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LETTER TO THE EDITOR

A highly sensitive bioluminescent method for measuring allergen-specific IgE in microliter samples

To the Editor,

Measurement of allergen-specific IgE (sIgE), along with the patient's clinical history and results of allergen provocation tests, is fundamental in diagnosing allergic disease.[1] The ImmunoCAP assay is used as a standard to quantify sIgE levels.[2] Although this method is sensitive (range, 0.1-100 kU_A/L), it requires relatively large amount of plasma (which can be problematic when testing young children), and is also limited by its cost and the need for a specific instrument to analyze the test's results. Therefore, further developments are needed to improve the sensitivity of sIgE detection methods, while markedly reducing the volume of sample required and the cost, without sacrificing assay robustness, reproducibility, and accuracy.

Here, we report development of a highly sensitive method for quantifying sIgE levels using a luciferase-linked immunosorbent assay (LuLISA). The IgE LuLISA allows bioluminescent detection of sIgE using an anti-IgE nanobody (single variable heavy

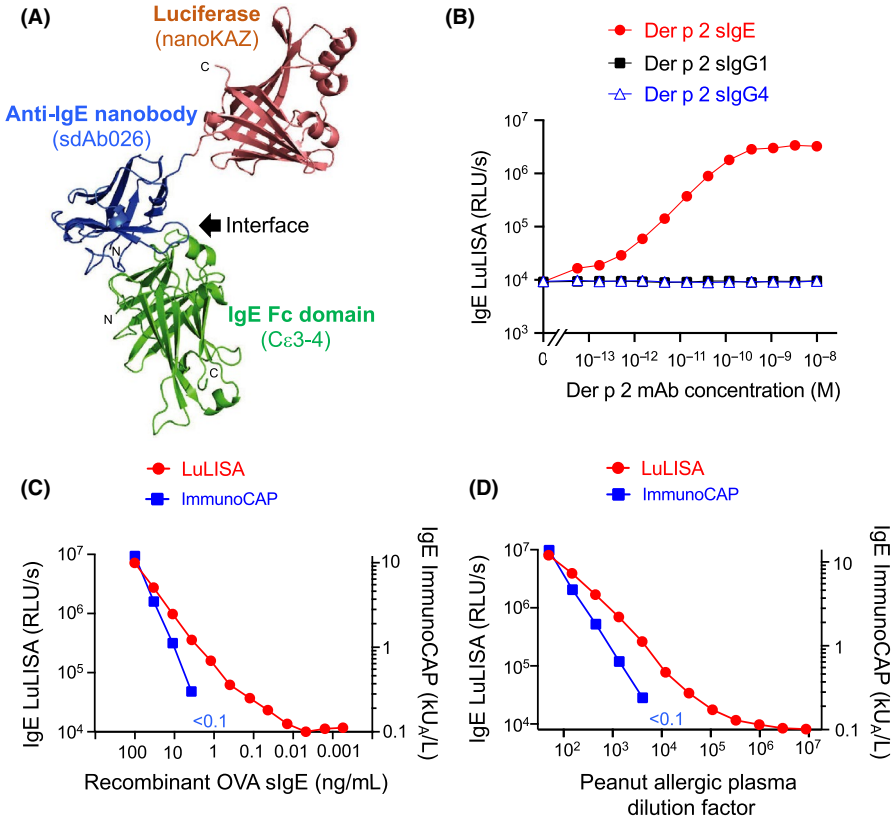


FIGURE 1 Sensitive and specific detection of allergen-specific IgE by LuLISA. A, Cartoon representation showing the anti-IgE nanobody-luciferase tandem (sdAb026-nanoKAZ) bound to the Fc portion of IgE (pink: nanoKAZ luciferase domain [PDB ID: 5B0U][3]; blue: anti-IgE nanobody sdAb026; green: IgE Fc portion Cε3-4 domains [PDB ID: 5NQW]).[9] B, Recombinant human anti-Der p 2 IgE, IgG1, and IgG4 were diluted in PBS at the indicated concentrations and incubated with plate-bound recombinant Der p 2. Bioluminescent detection of antibody levels was performed by LuLISA using the anti-IgE sdAb026-nanoKAZ. C and D, Recombinant anti-OVA IgE (C) or plasma from a peanut-allergic subject (D) were diluted in a pool of plasma from 30 healthy donors. Levels of OVA sIgE (C) or peanut sIgE (D) were assessed in aliquots from the same dilution sample using LuLISA or ImmunoCAP. All LuLISA data are from one experiment representative of three independent experiments. RLU, relative light unit

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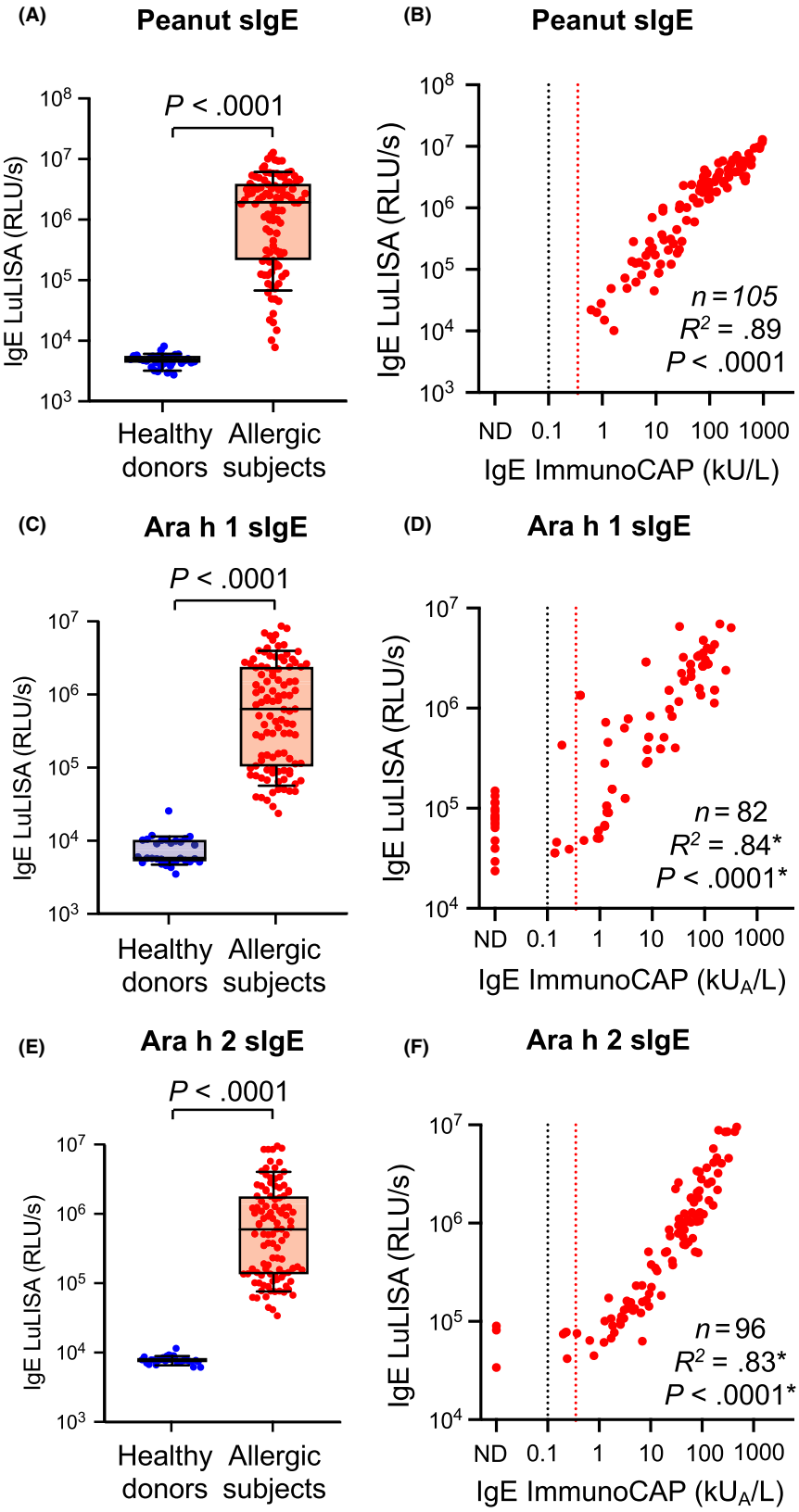


FIGURE 2 Detection of sIgE levels by LuLISA in 1 µL plasma samples from healthy donors and peanut-allergic subjects. A, C, E, Levels of peanut sIgE (A), Ara h 1 sIgE (C) or Ara h 2 sIgE (E) by LuLISA in 1 µL plasma samples from 31 healthy donors and 82-105 peanut-allergic subjects. Data in A, C, and E are shown as box and whisker plots (10th and 90th percentiles), and each circle represents an individual patient. *P* values were calculated by nonparametric Mann-Whitney test (2-tailed). B, D, F, Correlation between peanut sIgE (B), Ara h 1 sIgE (D), or Ara h 2 sIgE (F) by LuLISA vs ImmunoCAP. Black dashed line indicates ImmunoCAP cut-off level (0.1 kU_A/L); Red dashed line indicates cut-off level commonly used in clinical practice (0.35 kU_A/L). RLU: relative light unit. *Pearson's R^2 correlation coefficients and *P* values (two-tailed) were calculated using all samples above the ImmunoCAP cut-off level (0.1 kU_A/L)

immunoglobulin domain [sdAb] or V_HH) which recognizes the constant Cε3 region of human IgE[3] and is expressed in tandem with the catalytic domain of the enzyme luciferase (nanoKAZ)[4] (Figure 1A and Figure S1). The anti-IgE nanobody we used for this assay (sdAb026) has an affinity for IgE similar to that of the therapeutic anti-IgE antibody omalizumab (K_D 1.4 nM vs 2.6 nM, respectively[3,5]) and was reported to inhibit interactions between IgE and the two receptors FcεRI and CD23.[3]

To establish a proof-of-concept for the specific detection of sIgE using this method, we prepared dilution series in PBS of recombinant IgE, IgG1 (the major IgG subclass), or IgG4 (the main IgG subclass overproduced during allergen-specific immunotherapy) directed against the house dust mite allergen Der p 2 (Figure 1B). The three groups of samples were analyzed using IgE LuLISA. As expected, a concentration-dependent signal arose only for the sample containing anti-Der p 2 sIgE, with a detection limit of ~5 × 10^{-13} M sIgE (~1 pg/mL; ~0.0004 kU_A/L) (Figure 1B). We also obtained high sensitivity with recombinant anti-ovalbumin (OVA) IgE, which was detectable by LuLISA at concentrations as low as 5 pg/mL (~0.002 kU_A/L) (Figures S2 and S3). The sensitivity of LuLISA was also much higher than that of standard ELISA for the detection of sIgE with an extended dynamic range over four orders of magnitude instead of two (Figure S3).

Next, we compared the dynamic range and sensitivity of IgE LuLISA versus standard ImmunoCAP, using recombinant OVA sIgE diluted in plasma pooled from 30 healthy donors (Figure 1C). This head-to-head comparison revealed a markedly increased (≈250-fold) analytical sensitivity of LuLISA compared with ImmunoCAP (Figure 1C). We performed similar experiments with dilution series of a plasma sample from a highly peanut-allergic subject, which was again diluted in a pool of plasma from 30 healthy donors (Figure 1D). ImmunoCAP allowed detection of peanut sIgE in plasma diluted up to 4050 times, while peanut sIgE was still detected by LuLISA in allergic plasma diluted 100 000-300 000 times (Figure 1D). Dilution series of the anti-IgE nanobody-luciferase tandem gave a concentration-dependent signal at a fixed (1:50) dilution of this peanut allergic plasma sample and confirmed the very low bioluminescent background signal of the IgE LuLISA (Figure S4).

Altogether, these results indicate that the IgE LuLISA has a very high sensitivity and specificity, and could thus potentially be used to quantify IgE in samples from patients with very low sIgE. However, the cut-off level commonly used in clinical practice to define IgE positivity is 0.35 kU_A/L, which can be measured by ImmunoCAP and is much higher than the sensitivity of the IgE LuLISA. Thus, the main advantage of the IgE LuLISA over ImmunoCAP is that it requires extremely low volume of sample. In the case of the sample from the peanut-allergic patient used in Figure 1D, peanut sIgE could still be detected using <1 nanoliter of the initial patient's sample. Thus, very large screens of sIgE (automatable in 96- and 384-well plates) against arrays of potential allergens can be envisioned using IgE LuLISA, even when patient's sample sizes are limited.

We then sought to further validate this approach by measuring sIgE against total peanut extract, or against the major peanut allergens Ara h 1 and Ara h 2 using 1 µL of plasma from 31 healthy donors (obtained from the French blood bank EFS with unknown allergic status) and 82-105 peanut-allergic subjects (collected upon their enrollment into the institutional review board-approved peanut oral immunotherapy study: safety, efficacy, and discovery trial; ClinicalTrials.gov identifier: NCT02103270).[6] Dilution series from reference samples with titrated high peanut sIgE were used for assay calibration, to ensure that all plasma samples were analyzed within the linear range of detection of our method (Figure S5). As expected, significantly higher levels of peanut sIgE, Ara h 1 sIgE, and Ara h 2 sIgE were detected in plasma samples from peanut-allergic subjects as compared to healthy donors (Figure 2A,C,E). Head-to-head comparison between LuLISA and ImmunoCAP in allergic patients showed a high correlation between both methods (R^2 = .89, .84 and .83 for peanut sIgE, Ara h 1 sIgE and Ara h 2 sIgE, respectively) (Figure 2B,D,F). These correlations were calculated using all plasma samples for which sIgE levels were above the detection cut-off of ImmunoCAP (0.1 kU_A/L). This was the case for all samples for peanut sIgE. However, 17 out of 82 samples (19.7%) for Ara h 1 sIgE and 3 out of 96 samples (3.1%) for Ara h 2 sIgE were below the detection limit of ImmunoCAP (Figure 2B,D,F). However, all these subjects had clear clinical reactivity to peanut, as assessed by performing double-blind, placebo-controlled food challenge (DBPCFC), and skin prick tests (Table S1). Altogether, these results demonstrate that the IgE LuLISA is highly sensitive and accurate for the clinical detection of sIgE and requires very low volumes of plasma.

Besides ImmunoCAP, several other methods have been reported for the detection of sIgE, including IMMULITE and, more recently, isotype-specific agglutination-PCR (ISAP).[7,8] IMMULITE appears to be the closest method to LuLISA as it uses a chemiluminescent approach to detect sIgE. However, the reported detection limit for sIgE with IMMULITE is the same as for ImmunoCAP (0.1 kU_A/L).[7] Similarly to LuLISA, detection of sIgE by ISAP can be performed using 1 µL of clinical sample. Moreover, the two tests are based on different approaches as ISAP requires chemically synthesized allergen-DNA (for each type of allergen) and secondary anti-IgE antibody-DNA conjugate for the detection of sIgE by quantitative PCR.

In summary, the IgE LuLISA is a new method for the detection of sIgE of ultra-high sensitivity requiring only very small (1 µL or less) plasma sample volumes. The use of bioluminescence offers markedly increased sensitivity and extended dynamic range over classical colorimetric (ELISA) or fluorescent (ImmunoCAP) IgE detection methods. The method is fully automatable and uses commercialized plates and a standard luminometer for the bioluminescent detection of IgE. Thus, IgE LuLISA should be very cost-effective over conventional ImmunoCAP. Further tests will be performed to extend the potential use of IgE LuLISA for multiplexed detection of sIgE against arrays of allergens.

KEYWORDS

allergy diagnosis, bioluminescence, IgE, luciferase, nanobody

CONFLICTS OF INTEREST

Dr Nadeau reports grants and other from NIAID, other from Novartis, personal fees and other from Regeneron, grants and other from FARE, grants from EAT, other from Sanofi, other from Astellas, other from Nestle, other from BeforeBrands, other from Alladapt, other from ForTra, other from Genentech, other from AImmune Therapeutics, other from DBV Technologies, personal fees from Astrazeneca, personal fees from ImmuneWorks, personal fees from

Cour Pharmaceuticals, grants from Allergenis, grants from Ukko Pharma, other from AnaptysBio, other from Adare Pharmaceuticals, other from Stallergenes-Greer, other from NHLBI, other from NIEHS, other from EPA, other from WAO Center of Excellence, other from Iggenix, other from Probio, other from Vedanta, other from Centecor, other from Seed, from Immune Tolerance Network, from NIH, outside the submitted work; In addition, Dr Nadeau has a patent Inhibition of Allergic Reaction to Peanut Allergen using an IL-33 Inhibitor pending, a patent Special Oral Formula for Decreasing Food Allergy Risk and Treatment for Food Allergy pending, a patent Basophil Activation Based Diagnostic Allergy Test pending, a patent Granulocyte-based methods for detecting and monitoring immune system disorders pending, a patent Methods and Assays for Detecting and Quantifying Pure Subpopulations of White Blood Cells in Immune System Disorders pending, a patent Mixed Allergen Compositions and Methods for Using the Same pending, and a patent Microfluidic Device and Diagnostic Methods for Allergy Testing Based on Detection of Basophil Activation pending. Dr Galli reports grants from National Institutes of Health, USA, during the conduct of the study; other from Enable Biosciences, outside the submitted work; In addition, Dr Galli has a patent which discloses methods for performing a highly sensitive test for measuring IgE antibodies to certain allergens, using recombinant allergens. pending to Enable Biosciences. Dr Chinthrajah reports grants from NIAID, grants from CoFAR, grants from Aimmune, grants from DBV Technologies, grants from Astellas, grants from Regeneron, other from Alladapt, other from Genentech, other from Novartis, personal fees from Before Brands, outside the submitted work; Dr Reber reports grants from Institut National de la Santé et de la Recherche Médicale (INSERM), during the conduct of the study; grants and personal fees from Neovacs, outside the submitted work; In addition, Dr Reber has a patent WO2019197607 (A1) issued, a patent WO2019228674 (A1) issued, and a patent EP20315224.4 pending. Dr Goyard has a patent EP20315224.4 pending. Dr Janin has a patent EP20315224.4 pending. Dr Rose has a patent EP20315224.4 pending. Drs. Balbino, Liu, Poncet, and Bruhns have nothing to disclose.

AUTHOR CONTRIBUTIONS

SG involved in plasmid and reporter protein design and production. SG, LLR, and TR involved in experimental design. SG and TR involved in conducting experiments. BB, LLR, and PP involved in sample preparation. SG, BB, LLR, PP, and TR involved in acquiring data. RSC, SL, YJ, PB, SJG, and KCN involved in providing reagents and clinical samples. SG, LLR, and TR involved in statistical analysis. SG, LLR, and TR involved in formal analysis. SG, LLR, and TR involved in writing (original draft). All authors involved in writing (review and editing).

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REFERENCES

1. Canonica GW, Ansotegui IJ, Pawankar R, et al. A WAO - ARIA - GA(2)LEN consensus document on molecular-based allergy diagnostics. *World Allergy Organ J*. 2013;6(1):17.
2. van Hage M, Hamsten C, Valenta R. ImmunoCAP assays: pros and cons in allergology. *J Allergy Clin Immunol*. 2017;140(4):974-977.
3. Jabs F, Plum M, Laursen NS, et al. Trapping IgE in a closed conformation by mimicking CD23 binding prevents and disrupts FcepsilonRI interaction. *Nat Commun*. 2018;9(1):7.
4. Inouye S, Sato J, Sahara-Miura Y, Yoshida S, Hosoya T. Luminescence enhancement of the catalytic 19 kDa protein (KAZ) of Oplophorus luciferase by three amino acid substitutions. *Biochem Biophys Res Commun*. 2014;445(1):157-162.
5. Gasser P, Tarchevskaya SS, Guntern P, et al. The mechanistic and functional profile of the therapeutic anti-IgE antibody ligelizumab differs from omalizumab. *Nat Commun*. 2020;11(1):165.
6. Mukai K, Gaudenzio N, Gupta S, et al. Assessing basophil activation by using flow cytometry and mass cytometry in blood stored 24 hours before analysis. *J Allergy Clin Immunol*. 2017;139(3):889-899 e811.
7. Hamilton RG, Franklin Adkinson N Jr. In vitro assays for the diagnosis of IgE-mediated disorders. *J Allergy Clin Immunol*. 2004;114(2):213-225; quiz 226.
8. Tsai CT, Mukai K, Robinson PV, et al. Isotype-specific agglutination-PCR (ISAP): a sensitive and multiplex method for measuring allergen-specific IgE. *J Allergy Clin Immunol*. 2018;141(5):1901-1904 e1915.
9. Tomabechi Y, Hosoya T, Ehara H, Sekine SI, Shirouzu M, Inouye S. Crystal structure of nanoKAZ: The mutated 19 kDa component of Oplophorus luciferase catalyzing the bioluminescent reaction with coelenterazine. *Biochem Biophys Res Commun*. 2016;470(1):88-93.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Supplementary methods

Design and synthesis of plasmid encoding the anti-IgE nanobody-luciferase tandem

NanoKAZ (19 kDa) is an optimized sequence of the catalytic domain of the luciferase from the subtropical sea shrimp *Oplophorus gracilirostris*[1]. The gene *kaz* has been synthetized by Eurofins (Germany) with carboxy-end His_6-tag and flanking region corresponding to the pET23 sequence (Novagen). pET23 plasmid has been amplified with the forward and reverse oligonucleotides (Fwd:5'CTCGAGCACCACCACCACCACCAC3'; Rvr:5'GGTATATCTCCTTCTTAAAGTTAAAC3', Eurofins) using a Q5 DNA polymerase, dNTP mix (New England BioLabs). PCR product was purified by electrophoresis on agarose gel (1%, Macherey Nagel). Purified pET23 vector and the synthetic gene were assembled (pET23-*kaz*) using NEBuilder HiFi assembly master mix (New England BioLabs).

The Fcε3 IgE–binding moiety (Kd= 1.4 nM) is from a humanized heavy chain antibody of the camelid species llama (*lama glama*) selected against IgE (single-domain antibody, sdAb026)[2]. The gene *sdab026* has been synthetized by Eurofins with a flanking region corresponding to the pET23-*kaz* sequence. Synthetic gene *sdAb026* has been amplified with the corresponding forward and reverse oligonucleotides (Fwd: 5'ATGGTCTTCACACTCGAAGATTTC3'; Rvr: 5'CATGGTATATCTCCTTCTTAAAGTTAAA3'; Eurofins) using a Q5 DNA polymerase and dNTP mix. PCR products were purified by electrophoresis on agarose gel. Purified pET23-*kaz* vector and the synthetic gene *sdAb026* were assembled using NEBuilder HiFi assembly master mix. The assembled products (5 µL) were used to transform NEB 5-alpha competent *E. coli* and grown overnight on LB/Agar/ampicillin in a Petri dish. Isolated colonies were grown in liquid medium, plasmids were isolated and nucleotide sequence was performed to confirm the presence of the *sdabs026-kaz* insert. The full sequence of anti-IgE nanobody luciferase tandem

sdAb026-nanoKAZ ([nanobody anti-IgE]-[luciferase]-[His_6]) is shown in **Supplementary Fig 1**.

Expression, purification and validation of anti-IgE nanobody-Luciferase tandem

pET23-*sdab026-kaz* was used to transform *E.coli* BL21 (DE3) to achieve high expression in *E.coli.* Cells were grown at 18°C and IPTG was added to induce sdAb026-nanoKAZ production. After harvesting the cells by centrifugation (1.5 L), the pellet was resuspended in 50 mM Tris-HCl pH 8.0, 50 mM NaCl with protease inhibitor (Sigma) and lysozyme (0.1 mg/mL). Cells were disrupted by freezing-thawing cycle lysis method. DNase (Sigma-Aldrich) was then added to remove DNA from the sample.

The crude extract was centrifuged 30 min at 15000 g. The supernatant was collected and NaCl (500 mM), Imidazole (20 mM) and Triton (0.1 %) were added. The cleared lysate was loaded on a Hi-Trap 5 mL-column (GE-Healthcare) at 4 mL/min using an AKTA pure chromatography system (GE-Healthcare). The column was washed with 20 volumes of column with a running buffer (50 mM Tris-HCl pH 8.0, NaCl 50 mM, 20 mM imidazole) at 5 mL/min. The sdAb026-nanoKAZ was eluted with a gradient of imidazole from 20 mM to 200 mM in 50 mM Tris-HCl pH 8.0, 50 mM NaCl at 5 mL/min and fractions of 1 mL were collected in a 96-deepwell plate (GE-Heathcare). Catalytic activity of fractions was profiled using a luminometer Hydex by diluting 10^7 fold the fractions in PBS with 27 µM of furimazin (imidazo[1,2-*a*]pyrazin-3(7*H*)-ones). The fractions of high activity were pooled, and loaded on a 1 mL HiTrap Q column (GE-Healthcare) equilibrated in 50 mM Tris-HCl pH 8.0, NaCl 50 mM. The protein was eluted in 50 mM MES pH 6.5, 50 mM NaCl at 1 mL/min at 18°C using the AKTA pure chromatography system. The fractions of 500 µL were collected in 96-deepwell plates and their activities were assayed as described above. The fractions of high activity were pooled. The quality of the purified protein was assessed by loading an aliquot (10 µL) on a stain-free SDS gel (4–15%

Mini-PROTEAN® TGX Stain-Free™ Protein Gels, Bio-Rad. The gel was activated by UV trans-illumination for 5 min (Bio--Gel Doc XR Imaging System). Tryptophan residues undergo an UV-induced reaction with trihalo compounds and produce an imaged fluorescence signal (**Supplementary Fig 1, C**). An UV-spectrum (240-300 nm) was acquired for evaluating the concentration of sdAb026-nanoKAZ (MW 34,726 Da) from the solution absorption at 280 nm (ε = 50,780 $M^{-1}cm^{-1}$ estimated by the method of Gill and von Hippel (*Analytical Biochemistry*, **182**: 319-326, 1989)). The specific activity is about $4 \cdot 10^{17} \pm 0.5 \cdot 10^{17}$ $RLU \cdot s^{-1} \cdot g^{-1}$ of sdAb026-nanoKAZ.

IgE LuLISA

White 96-well plates with flat bottoms (Fluoronunc C96 Maxisorp Nunc) were coated with either 10 µg/mL of peanut extract (PE, F171, Greer laboratories), 5 µg/mL of ovalbumin (OVA, Sigma) or 1 µg/mL of Der p 2 (NA-DP2, Indoor Technology) in $NaHCO_3$ 50 mM buffer, 50 µL/well for 2 hours at room temperature. Wells were emptied and the coating was saturated with bovine serum albumin (Sigma) at 100 µg/mL in PBS for 1 hour at room temperature. Wells were washed four times with 100 µL of PBS/Tween 20 0.1%. Recombinant anti-OVA human chimeric IgE (clone X4A4D12/G9/H8, a kind gift from Arkab), recombinant human anti-Der p 2 IgE (2B12 CH-1, Indoor Technology), recombinant anti-Der p 2 human IgG1 (2B12 CH-2, 2B12, Indoor Technology), recombinant IgG4 (2B12 CH-3, Indoor Technology) or plasma from peanut allergic subjects were diluted in PBS or in a pool of plasma from healthy donors, as indicated. Sample dilutions were incubated 1 hour at room temperature in their respective allergen-coated wells, 50 µL/well. Wells were washed four times with 100 µL PBS/Tween 20 0.1%. Purified sdAb026-nanoKAZ 1 ng/mL ($400 \cdot 10^6$ $RLU \cdot s^{-1} \cdot mL^{-1}$) in PBS was loaded (50 µL/well) and incubated 30 min at room temperature. Wells were washed four times with 100

µL of PBS/Tween 20 0.1%. Plates can be stored at this step in PBS until measurements. Just before reading, each of the wells were emptied and loaded with 50 µL of furimazin (imidazo[1,2-a]pyrazin-3(7H)-ones) at 27 µM (about 10 times the K_M of nanoKAZ). The plate was orbitally shaken for 5 seconds and the collected photons were counted during 1 sec per well and measured 3 times in a plate luminometer (LB960 Centro, Berthold).

IgE ImmunoCAP

ImmunoCAP analysis was performed by Phadia, Thermo Fisher (Uppsala, Sweden and Villebon, France), 120 µL of plasma per analyzed sample.

IgE ELISA

Clear, flat bottomed, 96-well plates (Maxisorp, Nunc) were coated with either 10 µg/mL of peanut extract (PE, F171, Greer laboratories), 5 µg/mL of ovalbumin (Sigma) or 1 µg/mL of Der p 2 (NA-DP2, Indoor Technology) in $NaHCO_3$ 50 mM pH 9.5 (Sigma) and incubated for 2 h at room temperature. The plates were then emptied and loaded with BSA 100 µg/mL (Sigma) in $NaHCO_3$ 50 mM pH 9.5 (Sigma) and incubated for 1 h for well surface saturation at room temperature. Wells were washed four times with 100 µL of PBS/Tween 20 0.1%. Recombinant anti-OVA human chimeric IgE (100 ng/mL, clone X4A4D12/G9/H8, a kind gift from Arkab), recombinant human anti-Der p 2 IgE (20 ng/mL, CH1, Indoor Technology), or plasma from peanut allergic subjects (1 µL) were diluted in PBS (50 µL/well) and incubated for 1 hour at room temperature in their respective allergen-coated wells. Wells were washed four times with 100 µL of PBS/Tween 20 0.1%. Then, 50 µL of goat IgG anti-human IgE conjugated with alkaline phosphatase (dilution 1:700 in PBS, Sigma) were loaded in each well and incubated for 1 hour at room temperature. Wells were washed four times with 100 µL of

PBS/Tween 20 0.1%. All wells were emptied and 50 µL of phosphatase substrate pNPP 1 mg/mL (4-Nitrophenyl phosphate disodium salt**,** Sigma) were added per well and incubated for 3 h at room temperature or overnight at 4°C. Absorbances were measured at 405 nm with a plate spectrophotometer after 3 or 24 h of incubation (Sapphire, Tecan).

Statistical analysis

Differences in IgE levels between plasma samples from healthy donors and peanut allergic subjects were compared using an unpaired Mann-Whitney *U* test. *P* values < 0.05 are considered statistically significant. Correlation analyses between IgE LuLISA and IgE ImmunoCAP were performed using two-tailed non-parametric Spearman's tests.

Supplementary references

1. nouye S, Sato J, Sahara-Miura Y, Yoshida S, Hosoya T. Luminescence enhancement of the catalytic 19 kDa protein (KAZ) of Oplophorus luciferase by three amino acid substitutions. *Biochem Biophys Res Commun.* 2014;445(1):157-162.

2. Jabs F, Plum M, Laursen NS, et al. Trapping IgE in a closed conformation by mimicking CD23 binding prevents and disrupts FcepsilonRI interaction. *Nat Commun.* 2018;9(1):7.

3. Coutant EP, Gagnot G, Hervin V, Baatallah R, Goyard S, Jacob Y, Rose T, Janin YL. Bioluminescence Profiling of NanoKAZ/NanoLuc Luciferase Using a Chemical Library of Coelenterazine Analogues. *Chemistry*. 2020 Jan 16; 26(4):948-958.

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CTC GAG CAC CAC CAC CAC CAC CAC TGA
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B

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          10         20         30         40         50         60
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          70         80         90        100        110        120
HYADSVKGRF TISRDNAKNT LYLQMNSLRP EDTAVYWCAT DEEYALGPNE FDYYGQGTLV
         130        140        150        160        170        180
TVSSAAALEM VFTLEDFVGD WRQTAGYNLD QVLEQGGVSS LFQNLGVSVT PIQRIVLSGE
         190        200        210        220        230        240
NGLKIDIHVI IPYEGLSGDQ MGQIEKIFKV VYPVDDHHFK VILHYGTLVI DGVTPNMIDY
         250        260        270        280        290        300
FGRPYEGIAV FDGKKITVTG TLWNGNKIID ERLINPDGSL LFRVTINGVT GWRLCERILA

LEHHHHHH
```

C

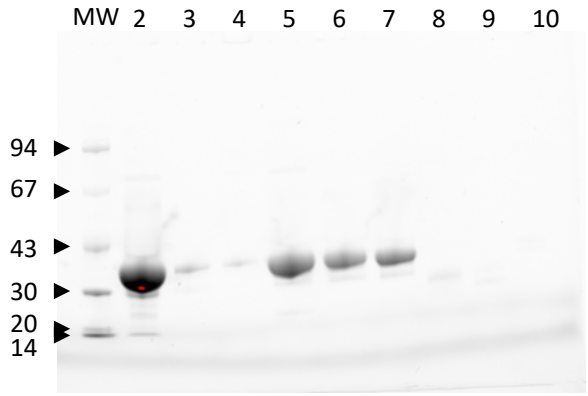


Figure S1. Sequence and characterization of the anti-IgE nanobody-luciferase tandem (sdAb026-KAZ). (**A** and **B**) DNA sequence of *sdAb026-kaz* (**A**), and corresponding amino acid sequence of sdAb026-KAZ (**B**). SdAb026 is a humanized heavy chain antibody of llama (single-domain antibody SdAb, nanobody or V_HH) directed against human IgE. KAZ is an optimized sequence of the catalytic domain of the luciferase from *Oplophorus gracilostris*. In red: sdAb026; In black: KAZ; In blue: His_6-Tag. (**C**) sdAb026-KAZ was produced in *E. coli* and purified using HiTrap chromatography column eluted in phosphate 50mM, imidazole 100mM, NaCl 150mM (lane 2), and DEAE eluted in MES 50mM NaCl 150mM pH 6.5 (successive 1mL-fractions from lanes 3 to 10). The purity and size of sdAb026-KAZ was assessed on SDS gel. The estimated molecular weight (MW) of sdAb026-KAZ calculated from sequence is 34.1kD. Protocols are detailed in supplementary ref. 3.

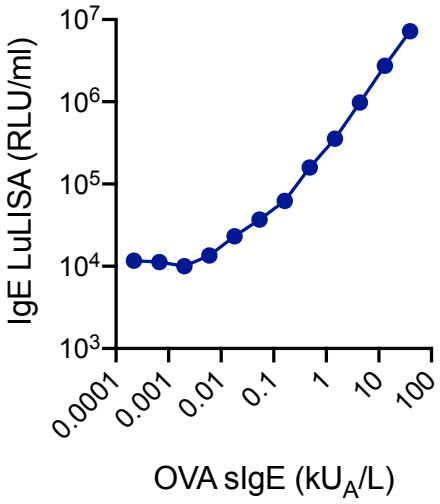


Figure S2. Correlation between LuLISA bioluminescent signal and sIgE concentration in international units. Recombinant human anti-ovalbumin (OVA) IgE were diluted in PBS at the indicated concentrations and incubated with plate-bound OVA. Bioluminescent detection of antibody levels was performed by LuLISA using the anti-IgE sdAb026-nanoKAZ. RLU: relative light unit.

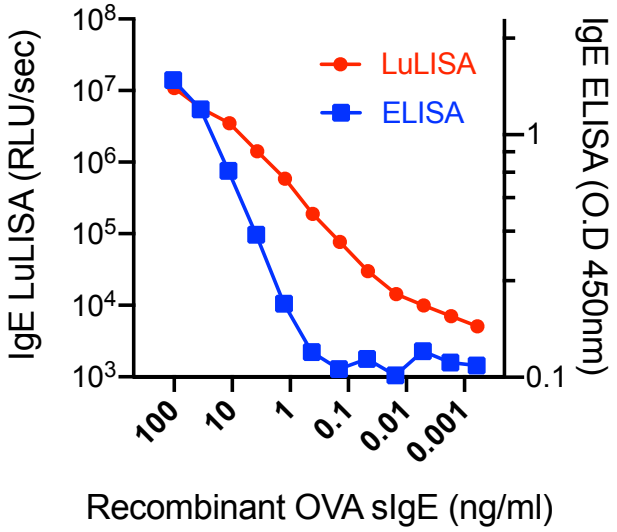


Figure S3. Comparison between IgE LuLISA and IgE ELISA. Recombinant anti-ovalbumin (OVA) IgE was diluted in PBS at the indicated concentrations. Levels of OVA sIgE were assessed in aliquots from the same dilution sample using LuLISA or ELISA. RLU: relative light unit.

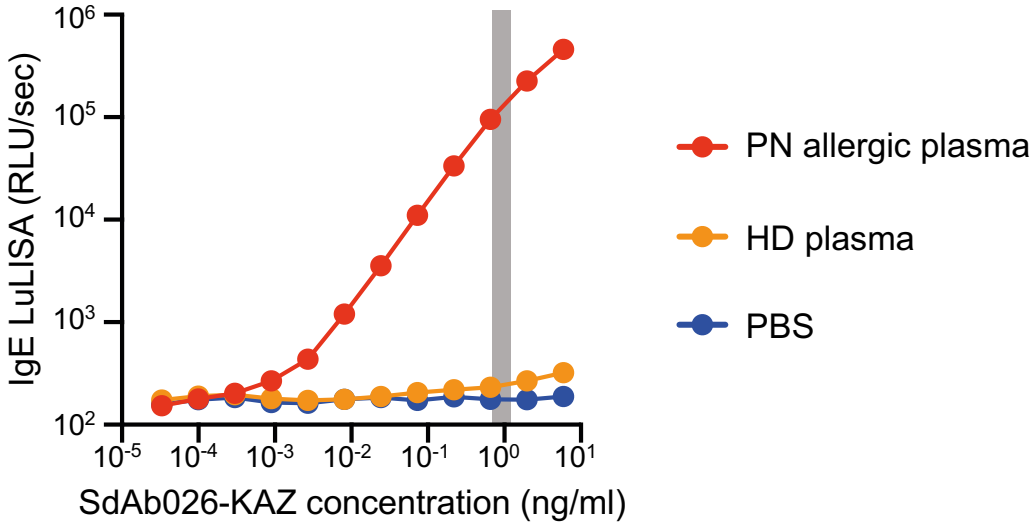


Figure S4. Influence of the concentration of the anti-IgE nanobody-luciferase tandem on bioluminescent detection of IgE by LuLISA. Plasma from a peanut allergic subject ("PN allergic plasma"; peanut-specific IgE ImmunoCAP value: 2186 kU/L) was diluted 2000 times in PBS and incubated in triplicate with plate-bound peanut extract. Plasma from a healthy donor ("HD plasma") diluted 2000 times in PBS or PBS alone were used as controls. Bioluminescent detection of peanut-specific IgE levels was performed by LuLISA at the indicated concentrations of anti-IgE nanobody-luciferase tandem (sdAb026-nanoKAZ). Each point represents average values from triplicates. Area in grey shows values obtained at the sdAb026-nanoKAZ concentrations used for all other experiments in this study. RLU: relative light unit.

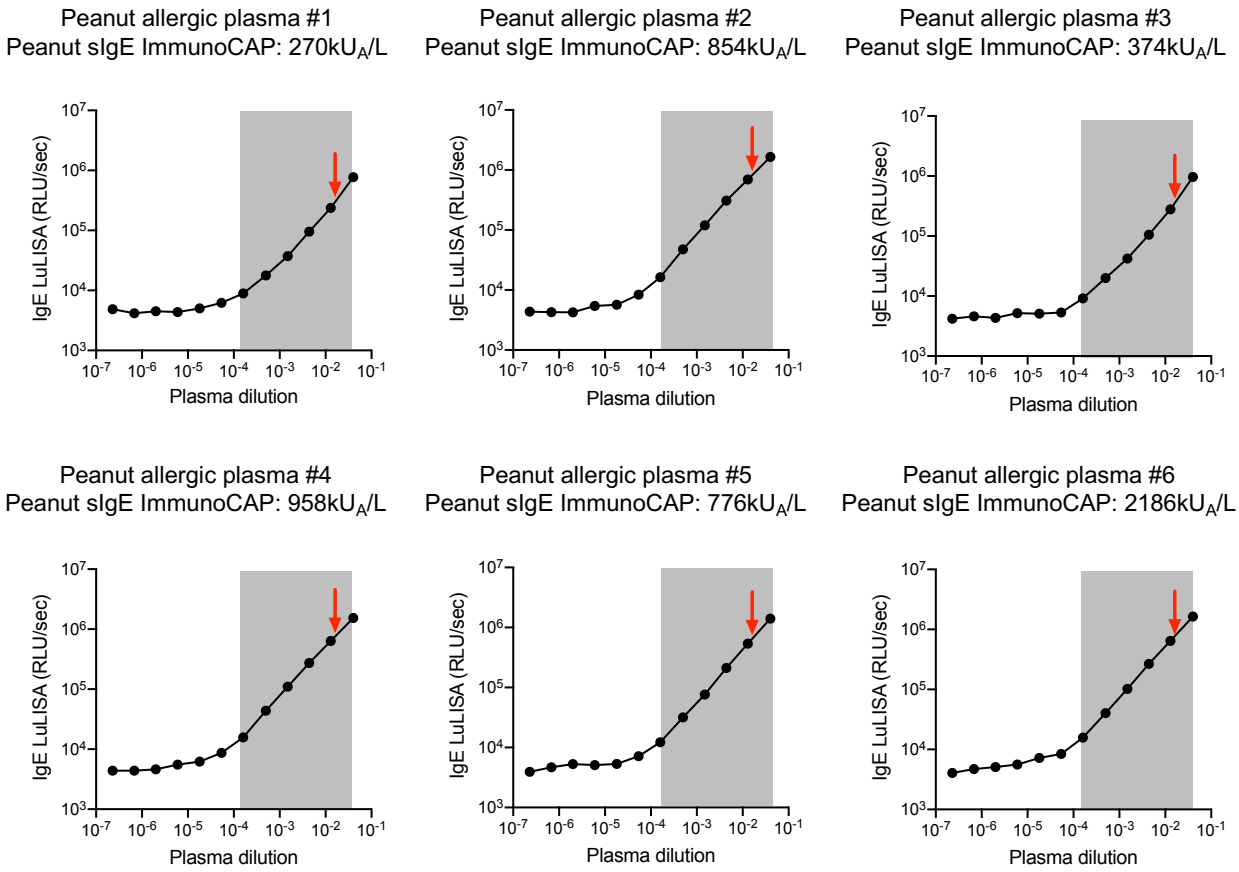


Figure S5. Detection of peanut sIgE by LuLISA using dilution series of plasma from peanut allergic subjects. Plasma specimens from six peanut allergic subjects were diluted in PBS at the indicated concentrations and incubated with plate-bound peanut extract. Bioluminescent detection of peanut sIgE levels was performed by LuLISA. Red arrows indicate plasma dilution used in Figure 2A. Grey areas show the linear range of detection of the peanut sIgE LuLISA. RLU: relative light unit.

patient's ID#	Peanut DBPCFC cumulated tolerated dose (mg)	Skin prick test peanut wheal diameter (mm)	ImmunoCAP Total IgE (IUA/L)	ImmunoCAP peanut specific IgE (IUA/L)	ImmunoCAP Ara h 1 sIgE (IUA/L)	ImmunoCAP Ara h 2 sIgE (IUA/L)
P003	75	7.5	985	4.27	<0.1	2.72
P004	175	15.5	470	3.82	<0.1	3.63
P006	375	19	243	7.67	<0.1	5.25
P013	275	8.5	274	30.4	<0.1	0.37
P025	75	20	410	4.18	<0.1	3.03
P030	275	8.5	35	6.49	<0.1	3.54
P033	375	4	550	1.65	<0.1	<0.1
P055	25	11	333	3.58	<0.1	1.54
P059	5	16.5	449	1.46	<0.1	1.29
P060	75	8	240	0.41	<0.1	0.2
P067	5	10.5	73	0.79	0.5	<0.1
P070	5	19.5	443	13.6	<0.1	10.3
P085	25	12	316	2.89	<0.1	1.71
P097	5	14.5	281	5.43	<0.1	1.72
P102	5	5	87	0.62	<0.1	<0.1
P103	5	17.5	102	0.95	<0.1	0.79
P109	0	6	242	25	<0.1	0.24
P111	0	23	1529	1.1	<0.1	1.25

Table S1. Clinical data from all peanut allergic subjects with Ara h 1 and/or Ara h 2 sIgE below detection limit by ImmunoCAP (0.1 kU_A/L). DBPCFC: double-blind, placebo-controlled food challenge.