostic performance and, showed comparable AUC's for both s-IgE levels and SA to peanut, and peanut components<sup>35</sup>. In our study, we report a significant increase in AUC's with peanut SA compared to standalone peanut-sIgE, suggesting that determining sIgE:tlgE ratios may have diagnostic benefits. Moreover, the increased influence on effector cell activation shown by incorporating peanut SA, over peanut-sIgE, in multivariable regression models also supports the utility of SA as a better predictor of allergic reactivity. The direct comparison of peanut-sIgE and SA in single models, S2 and S9 (**Table E3 and E6**), further substantiates SA's dominance as an influential characteristic over standalone sIgE titres. Conceptually, an increase in IgE SA or ratio of relevant sIgE to irrelevant sIgE acts to increase the probability

that two IgE antibodies attached to FcεRI will become complexed with an allergenic molecule, thus inducing degranulation.

It is well established that high affinity binding of IgE to allergen is a characteristic that influences the mechanisms of allergic reactions, and has been demonstrated across both experimental and clinical studies<sup>18, 36</sup>. It has recently been reported that production of high affinity IgE is regulated by a distinct subset of previously unidentified T follicular helper cells, characterised by their high IL-13 expression<sup>37</sup>. High affinity allergen-sIgE is required to outcompete lower affinity allergen specific-IgG (sIgG) and initiate the allergic response<sup>38</sup>. Despite this, a role for low affinity interactions has been previously demonstrated using both high and low affinity IgE of same specificity and showing marked reduction of effector cell degranulation in response to inhibition of only low affinity sIgE<sup>39, 40</sup>. This implies that summative avidity of allergen-sIgE is a potentially more important consideration than the affinity of individual IgE molecules.

The development of avidity ELISA's has enabled the estimation of avidity in polyclonal samples, by employing different chaotropic agents<sup>22, 41-44</sup>. We applied these principles using the well-established, validated and fully quantitative ImmunoCAP technology. We showed a high degree of significant discrimination between PA and PS individuals, in which peanut-sIgE from PA individuals was far more tolerant of NaSCN addition, with a greater proportion remaining bound to peanut ImmunoCAP than peanut-sIgE from PS individuals. A recently published study suggested that this difference in avidity between clinically distinct sensitized populations could be a consequence of allergen exposure, with chronic allergen exposure leading to the accumulation of long lived plasma cells, generating higher affinity IgE<sup>45</sup>. We expected that our data for peanut sIgE avidity would have higher impact on effector cell activation. This may be a consequence of limitations within the variable itself. We proposed a fully quantitative method for determining IgE avidity in patient samples, in which removal of low avidity interactions in the presence of NaSCN, were not influenced by the levels of sIgE in the sample. However, unlike the other variables tested, the avidity score is presented as relative inhibition, and therefore relies heavily on the original peanut-sIgE level, determined in the absence of NaSCN. We believe that IgE avidity can become more influential in cases where IgE levels/concentrations and diversity are comparable between allergic and non-allergic individuals.

The diversity of one's IgE repertoire as an influential characteristic in the mechanisms of allergy is lesser established. Allergen-sIgE levels, from both the whole allergen and the components perspectives, are exceptionally informative for the diagnosis and mechanistic understanding of peanut allergy; however, we do not normally consider the specificity of remaining IgE. Over recent years the phenomena of 'epitope/molecular spreading' has been described in allergy, whereby IgE specificity and concentration, to components/epitopes within clinically relevant allergens, diversifies over time and creates a more heterogenous pool of allergen-sIgE<sup>15, 46, 47</sup>. We employed ImmunoCAP-ISAC technology and designed variables, based on Shannon's diversity, to reflect the diversity and concentration of IgE to both a wide array of allergens (WD) and allergen components within peanut (PD)<sup>48, 49</sup>. When applying Shannon's diversity principles' to our population, WD did not discriminate between PA and PS individuals. This was not an unexpected finding, as most children in our cohort were highly atopic presenting with multiple allergic comorbidities. However, as both PA and PS individuals had detectable IgE to peanut and peanut major allergens, we could have expected a similar outcome with PD, but this was not the case as PD was able to discriminate between these cohorts in a highly significant manner. Moreover, PD showed unusually high diagnostic value and correlated well with mast cell activation. Perhaps most impressive was the influence that PD had on effector cell activation, where simple logistic regression models, PD reported the outright highest standardised  $\beta$ -coefficients ( $P < 0.0001$ ), reflecting influence on both mast cell and basophil activation. In multivariable mechanistic models, PD and peanut SA outperformed avidity in model 2, reporting comparably significant influence on mast cell activation, but with PD reporting slightly more significant impact on basophil activation. When plotted in isolation with peanut SA, PD reported a slightly greater standardised  $\beta$  coefficient, considering both mast cell and basophil activation. The molecular spreading phenomena is the most viable rationale for higher PD, in PA compared to PS individuals. This phenomenon has been described in children with hay fever, whereby *Phleum pratense* sensitization across different allergenic molecules increases/diversifies temporally, following initial onset of disease<sup>47</sup>. Under the assumption that some PS children eventually develop clinical peanut allergy, we could predict an increase in their IgE diversity to peanut allergens over time with progression of their allergy. Similar observations have been reported in the LEAP study<sup>50</sup>.

Overall, for each of the IgE characteristics measured, there was a significant difference between PA and PS individuals, all showing improved diagnostic utility over peanut-sIgE levels and influenced effector cell activation. Between many of these variables significant correlation was reported, reflecting the fact that allergic individuals tend to report high levels/scores across different functional IgE characteristics, namely higher levels of peanut-specific IgE, higher peanut SA, higher peanut diversity and higher avidity for peanut allergens. Similarly, as discussed, many peanut allergic individuals also report polysensitisation to the different peanut allergens. This can often introduce concerns of multicollinearity when building multivariable models. In our study, IgE characteristics were only included in the same model if dissimilar in nature and acquisition, with collinearity presumed minimal as demonstrated by the low variance inflation factor (VIF) scores. Our conclusions require, nevertheless, verification in future studies. In our cohort of 90 subjects with detectable peanut-sIgE, PD and peanut SA held the most influence on effector cell activation, with SA offering improved diagnostic accuracy over peanut-sIgE levels. It is likely that mast cell reactivity, and thus clinical peanut allergy, is a consequence of multiple IgE characteristics and none should be discounted in further investigations into the mechanisms of food allergy. Other IgE characteristics, such as glycosylation/post-translational modifications, should be investigated, with the inhibitory effect of desialylation of glycans on IgE from allergic individuals potentially leading to therapeutic benefits<sup>51</sup>. The clinical implications of IgE characteristics may extend to the effect of immunomodulatory treatments, such as allergen-specific immunotherapy<sup>52</sup> and biologics, and could be used as biomarkers of response to treatment.

#### **Acknowledgements:**

The authors wish to thank Professor George Du Toit, Dr Suzana Radulovic and the Paediatric Allergy Team at the Evelina London Children's Hospital for their effort in recruiting participants and collecting samples to the study, and acknowledge support by the UK National Institute for Health Research comprehensive Biomedical Research Centre award to Guy's and St Thomas' National Health Service (NHS) Foundation Trust, in partnership with the King's College London and King's College Hospital NHS Foundation Trust.

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**Tables:****Table 1. Demographic and clinical features and serum specific IgE levels of the study population (n=100). Median and range are indicated.**

| Demographic, clinical and immunological features | Peanut Allergic (n=50) <sup>†</sup> | Peanut Sensitized but Tolerant (n=40) <sup>†</sup> | Non sensitized Non-Allergic (n=10) <sup>†</sup> | P Value |
|--------------------------------------------------|-------------------------------------|----------------------------------------------------|-------------------------------------------------|---------|
| Age                                              | 8.31 (1.68-17.91)                   | 5.93 (0.52-15.81)                                  | 5.96 (0.77-12.58)                               | 0.003   |
| Males                                            | 36 (72%)                            | 25 (62.5%)                                         | 8 (80%)                                         | 0.371   |
| Atopic eczema (%)                                | 17 (34%)                            | 18 (45%)                                           | 2 (20%)                                         | 0.287   |
| Asthma / wheezing (%)                            | 29 (58%)                            | 13 (33%)                                           | 1 (10%)                                         | 0.016   |
| Allergic rhinitis (%)                            | 33 (66%)                            | 14 (35%)                                           | 1 (10%)                                         | 0.003   |
| Other food allergy (%)                           | 38 (76%)                            | 33 (83%)                                           | 2 (20%)                                         | 0.453   |
| SPT to peanut (mm)                               | 9 (1-34)*                           | 3 (0-12)*                                          | 0 (0-0)                                         | <0.0001 |
| Total IgE (kU <sub>A</sub> /L)                   | 297.5 (4-3550)                      | 469 (7-10714)                                      | 29.5 (6-397)                                    | 0.5757  |
| Specific IgE to Peanut (kU <sub>A</sub> /L)      | 12 (0.2-568)                        | 2.41 (0.04-128)                                    | 0.01 (0-0.9)                                    | 0.0019  |
| Specific IgE to Ara h 1 (kU <sub>A</sub> /L)     | 0.80 (0-199)                        | 0.11 (0-88.3)                                      | 0 (0-0.01)                                      | 0.0838  |
| Specific IgE to Ara h 2 (kU <sub>A</sub> /L)     | 2.54 (0.01-278)                     | 0.07 (0-82.3)                                      | 0.04 (0.01-0.07)                                | <0.0001 |
| Specific IgE to Ara h 3 (kU <sub>A</sub> /L)     | 0.36 (0-89.6)                       | 0.06 (0-7.28)                                      | 0.01 (0-0.04)                                   | 0.0456  |
| Specific IgE to Ara h 6 (kU <sub>A</sub> /L)     | 3.835 (0-155)                       | 0.03 (0-86)                                        | 0 (0-0.29)                                      | <0.0001 |
| Specific IgE to Ara h 8 (kU <sub>A</sub> /L)     | 0.09 (0-185)                        | 0.04 (0-88.6)                                      | 0 (0-0.02)                                      | 0.4022  |
| Specific IgE to Ara h 9 (kU <sub>A</sub> /L)     | 0.02 (0-871)                        | 0.05 (0-43.9)                                      | 0.01 (0-0.02)                                   | 0.0834  |
| Peanut Specific Activity (%)                     | 5.03 (0.06-81.71)                   | 0.82 (0.02-7.68)                                   | 0.03 (0-0.18)                                   | <0.0001 |
| Ara h 1 Specific Activity (%)                    | 0.37 (0-24.34)                      | 0.03 (0-2.12)                                      | 0 (0-0.04)                                      | 0.0038  |
| Ara h 2 Specific Activity (%)                    | 2.48 (0.01-55.26)                   | 0.012 (0-1.86)                                     | 0.09 (0.01-0.83)                                | <0.0001 |
| Ara h 3 Specific Activity (%)                    | 0.14 (0-9.98)                       | 0.019 (0-0.90)                                     | 0.01 (0-0.67)                                   | 0.0002  |
| Ara h 6 Specific Activity (%)                    | 1.91 (0-25.63)                      | 0.01 (0-1.06)                                      | 0 (0-0.41)                                      | <0.0001 |
| Ara h 8 Specific Activity (%)                    | 0.05 (0-11.90)                      | 0.01 (0-7.77)                                      | 0 (0-0.04)                                      | 0.2862  |
| Ara h 9 Specific Activity (%)                    | 0.01 (0-1.5)                        | 0.01 (0-4.72)                                      | 0.01 (0-0.17)                                   | 0.3529  |
| Peanut Diversity                                 | 0.97 (0-1.41)                       | 0 (0-1.35)                                         | 0 (0-0)                                         | <0.0001 |
| Whole Diversity                                  | 1.985 (0-3.39)                      | 1.82 (0-3.1)                                       | 0 (0-1.32)                                      | 0.4945  |
| Avidity (%inhibition) ‡                          | 41.07 (15.94-72.5)                  | 28.63 (7.43-50)                                    | -                                               | <0.0001 |
| MAT to peanut (%CD63+ LAD2 cells)                | 24.6 (0-79.9)                       | 4.18 (0-31.65)                                     | 1.09 (0-3.9)                                    | <0.0001 |

|                                       |                    |               |               |         |
|---------------------------------------|--------------------|---------------|---------------|---------|
| BAT to peanut +<br>(%CD63+ basophils) | 37.55 (1.75-90.79) | 1.57 (0-16.6) | 0.36 (0-0.99) | <0.0001 |
|---------------------------------------|--------------------|---------------|---------------|---------|

Footnote: Values are expressed as no. (%) or median (range). P value refers to the comparison between Peanut Allergic and Peanut Sensitised but tolerant patients. (\*) SPT= PA (n=49), PS (n=38) PA+PS (n=87). (†) Population sizes differ when considering BAT data, they are as follows; Peanut sensitized (n=54), Peanut Allergic (n=23), Peanut sensitized but tolerant (n=31) and Non sensitized Non-Allergic (n=7). (‡) For avidity analyses n=60, with Peanut Allergic (n=36) and Peanut sensitised but tolerant (n=24). LAD2 mast cells, Laboratory of Allergic Diseases mast cells.

**Table 2. A summary table reporting the discriminative ability and association of each characteristic with mast cell activation (%CD63+ LAD2 cells).**

Discriminative ability was assessed using Mann Whitney T test and associations with mast cell activation are reported as both spearman's rank (R value) correlation and logistic regression ( $\beta$  coefficient).

| Characteristic                    | PA vs PS T-test P value (adjusted for multiple comparisons*) | Correlation    |                |                    |                    |                    |                    | Linear regression (n=90) |                        |                                  |         |                |
|-----------------------------------|--------------------------------------------------------------|----------------|----------------|--------------------|--------------------|--------------------|--------------------|--------------------------|------------------------|----------------------------------|---------|----------------|
|                                   |                                                              | R value (n=90) | P value (n=90) | R value (PA, n=50) | P value (PA, n=50) | R value (PS, n=40) | P value (PS, n=40) | $\beta$ coefficient      | Coefficient Std. Error | Standardised $\beta$ coefficient | P value | R square value |
| Peanut slgE (kU <sub>A</sub> /L)  | 0.0019 (0.030)                                               | 0.787          | <0.0001        | 0.716              | <0.0001            | 0.702              | <0.0001            | 0.094                    | 0.016                  | 0.521                            | <0.0001 | 0.271          |
| Ara h 1 slgE (kU <sub>A</sub> /L) | 0.0838 (1)                                                   | 0.621          | <0.0001        | 0.637              | <0.0001            | 0.475              | 0.0019             | 0.307                    | 0.050                  | 0.551                            | <0.0001 | 0.304          |
| Ara h 2 slgE (kU <sub>A</sub> /L) | <0.0001 (<0.0001)                                            | 0.666          | <0.0001        | 0.641              | <0.0001            | 0.208              | 0.1977             | 0.212                    | 0.037                  | 0.526                            | <0.0001 | 0.268          |
| Ara h 3 slgE (kU <sub>A</sub> /L) | 0.0456 (0.7296)                                              | 0.613          | <0.0001        | 0.587              | <0.0001            | 0.556              | 0.0002             | 0.490                    | 0.118                  | 0.405                            | <0.0001 | 0.164          |
| Ara h 6 slgE (kU <sub>A</sub> /L) | <0.0001 (<0.0001)                                            | 0.658          | <0.0001        | 0.625              | <0.0001            | 0.205              | 0.2042             | 0.384                    | 0.074                  | 0.484                            | <0.0001 | 0.234          |
| Ara h 8 slgE (kU <sub>A</sub> /L) | 0.4022 (1)                                                   | 0.385          | 0.0001         | 0.311              | 0.0280             | 0.444              | 0.0041             | -0.065                   | 0.091                  | -0.078                           | 0.463   | 0.006          |
| Ara h 9 slgE (kU <sub>A</sub> /L) | 0.0834 (1)                                                   | 0.219          | 0.0385         | 0.237              | 0.0975             | 0.445              | 0.0036             | 0.146                    | 0.410                  | 0.038                            | 0.722   | 0.001          |
| Peanut Specific Activity (%)      | <0.0001 (<0.0001)                                            | 0.659          | <0.0001        | 0.604              | <0.0001            | 0.485              | 0.0015             | 0.980                    | 0.129                  | 0.628                            | <0.0001 | 0.395          |
| Ara h 1 Specific Activity (%)     | 0.0038 (0.0608)                                              | 0.502          | <0.0001        | 0.580              | <0.0001            | 0.158              | 0.3316             | 2.722                    | 0.361                  | 0.626                            | <0.0001 | 0.386          |
| Ara h 2 Specific Activity (%)     | <0.0001 (<0.0001)                                            | 0.515          | <0.0001        | 0.523              | 0.0001             | -0.209             | 0.1950             | 1.494                    | 0.245                  | 0.545                            | <0.0001 | 0.297          |
| Ara h 3 Specific Activity (%)     | 0.0002 (0.0032)                                              | 0.418          | <0.0001        | 0.457              | 0.0008             | 0.024              | 0.8847             | 4.231                    | 1.224                  | 0.346                            | <0.0001 | 0.120          |
| Ara h 6 Specific Activity (%)     | <0.0001 (0.0001)                                             | 0.513          | <0.0001        | 0.411              | 0.0030             | -0.031             | 0.8533             | 2.194                    | 0.455                  | 0.455                            | <0.0001 | 0.209          |
| Ara h 8 Specific Activity (%)     | 0.2862 (1)                                                   | 0.146          | 0.169          | 0.012              | 0.2957             | 0.194              | 0.2302             | 0.045                    | 1.055                  | 0.005                            | 0.966   | 0.000          |
| Ara h 9 Specific Activity (%)     | 0.3529 (1)                                                   | -0.008         | 0.942          | -0.081             | 0.6262             | 0.248              | 0.1219             | -2.850                   | 3.569                  | -0.085                           | 0.427   | 0.007          |
| Peanut Diversity                  | <0.0001 (<0.0001)                                            | 0.649          | <0.0001        | 0.565              | <0.0001            | 0.233              | 0.1477             | 23.763                   | 3.085                  | 0.635                            | <0.0001 | 0.396          |
| Whole Diversity                   | 0.4945 (1)                                                   | 0.494          | <0.0001        | 0.442              | 0.0006             | 0.545              | 0.0002             | 8.362                    | 2.525                  | 0.333                            | 0.001   | 0.111          |

|                            |         |       |       |        |        |            |        |       |       |       |       |       |
|----------------------------|---------|-------|-------|--------|--------|------------|--------|-------|-------|-------|-------|-------|
|                            |         |       | 1     |        |        |            |        |       |       |       |       |       |
| Avidity (%inhibition)<br>* | <0.0001 | 0.236 | 0.069 | -0.207 | 0.1168 | -<br>0.344 | 0.0999 | 0.267 | 0.177 | 0.174 | 0.183 | 0.036 |

## Footnote:

P value refers to the comparison between Peanut Allergic and Peanut Sensitised but tolerant patients. \* For avidity analyses n=60, with Peanut Allergic (n=36) and Peanut sensitised but tolerant (n=24).

\*Adjustment for multiple comparisons was done with Bonferroni-Dunn

**Table 3. A summary of mechanistic multivariable linear regression models (n=60), in which combinations of functional IgE characteristics were used to predict %CD63+ LAD2 cell activation.** Unstandardized and standardized beta coefficients are reported alongside significance and variance inflation factor (VIF). VIF>10 was used to indicate any collinearity.

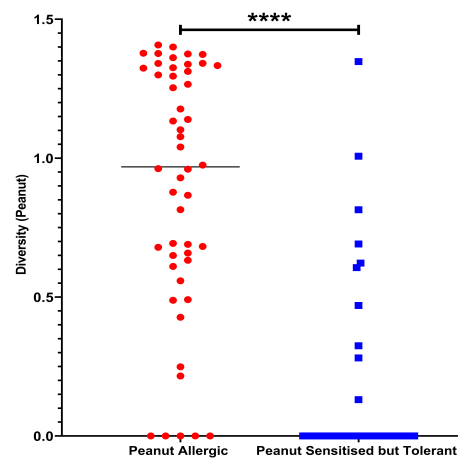
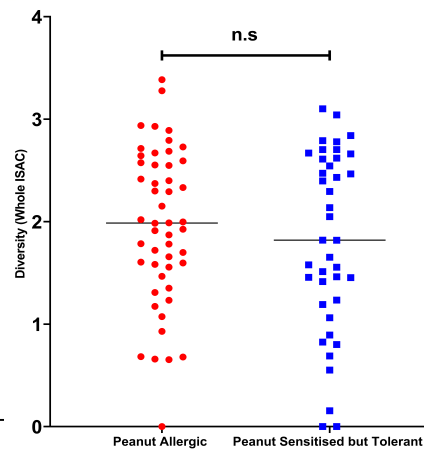
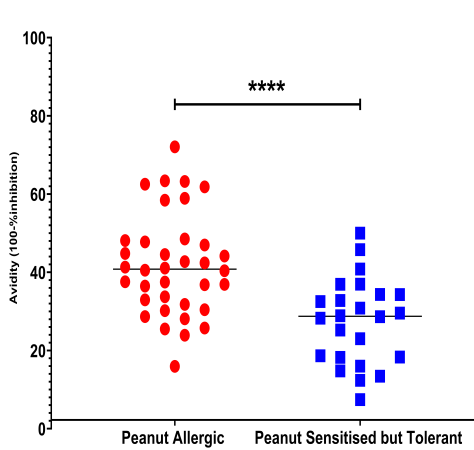
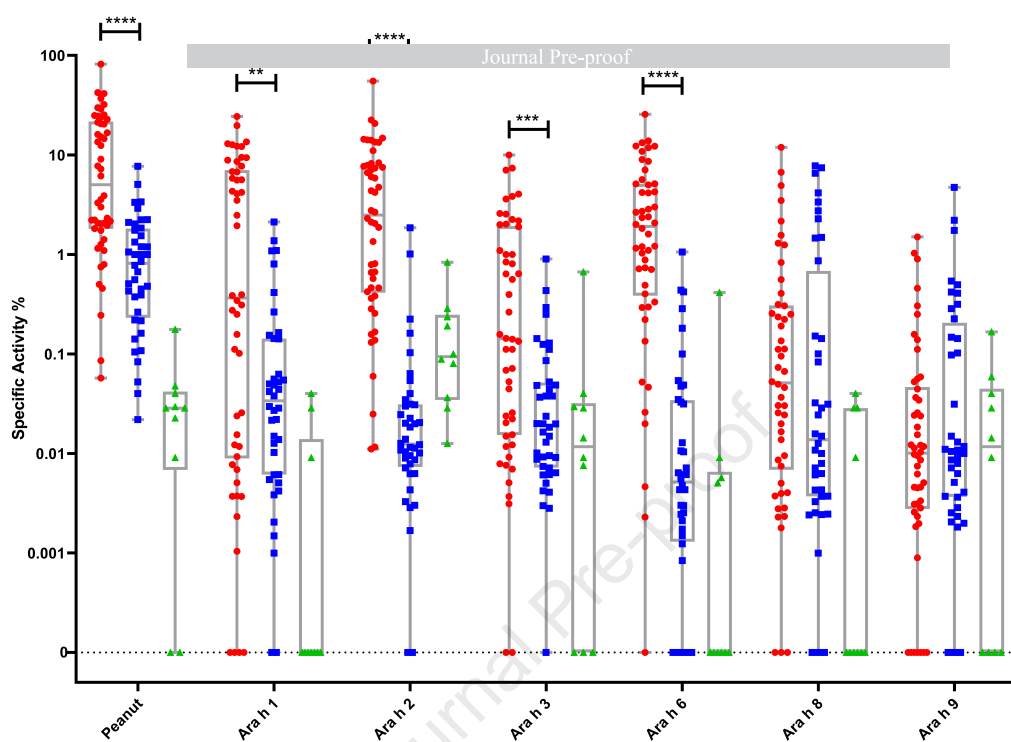
| Model No. | Characteristic   | Unstandardized $\beta$ Coefficient | Coefficient Std. Error | Standardized $\beta$ Coefficient | P Value | R Square Value | VIF   |
|-----------|------------------|------------------------------------|------------------------|----------------------------------|---------|----------------|-------|
| 1         | Peanut slgE      | 0.037                              | 0.020                  | 0.225                            | 0.066   | 0.415          | 1.384 |
|           | Peanut Diversity | 20.528                             | 5.203                  | 0.534                            | <0.0001 |                | 1.750 |
|           | Avidity          | -0.141                             | 0.180                  | -0.092                           | 0.436   |                | 1.322 |
| 2         | Peanut SA        | 0.664                              | 0.156                  | 0.447                            | <0.0001 | 0.530          | 1.314 |
|           | Peanut Diversity | 16.940                             | 4.470                  | 0.440                            | <0.0001 |                | 1.608 |
|           | Avidity          | -0.181                             | 0.158                  | -0.118                           | 0.258   |                | 1.275 |
| 3         | Peanut slgE      | 0.077                              | 0.019                  | 0.472                            | <0.0001 | 0.255          | 1.008 |
|           | Whole Diversity  | 2.171                              | 4.306                  | 0.057                            | 0.628   |                | 1.035 |
|           | Avidity          | 0.223                              | 0.180                  | 0.145                            | 0.224   |                | 1.043 |
| 4         | Peanut SA        | 0.993                              | 0.154                  | 0.668                            | <0.0001 | 0.447          | 1.084 |
|           | Whole Diversity  | 7.383                              | 3.786                  | 0.201                            | 0.056   |                | 1.078 |
|           | Avidity          | 0.121                              | 0.157                  | 0.079                            | 0.444   |                | 1.063 |

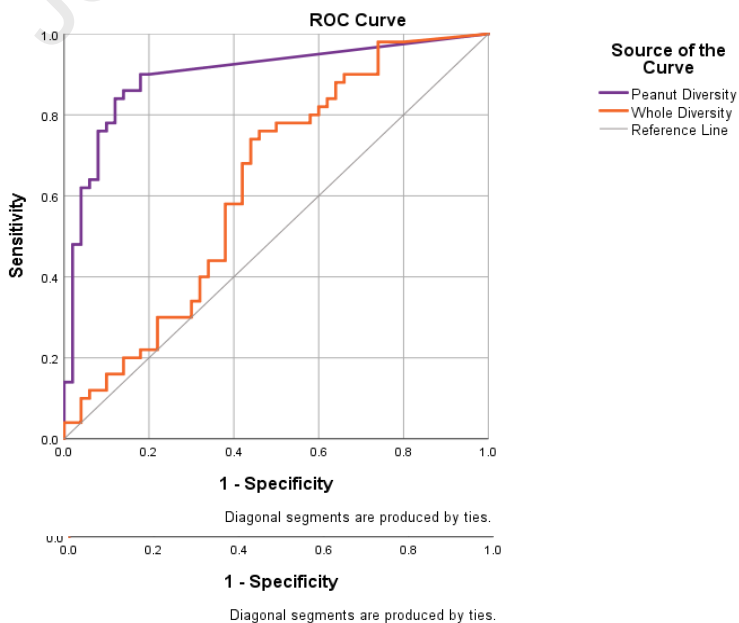
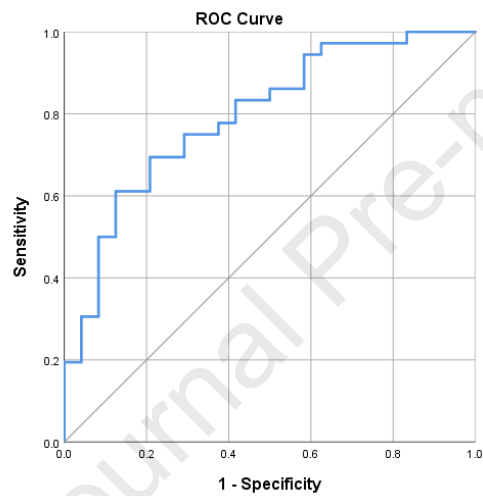
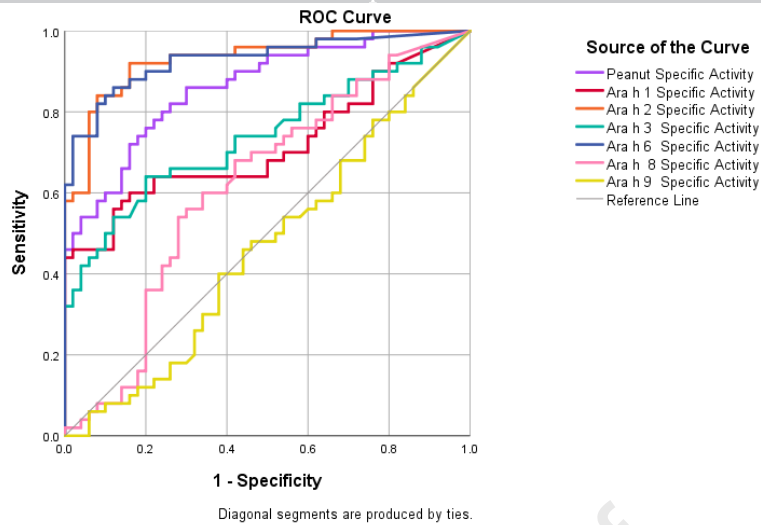
**Figure legends:**

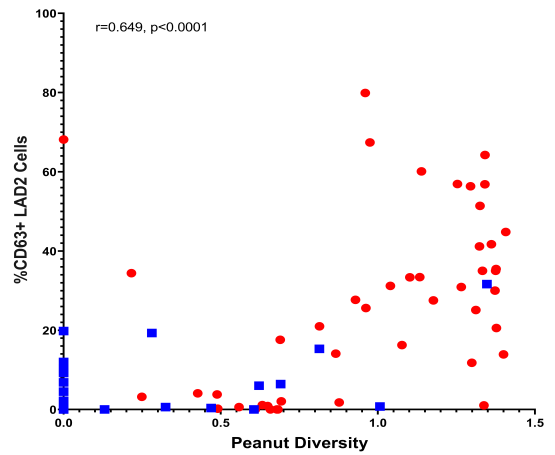
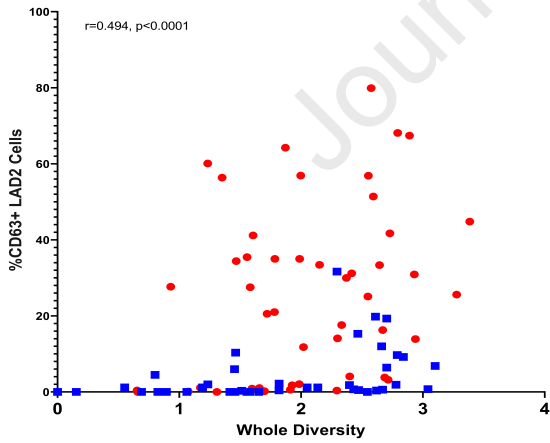
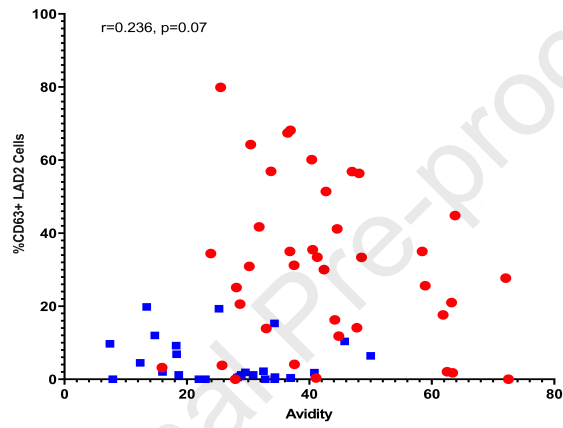
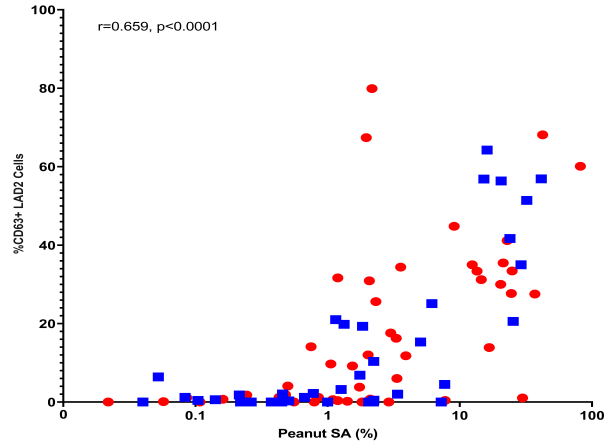
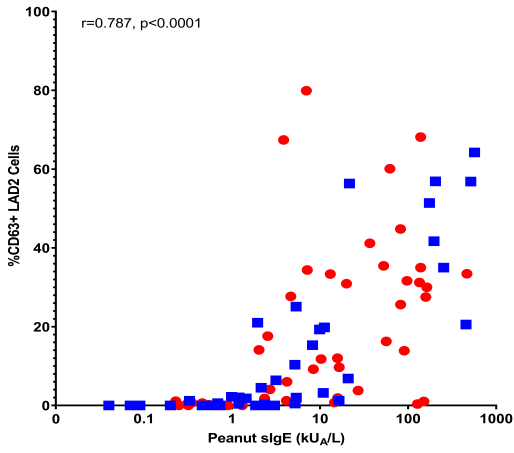
**Figure 1.** Distribution of IgE (A) specific activity, (B) peanut avidity and (C) diversity in individuals with peanut allergy (n = 50), individuals sensitized to but tolerant of peanut (n = 40), and individuals neither sensitized nor allergic to peanut (n = 10). The Mann-Whitney U test was used for the comparison between individuals with peanut allergy and individuals sensitized to but tolerant of peanut. \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; \*\*\*\*P < 0.0001.

**Figure 2.** Receiver operator characteristic (ROC) curves for IgE (A) specific activity, (B) peanut avidity and (C) diversity across whole study population (n=100). For corresponding areas under the curves, see **Table 2**. Labels have been included in the figure, where necessary.

**Figure 3.** Spearman's correlation between (A) peanut specific IgE (sIgE) levels (B) peanut specific activity, (C) peanut avidity, (D) whole diversity and (E) peanut diversity, and mast cell activation. %CD63+ LAD 2 cells, represents cell activation following the mast cell activation test. Correlation reported across peanut sensitised population (n=90), with peanut allergic individuals (red) and Peanut sensitized but tolerant individuals (blue). R values have been displayed on the figure and in table 2.







## Online Supplementary Material

### Peanut diversity and specific activity are the dominant IgE characteristics for effector cell activation in children

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## References

1. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the Areas under Two or More Correlated Receiver Operating Characteristic Curves: A Nonparametric Approach. *Biometrics*. 1988;44(3):837-845.

**Table E1. Demographic/clinical features and serum specific-IgE levels of the study population, used explicitly in Avidity assays (n=60). Median and range are**

indicated.

|                                 | Peanut Allergic (n=36) | Peanut Sensitized but Tolerant (n=24) | P Value* |
|---------------------------------|------------------------|---------------------------------------|----------|
| Age                             | 8.37 (2.06–16.27)      | 6.05 (0.52–13.71)                     | 0.0155   |
| Males                           | 24 (66.67%)            | 15 (62.5%)                            | ----     |
| SPT to peanut (mm)              | 10 (1-34)              | 3 (0-9)*                              | <0.0001  |
| Total IgE (kUA/L)               | 518 (19-3550)          | 547 (28 -6036)                        | 0.5845   |
| Specific IgE to Peanut (kUA/L)  | 31.95 (1.2-568)        | 5.195 (0.99-20.9)                     | 0.0005   |
| Specific IgE to Ara h 1 (kUA/L) | 8.92 (0-199)           | 0.16 (0-11.4)                         | 0.0099   |
| Specific IgE to Ara h 2 (kUA/L) | 12.3375 (0.05-278)     | 0.07 (0-0.52)                         | <0.0001  |
| Specific IgE to Ara h 3 (kUA/L) | 1.475 (0-89.6)         | 0.07 (0.02-7.28)                      | 0.0115   |
| Specific IgE to Ara h 6 (kUA/L) | 10.17 (0-155)          | 0.03 (0-0.49)                         | <0.0001  |
| Specific IgE to Ara h 8 (kUA/L) | 0.215 (0-185)          | 0.11 (0-88.6)                         | 0.7787   |
| Specific IgE to Ara h 9 (kUA/L) | 0.04 (0-8.71)          | 0.13 (0-13.9)                         | 0.0354   |

Footnote: Values are expressed as no. (%) or median (range). P value refers to the comparison between PA and PS patients and were generated using Mann-Whitney T tests. (\*) SPT in PS individuals (n=23).

**Table E2: Diagnostic performance of each characteristic in the determination of peanut allergy.** The following AUC's, respective 95% CI's, optimal cut-off's, sensitivity, and specificity have been reported alongside. \* Avidity cohort, n =60.

| Characteristic                    | AUC   | Lower Bound (95% CI) | Upper Bound (95% CI) | Optimal Cut-off | Sensitivity (%) | Specificity (%) |
|-----------------------------------|-------|----------------------|----------------------|-----------------|-----------------|-----------------|
| Peanut sIgE (kU <sub>A</sub> /L)  | 0.752 | 0.658                | 0.845                | 21.25           | 32.29           | 93.10           |
| Ara h 1 sIgE (kU <sub>A</sub> /L) | 0.668 | 0.559                | 0.777                | 2.68            | 32.86           | 90.00           |
| Ara h 2 sIgE (kU <sub>A</sub> /L) | 0.916 | 0.857                | 0.975                | 0.28            | 82.00           | 94.00           |
| Ara h 3 sIgE (kU <sub>A</sub> /L) | 0.672 | 0.563                | 0.780                | 1.10            | 33.33           | 87.10           |
| Ara h 6 sIgE (kU <sub>A</sub> /L) | 0.908 | 0.846                | 0.970                | 0.32            | 82.00           | 90.00           |
| Ara h 8 sIgE (kU <sub>A</sub> /L) | 0.624 | 0.511                | 0.737                | 0.02            | 60.00           | 31.43           |
| Ara h 9 sIgE (kU <sub>A</sub> /L) | 0.468 | 0.354                | 0.581                | 0.86            | 14.58           | 82.69           |
| Peanut Specific Activity (%)      | 0.862 | 0.793                | 0.932                | 1.79            | 76.00           | 80.00           |
| Ara h 1 Specific Activity (%)     | 0.716 | 0.613                | 0.820                | 2.29            | 44.00           | 100.00          |
| Ara h 2 Specific Activity (%)     | 0.933 | 0.885                | 0.981                | 0.12            | 92.00           | 84.00           |
| Ara h 3 Specific Activity (%)     | 0.738 | 0.639                | 0.837                | 0.05            | 64.00           | 80.00           |
| Ara h 6 Specific Activity (%)     | 0.930 | 0.878                | 0.981                | 0.29            | 82.00           | 92.00           |
| Ara h 8 Specific Activity (%)     | 0.618 | 0.507                | 0.730                | 0.043           | 54.00           | 72.00           |
| Ara h 9 Specific Activity (%)     | 0.465 | 0.351                | 0.578                | 0.015           | 40.00           | 62.00           |
| Peanut Diversity                  | 0.898 | 0.831                | 0.964                | 0.48            | 84.00           | 88.00           |
| Whole Diversity                   | 0.628 | 0.517                | 0.739                | 1.58            | 74.00           | 56.00           |
| Avidity (%inhibition)<br>*        | 0.795 | 0.681                | 0.910                | 37.23           | 61.10           | 87.50           |

**Table E3: Diagnostic performance of selected characteristics in the determination of peanut allergy, in reduced study cohort used for avidity assays, as governed by the Area's under the Receiver Operator Characteristic curve (ROC).** The following AUC's and their respective 95% CI's were generated in the cohort described in supplementary table 1 and allows for comparison of diagnostic performance in the reduced study cohort.

|                                 | Area under the Curve | Lower Bound (95% CI) | Upper Bound (95% CI) |
|---------------------------------|----------------------|----------------------|----------------------|
| Specific IgE to Peanut (kUA/L)  | 0.760                | 0.641                | 0.880                |
| Specific IgE to Ara h 2 (kUA/L) | 0.944                | 0.885                | 1.000                |
| Specific IgE to Ara h 6 (kUA/L) | 0.947                | 0.889                | 1.000                |
| Peanut Specific Activity (%)    | 0.843                | 0.746                | 0.939                |
| Ara h 2 Specific Activity (%)   | 0.970                | 0.933                | 1.000                |
| Ara h 6 Specific Activity (%)   | 0.954                | 0.899                | 1.000                |
| Peanut Diversity                | 0.931                | 0.866                | 0.995                |
| Whole Diversity                 | 0.505                | 0.348                | 0.661                |
| Avidity (%inhibition)           | 0.795                | 0.681                | 0.910                |

Table E4a: Correlation matrix for each characteristic assessed in this study across PA and PS individuals (n=90), including peanut avidity (n=60). R values from Spearman's correlation have been reported here; for corresponding P values, see table E4b below.

| 0                       | Age     | MAT (%CD63+ LAD2 Cells) | Peanut slgE | Ara h 1 slgE | Ara h 2 slgE | Ara h 3 slgE | Ara h 6 slgE | Ara h 8 slgE | Ara h 9 slgE | Peanut SA | Ara h 1 SA | Ara h 2 SA | Ara h 3 SA | Ara h 6 SA | Ara h 8 SA | Ara h 9 SA | Whole Diversity | Peanut Diversity | Peanut Avidity |
|-------------------------|---------|-------------------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|-----------|------------|------------|------------|------------|------------|------------|-----------------|------------------|----------------|
| Age                     | 1.000   | 0.357                   | 0.288       | 0.116        | 0.431        | 0.192        | 0.345        | 0.425        | - 0.038      | 0.069     | - 0.024    | 0.311      | - 0.053    | 0.239      | 0.265      | - 0.269    | 0.370           | 0.363            | 0.249          |
| MAT (%CD63+ LAD2 Cells) | 0.357   | 1.000                   | 0.787       | 0.621        | 0.666        | 0.613        | 0.658        | 0.383        | 0.219        | 0.659     | 0.502      | 0.515      | 0.418      | 0.513      | 0.146      | - 0.008    | 0.494           | 0.649            | 0.236          |
| Peanut slgE             | 0.288   | 0.787                   | 1.000       | 0.820        | 0.692        | 0.865        | 0.670        | 0.438        | 0.416        | 0.707     | 0.584      | 0.448      | 0.547      | 0.444      | 0.046      | 0.006      | 0.580           | 0.645            | -0.020         |
| Ara h 1 slgE            | 0.116   | 0.621                   | 0.820       | 1.000        | 0.567        | 0.874        | 0.535        | 0.126        | 0.386        | 0.623     | 0.859      | 0.418      | 0.677      | 0.384      | -0.214     | 0.068      | 0.348           | 0.551            | 0.159          |
| Ara h 2 slgE            | 0.431   | 0.666                   | 0.692       | 0.567        | 1.000        | 0.582        | 0.880        | 0.268        | 0.066        | 0.599     | 0.473      | 0.841      | 0.462      | 0.736      | -0.002     | - 0.189    | 0.351           | 0.809            | 0.490          |
| Ara h 3 slgE            | 0.192   | 0.613                   | 0.865       | 0.874        | 0.582        | 1.000        | 0.527        | 0.270        | 0.426        | 0.589     | 0.666      | 0.353      | 0.718      | 0.319      | -0.118     | - 0.001    | 0.430           | 0.547            | 0.021          |
| Ara h 6 slgE            | 0.345   | 0.658                   | 0.670       | 0.535        | 0.880        | 0.527        | 1.000        | 0.242        | 0.020        | 0.616     | 0.479      | 0.790      | 0.474      | 0.891      | 0.021      | - 0.147    | 0.296           | 0.832            | 0.470          |
| Ara h 8 slgE            | 0.425   | 0.383                   | 0.438       | 0.126        | 0.268        | 0.270        | 0.242        | 1.000        | 0.442        | -0.001    | - 0.164    | - 0.036    | - 0.160    | - 0.017    | 0.779      | 0.101      | 0.842           | 0.216            | -0.106         |
| Ara h 9 slgE            | - 0.038 | 0.219                   | 0.416       | 0.386        | 0.066        | 0.426        | 0.020        | 0.442        | 1.000        | -0.033    | 0.089      | - 0.235    | 0.030      | - 0.231    | 0.138      | 0.692      | 0.490           | 0.075            | -0.213         |
| Peanut SA               | 0.069   | 0.659                   | 0.707       | 0.623        | 0.599        | 0.589        | 0.616        | - 0.001      | - 0.033      | 1.000     | 0.749      | 0.723      | 0.780      | 0.687      | 0.006      | 0.025      | 0.135           | 0.628            | 0.166          |
| Ara h 1 SA              | - 0.024 | 0.502                   | 0.584       | 0.859        | 0.473        | 0.666        | 0.479        | - 0.164      | 0.089        | 0.749     | 1.000      | 0.579      | 0.834      | 0.539      | -0.211     | 0.131      | 0.047           | 0.512            | 0.210          |
| Ara h 2 SA              | 0.311   | 0.515                   | 0.448       | 0.418        | 0.841        | 0.353        | 0.790        | - 0.036      | - 0.235      | 0.723     | 0.579      | 1.000      | 0.586      | 0.871      | -0.012     | - 0.141    | 0.017           | 0.736            | 0.491          |
| Ara h 3 SA              | - 0.053 | 0.418                   | 0.547       | 0.677        | 0.462        | 0.718        | 0.474        | - 0.160      | 0.030        | 0.780     | 0.834      | 0.586      | 1.000      | 0.545      | -0.154     | 0.088      | -0.031          | 0.517            | 0.067          |
| Ara h 6                 | 0.239   | 0.513                   | 0.444       | 0.384        | 0.736        | 0.319        | 0.891        | -            | -            | 0.687     | 0.539      | 0.871      | 0.545      | 1.000      | 0.007      | -          | 0.014           | 0.744            | 0.493          |

|                     |            |        |        |            |            |            |            |            |            |       |            |            |            |            |        |            |        |        |        |
|---------------------|------------|--------|--------|------------|------------|------------|------------|------------|------------|-------|------------|------------|------------|------------|--------|------------|--------|--------|--------|
| SA                  |            |        |        |            |            |            |            | 0.017      | 0.231      |       |            |            |            |            |        | 0.117      |        |        |        |
| Ara h 8<br>SA       | 0.265      | 0.146  | 0.046  | -<br>0.214 | -<br>0.002 | -<br>0.118 | 0.021      | 0.779      | 0.138      | 0.006 | -<br>0.211 | -<br>0.012 | -<br>0.154 | 0.007      | 1.000  | 0.237      | 0.537  | 0.015  | -0.061 |
| Ara h 9<br>SA       | -<br>0.269 | -0.008 | 0.006  | 0.068      | -<br>0.189 | -<br>0.001 | -<br>0.147 | 0.101      | 0.692      | 0.025 | 0.131      | -<br>0.141 | 0.088      | -<br>0.117 | 0.237  | 1.000      | 0.123  | -0.083 | -0.094 |
| Whole<br>Diversity  | 0.370      | 0.494  | 0.580  | 0.348      | 0.351      | 0.430      | 0.296      | 0.842      | 0.490      | 0.135 | 0.047      | 0.017      | -<br>0.031 | 0.014      | 0.537  | 0.123      | 1.000  | 0.285  | -0.239 |
| Peanut<br>Diversity | 0.363      | 0.649  | 0.645  | 0.551      | 0.809      | 0.547      | 0.832      | 0.216      | 0.075      | 0.628 | 0.512      | 0.736      | 0.517      | 0.744      | 0.015  | -<br>0.083 | 0.285  | 1.000  | 0.414  |
| Peanut<br>Avidity   | 0.249      | 0.236  | -0.020 | 0.159      | 0.490      | 0.021      | 0.470      | -<br>0.106 | -<br>0.213 | 0.166 | 0.210      | 0.491      | 0.067      | 0.493      | -0.061 | -<br>0.094 | -0.239 | 0.414  | 1.000  |

**Table E4b: Correlation matrix for each characteristic assessed in this study across PA and PS individuals (n=90), including peanut avidity (n=60).** P values from Spearman's correlation have been reported here; for corresponding R values, see table E4 above.

|                                      | Age         | MAT<br>(%CD6<br>3+<br>LAD2<br>Cells) | Peanu<br>t slgE | Ara h<br>1 slgE | Ara h<br>2 slgE | Ara h<br>3 slgE | Ara h<br>6 slgE | Ara h<br>8 slgE | Ara h<br>9 slgE | Peanu<br>t SA | Ara h<br>1 SA | Ara h<br>2 SA | Ara h<br>3 SA | Ara h<br>6 SA | Ara h<br>8 SA | Ara h<br>9 SA | Whole<br>Diversi<br>ty | Peanut<br>Diversi<br>ty | Peanu<br>t<br>Avidity |
|--------------------------------------|-------------|--------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|------------------------|-------------------------|-----------------------|
| Age                                  |             | 0.0005<br>6                          | 0.005<br>96     | 0.274<br>44     | 0.000<br>02     | 0.069<br>55     | 0.000<br>86     | 0.000<br>03     | 0.723<br>18     | 0.517<br>60   | 0.825<br>89   | 0.002<br>83   | 0.619<br>99   | 0.023<br>49   | 0.011<br>69   | 0.010<br>21   | 0.0003<br>4            | 0.0004<br>4             | 0.055<br>03           |
| MAT<br>(%CD6<br>3+<br>LAD2<br>Cells) | 0.000<br>56 |                                      | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.000<br>19     | 0.038<br>53     | 0.000<br>00   | 0.000<br>00   | 0.000<br>00   | 0.000<br>04   | 0.000<br>00   | 0.169<br>17   | 0.941<br>74   | 0.0000<br>0            | 0.0000<br>0             | 0.068<br>97           |
| Peanut<br>slgE                       | 0.005<br>96 | 0.0000<br>0                          |                 | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.000<br>02     | 0.000<br>04     | 0.000<br>00   | 0.000<br>00   | 0.000<br>01   | 0.000<br>00   | 0.000<br>01   | 0.667<br>37   | 0.952<br>23   | 0.0000<br>0            | 0.0000<br>0             | 0.878<br>07           |
| Ara h 1<br>slgE                      | 0.274<br>44 | 0.0000<br>0                          | 0.000<br>00     |                 | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.236<br>74     | 0.000<br>17     | 0.000<br>00   | 0.000<br>00   | 0.000<br>04   | 0.000<br>00   | 0.000<br>19   | 0.042<br>91   | 0.521<br>20   | 0.0007<br>8            | 0.0000<br>0             | 0.226<br>35           |
| Ara h 2<br>slgE                      | 0.000<br>02 | 0.0000<br>0                          | 0.000<br>00     | 0.000<br>00     |                 | 0.000<br>00     | 0.000<br>00     | 0.010<br>59     | 0.533<br>94     | 0.000<br>00   | 0.000<br>00   | 0.000<br>00   | 0.000<br>00   | 0.000<br>00   | 0.986<br>54   | 0.074<br>82   | 0.0006<br>9            | 0.0000<br>0             | 0.000<br>07           |
| Ara h 3<br>slgE                      | 0.069<br>55 | 0.0000<br>0                          | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     |                 | 0.000<br>00     | 0.010<br>07     | 0.000<br>03     | 0.000<br>00   | 0.000<br>00   | 0.000<br>64   | 0.000<br>00   | 0.002<br>20   | 0.267<br>68   | 0.995<br>53   | 0.0000<br>2            | 0.0000<br>0             | 0.875<br>82           |
| Ara h 6<br>slgE                      | 0.000<br>86 | 0.0000<br>0                          | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     |                 | 0.021<br>66     | 0.849<br>25     | 0.000<br>00   | 0.000<br>00   | 0.000<br>00   | 0.000<br>00   | 0.000<br>00   | 0.845<br>75   | 0.168<br>04   | 0.0046<br>9            | 0.0000<br>0             | 0.000<br>15           |
| Ara h 8<br>slgE                      | 0.000<br>03 | 0.0001<br>9                          | 0.000<br>02     | 0.236<br>74     | 0.010<br>59     | 0.010<br>07     | 0.021<br>66     |                 | 0.000<br>01     | 0.989<br>97   | 0.121<br>95   | 0.737<br>53   | 0.131<br>51   | 0.877<br>23   | 0.000<br>00   | 0.341<br>42   | 0.0000<br>0            | 0.0405<br>5             | 0.421<br>42           |
| Ara h 9<br>slgE                      | 0.723<br>18 | 0.0385<br>3                          | 0.000<br>04     | 0.000<br>17     | 0.533<br>94     | 0.000<br>03     | 0.849<br>25     | 0.000<br>01     |                 | 0.758<br>74   | 0.404<br>62   | 0.025<br>67   | 0.781<br>22   | 0.028<br>19   | 0.193<br>07   | 0.000<br>00   | 0.0000<br>0            | 0.4794<br>2             | 0.102<br>18           |
| Peanut<br>SA                         | 0.517<br>60 | 0.0000<br>0                          | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.989<br>97     | 0.758<br>74     |               | 0.000<br>00   | 0.000<br>00   | 0.000<br>00   | 0.000<br>00   | 0.958<br>36   | 0.814<br>83   | 0.2034<br>8            | 0.0000<br>0             | 0.205<br>89           |
| Ara h 1<br>SA                        | 0.825<br>89 | 0.0000<br>0                          | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.000<br>00     | 0.121<br>95     | 0.404<br>62     | 0.000<br>00   |               | 0.000<br>00   | 0.000<br>00   | 0.000<br>00   | 0.045<br>89   | 0.219<br>51   | 0.6632<br>5            | 0.0000<br>0             | 0.107<br>09           |
| Ara h 2                              | 0.002       | 0.0000                               | 0.000           | 0.000           | 0.000           | 0.000           | 0.000           | 0.737           | 0.025           | 0.000         | 0.000         |               | 0.000         | 0.000         | 0.907         | 0.183         | 0.8708                 | 0.0000                  | 0.000                 |

|                         |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |             |
|-------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| SA                      | 83          | 0           | 01          | 04          | 00          | 64          | 00          | 53          | 67          | 00          | 00          |             | 00          | 00          | 42          | 84          | 0           | 0           | 07          |
| Ara h 3<br>SA           | 0.619<br>99 | 0.0000<br>4 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 | 0.131<br>51 | 0.781<br>22 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 |             | 0.000<br>00 | 0.148<br>27 | 0.410<br>57 | 0.7701<br>2 | 0.0000<br>0 | 0.608<br>84 |
| Ara h 6<br>SA           | 0.023<br>49 | 0.0000<br>0 | 0.000<br>01 | 0.000<br>19 | 0.000<br>00 | 0.002<br>20 | 0.000<br>00 | 0.877<br>23 | 0.028<br>19 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 |             | 0.945<br>63 | 0.271<br>25 | 0.8986<br>6 | 0.0000<br>0 | 0.000<br>06 |
| Ara h 8<br>SA           | 0.011<br>69 | 0.1691<br>7 | 0.667<br>37 | 0.042<br>91 | 0.986<br>54 | 0.267<br>68 | 0.845<br>75 | 0.000<br>00 | 0.193<br>07 | 0.958<br>36 | 0.045<br>89 | 0.907<br>42 | 0.148<br>27 | 0.945<br>63 |             | 0.024<br>39 | 0.0000<br>0 | 0.8865<br>7 | 0.641<br>15 |
| Ara h 9<br>SA           | 0.010<br>21 | 0.9417<br>4 | 0.952<br>23 | 0.521<br>20 | 0.074<br>82 | 0.995<br>53 | 0.168<br>04 | 0.341<br>42 | 0.000<br>00 | 0.814<br>83 | 0.219<br>51 | 0.183<br>84 | 0.410<br>57 | 0.271<br>25 | 0.024<br>39 |             | 0.2468<br>7 | 0.4353<br>0 | 0.472<br>94 |
| Whole<br>Diversi<br>ty  | 0.000<br>34 | 0.0000<br>0 | 0.000<br>00 | 0.000<br>78 | 0.000<br>69 | 0.000<br>02 | 0.004<br>69 | 0.000<br>00 | 0.000<br>00 | 0.203<br>48 | 0.663<br>25 | 0.870<br>80 | 0.770<br>12 | 0.898<br>66 | 0.000<br>00 | 0.246<br>87 |             | 0.0063<br>8 | 0.065<br>95 |
| Peanut<br>Diversi<br>ty | 0.000<br>44 | 0.0000<br>0 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 | 0.040<br>55 | 0.479<br>42 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 | 0.000<br>00 | 0.886<br>57 | 0.435<br>30 | 0.0063<br>8 |             | 0.001<br>00 |
| Peanut<br>Avidity       | 0.055<br>03 | 0.0689<br>7 | 0.878<br>07 | 0.226<br>35 | 0.000<br>07 | 0.875<br>82 | 0.000<br>15 | 0.421<br>42 | 0.102<br>18 | 0.205<br>89 | 0.107<br>09 | 0.000<br>07 | 0.608<br>84 | 0.000<br>06 | 0.641<br>15 | 0.472<br>94 | 0.0659<br>5 | 0.0010<br>0 |             |

**Table E5. Simple multivariable logistic regression models (n=90), in which combinations of functional IgE characteristics were used to predict %CD63+ LAD2 cell activation.** Unstandardized and standardized beta coefficients are reported alongside significance and variance inflation factor (VIF). VIF>10 was used to indicate any collinearity.

| Supplementary Model No. | Characteristic   | Unstandardized Beta Coefficient | Coefficient Std. Error | Standardized $\beta$ Coefficient | P Value | R Square Value | VIF   |
|-------------------------|------------------|---------------------------------|------------------------|----------------------------------|---------|----------------|-------|
| S1                      | Peanut Diversity | 22.005                          | 3.196                  | 0.588                            | <0.0001 | 0.411          | 1.101 |
|                         | Whole Diversity  | 3.894                           | 2.143                  | 0.155                            | 0.073   |                | 1.101 |
| S2                      | Peanut sIgE      | 0.050                           | 0.016                  | 0.279                            | 0.003   | 0.454          | 1.322 |
|                         | Peanut SA        | 0.765                           | 0.142                  | 0.491                            | <0.0001 |                | 1.322 |
| S3                      | Peanut Diversity | 15.752                          | 3.260                  | 0.421                            | <0.0001 | 0.523          | 1.381 |
|                         | Peanut SA        | 0.635                           | 0.136                  | 0.407                            | <0.0001 |                | 1.381 |

**Table E6. Pairwise comparison of ROC analyses in the same sample cohort (n=100).** Comparison of AUC paired data ROC curves as described by deLong *et al.*<sup>1</sup>

| Characteristic Comparisons                                        | Diagnostic Performance (ROC analyses) |                      |                      |       |                      |                      | Pairwise comparison of AUC's (p value) |
|-------------------------------------------------------------------|---------------------------------------|----------------------|----------------------|-------|----------------------|----------------------|----------------------------------------|
|                                                                   | AUC                                   | Lower Bound (95% CI) | Upper Bound (95% CI) | AUC   | Lower Bound (95% CI) | Upper Bound (95% CI) |                                        |
| Peanut sIgE (kU <sub>A</sub> /L) - Peanut Specific Activity (%)   | 0.752                                 | 0.658                | 0.845                | 0.862 | 0.793                | 0.932                | 0.0038                                 |
| Ara h 1 sIgE (kU <sub>A</sub> /L) - Ara h 1 Specific Activity (%) | 0.668                                 | 0.559                | 0.777                | 0.716 | 0.613                | 0.820                | 0.0898                                 |
| Ara h 2 sIgE (kU <sub>A</sub> /L) - Ara h 2 Specific Activity (%) | 0.916                                 | 0.857                | 0.975                | 0.933 | 0.885                | 0.981                | 0.5415                                 |
| Ara h 3 sIgE (kU <sub>A</sub> /L) - Ara h 3 Specific Activity (%) | 0.672                                 | 0.563                | 0.780                | 0.738 | 0.639                | 0.837                | 0.0664                                 |
| Ara h 6 sIgE (kU <sub>A</sub> /L) - Ara h 6 Specific Activity (%) | 0.908                                 | 0.846                | 0.970                | 0.930 | 0.878                | 0.981                | 0.2420                                 |
| Ara h 8 sIgE (kU <sub>A</sub> /L) - Ara h 8 Specific Activity (%) | 0.624                                 | 0.511                | 0.737                | 0.618 | 0.507                | 0.730                | 0.8743                                 |
| Ara h 9 sIgE (kU <sub>A</sub> /L) - Ara h 9 Specific Activity (%) | 0.468                                 | 0.354                | 0.581                | 0.465 | 0.351                | 0.578                | 0.9552                                 |
| Whole Diversity - Peanut Diversity                                | 0.628                                 | 0.517                | 0.739                | 0.898 | 0.831                | 0.964                | < 0.0001                               |

**Table E7. Simple linear regression models, in which functional IgE characteristics were used to predict %CD63+ Basophil activation.** Unstandardized and standardised beta coefficients are reported alongside significance (n=54).

| Characteristic   | Unstandardized $\beta$<br>Coefficient | Coefficient Std. Error | Standardized $\beta$<br>Coefficient | P Value | R Square<br>Value |
|------------------|---------------------------------------|------------------------|-------------------------------------|---------|-------------------|
| Peanut sIgE      | 0.151                                 | 0.019                  | 0.737                               | <0.0001 | 0.543             |
| Ara h 1 sIgE     | 0.392                                 | 0.067                  | 0.632                               | <0.0001 | 0.399             |
| Ara h 2 sIgE     | 0.338                                 | 0.041                  | 0.756                               | <0.0001 | 0.564             |
| Ara h 3 sIgE     | 0.875                                 | 0.130                  | 0.682                               | <0.0001 | 0.465             |
| Ara h 6 sIgE     | 0.674                                 | 0.091                  | 0.717                               | <0.0001 | 0.514             |
| Ara h 8 sIgE     | -0.232                                | 0.280                  | -0.114                              | 0.410   | 0.130             |
| Ara h 9 sIgE     | -0.011                                | 1.783                  | -0.001                              | 0.995   | 0.000             |
| Peanut SA        | 1.434                                 | 0.169                  | 0.762                               | <0.0001 | 0.581             |
| Ara h 1 SA       | 3.772                                 | 0.584                  | 0.584                               | <0.0001 | 0.445             |
| Ara h 2 SA       | 2.119                                 | 0.322                  | 0.674                               | <0.0001 | 0.455             |
| Ara h 3 SA       | 11.606                                | 1.555                  | 0.719                               | <0.0001 | 0.517             |
| Ara h 6 SA       | 5.681                                 | 0.908                  | 0.655                               | <0.0001 | 0.429             |
| Ara h 8 SA       | -1.692                                | 2.109                  | -0.111                              | 0.426   | 0.012             |
| Ara h 9 SA       | -2.567                                | 5.088                  | -0.070                              | 0.616   | 0.005             |
| Whole Diversity  | 8.378                                 | 4.315                  | 0.260                               | 0.058   | 0.068             |
| Peanut Diversity | 40.432                                | 3.578                  | 0.843                               | <0.0001 | 0.711             |
| Avidity          | 0.635                                 | 0.378                  | 0.281                               | 0.102   | 0.079             |

**Table E8. Simple multivariable linear regression models, in which combinations of functional IgE characteristics were used to predict %CD63+ Basophil activation (n=54).** Unstandardized and standardised beta coefficients are reported alongside significance and variance inflation factor (VIF). VIF>10 was used to indicate any collinearity. For models containing avidity as an input characteristic, n=35 is observed.

| Model No. | Characteristic   | Unstandardized $\beta$ Coefficient | Coefficient Std. Error | Standardized $\beta$ Coefficient | P Value | R Square Value | VIF   |
|-----------|------------------|------------------------------------|------------------------|----------------------------------|---------|----------------|-------|
| S4*       | Peanut slgE      | 0.043                              | 0.025                  | 0.213                            | 0.098   | 0.765          | 2.045 |
|           | Peanut Diversity | 38.851                             | 7.119                  | 0.751                            | <0.0001 |                | 2.50  |
|           | Avidity          | -0.230                             | 0.227                  | -0.102                           | 0.317   |                | 1.326 |
| S5*       | Peanut SA        | 0.616                              | 0.187                  | 0.335                            | 0.002   | 0.809          | 1.690 |
|           | Peanut Diversity | 35.673                             | 5.787                  | 0.690                            | <0.0001 |                | 2.037 |
|           | Avidity          | -0.243                             | 0.200                  | -0.108                           | 0.234   |                | 1.280 |
| S6*       | Peanut slgE      | 0.138                              | 0.025                  | 0.688                            | <0.0001 | 0.540          | 1.041 |
|           | Whole Diversity  | 1.372                              | 6.740                  | 0.025                            | 0.840   |                | 1.007 |
|           | Avidity          | 0.352                              | 0.280                  | 0.156                            | 0.219   |                | 1.037 |
| S7*       | Peanut SA        | 1.374                              | 0.214                  | 0.748                            | <0.0001 | 0.607          | 1.071 |
|           | Whole Diversity  | 9.911                              | 6.270                  | 0.180                            | 0.124   |                | 1.021 |
|           | Avidity          | 0.280                              | 0.260                  | 0.124                            | 0.290   |                | 1.049 |
| S8        | Peanut Diversity | 41.936                             | 3.885                  | 0.874                            | <0.0001 | 0.716          | 1.179 |
|           | Whole Diversity  | -2.594                             | 2.610                  | -0.81                            | 0.325   |                | 1.179 |
| S9        | Peanut slgE      | 0.098                              | 0.016                  | 0.482                            | <0.0001 | 0.760          | 1.299 |
|           | Peanut SA        | 0.998                              | 0.147                  | 0.531                            | <0.0001 |                | 1.299 |
| S10       | Peanut Diversity | 28.841                             | 3.927                  | 0.601                            | <0.0001 | 0.796          | 1.680 |
|           | Peanut SA        | 0.714                              | 0.154                  | 0.380                            | <0.0001 |                | 1.680 |

Footnote: \* For models that include avidity analyses n=35

**Supplementary figure legends:**

**Supplementary figure E1. Spearman's correlation between (A) Ara h 1, (B) Ara h 2, (C) Ara h 6 specific (sIgE) levels and mast cell activation.** %CD63+ LAD 2 cells, represents cell activation following the mast cell activation test. Correlation reported across peanut sensitised population (n=90), with peanut allergic individuals (red) and Peanut sensitized but tolerant individuals (blue). R values have been displayed on the figure and in table 2.

**Supplementary figure E2. Spearman's correlation between (A) Ara h 1, (B) Ara h 2, (C) Ara h 6 IgE specific activity and mast cell activation.** %CD63+ LAD 2 cells, represents cell activation following the mast cell activation test. Correlation reported across peanut sensitised population (n=90), with peanut allergic individuals (red) and Peanut sensitized but tolerant individuals (blue). R values have been displayed on the figure and in table 2.

**Supplementary Figure E3. Avidity ImmunoCAP development assay incorporating two peanut allergic individuals and one peanut sensitised but tolerant individual.** Titration of sodium thiocyanate (NaSCN) using the following concentrations: 0M (PBS), 0.5M, 1M, 2M, 4M, 6M and 8M.

**Supplementary Figure E4. Receiver operator characteristic (ROC) curves for the most discriminative characteristics, allowing for comparison, in the sub population of (n=60), initially selected for Avidity analyses.** Displayed are ROC curves for peanut sIgE (purple), Ara h 2 sIgE (orange), Ara h 6 sIgE (blue), peanut SA (red), Ara h 2 SA (yellow), Ara h 6 SA (turquoise), Avidity (pink), peanut diversity (light green) and whole diversity (grey). For corresponding areas under the curves, see Table E2.

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