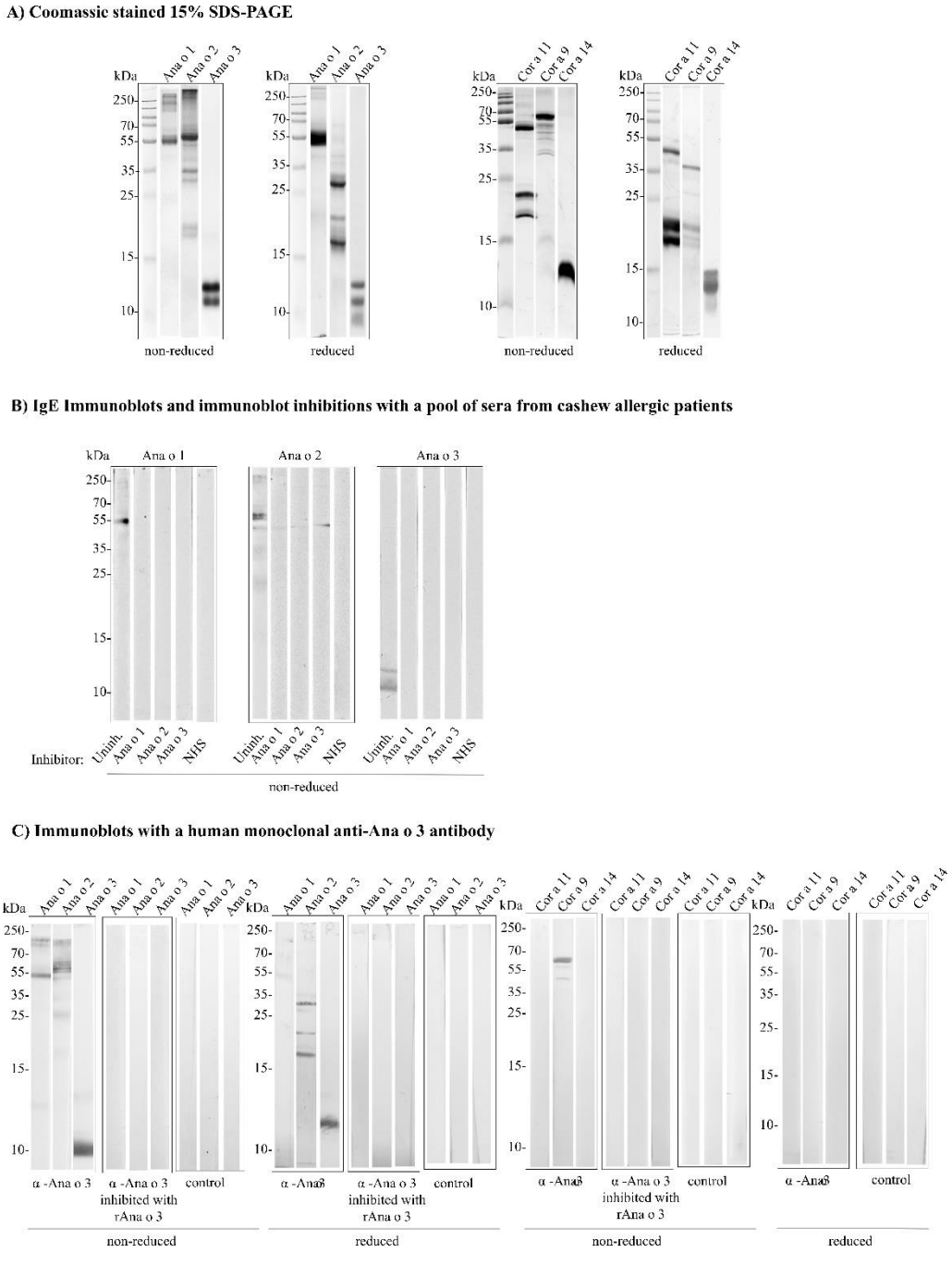
SUPPLEMENTARY MATERIAL

Figure S1 A) Coomassie-stained 15% SDS-PAGE of purified cashew nut and hazelnut allergens run under non-reducing and reducing conditions. B) IgE immunoblot and immunoblot inhibitions of cashew nut allergens. Purified allergens were separated by 15% SDS-PAGE under non-reducing conditions. For IgE immunoblotting, pooled sera from 3 cashew nut allergic patients (CA 1, 2 and 8; Table IA) were used. Uninh.: uninhibited serum pool. NHS: normal human serum of a non-atopic patient was used as a control **C) Immunoblots and immunoblot inhibitions of cashew nut allergens with a human monoclonal anti-Ana o 3 antibody.** Purified allergens were separated by 15% SDS-PAGE under reducing and non-reducing conditions. Inhibitions of binding of anti-Ana o 3 antibody to cashew and hazelnut allergens were performed with 10 µg/ml of rAna o 3.



doi: 10.18176/jiaci.0867

Figure S2 IgE cross-reactivity between Ana o 1, Ana o 2, and Ana o 3 tested using an IgE ELISA inhibition assay. Cross-inhibitions were performed in a dose-dependent manner (0.01 - 10 µg/ml) with 10 sera from patients with cashew nut allergy. Values of 5 representative sera (CA 1-5) are shown.

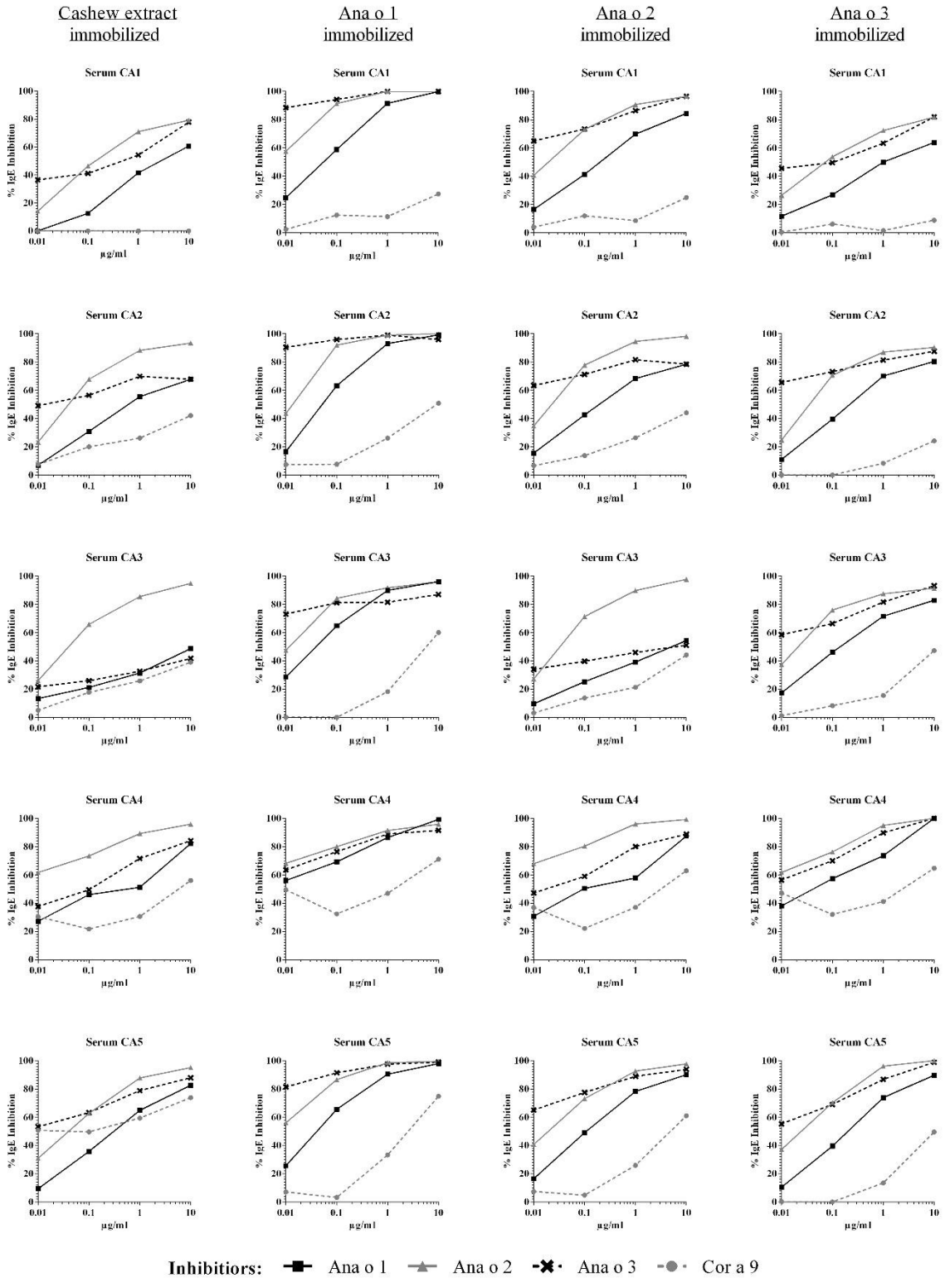


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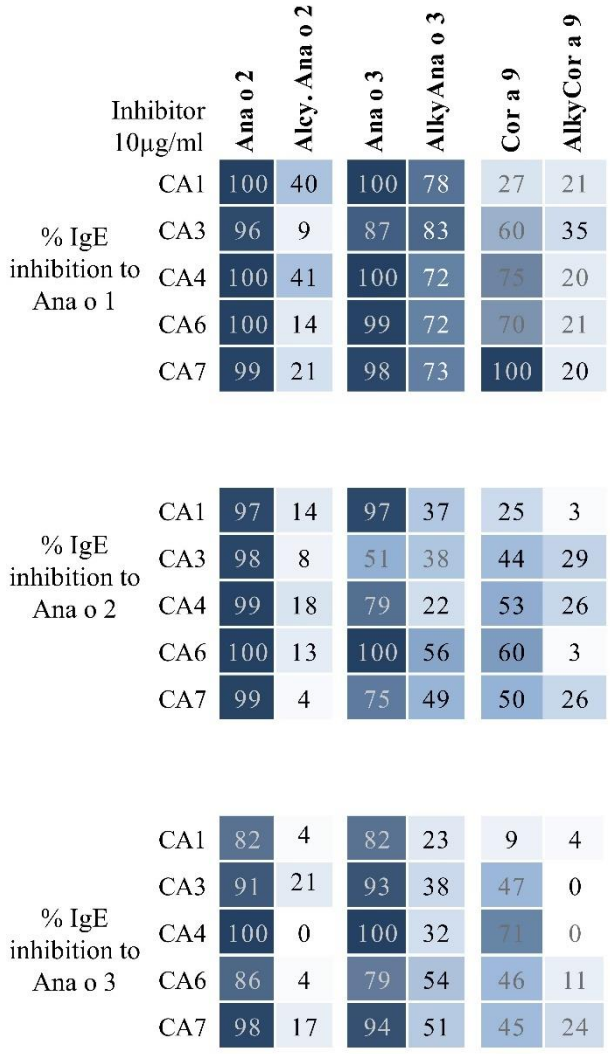
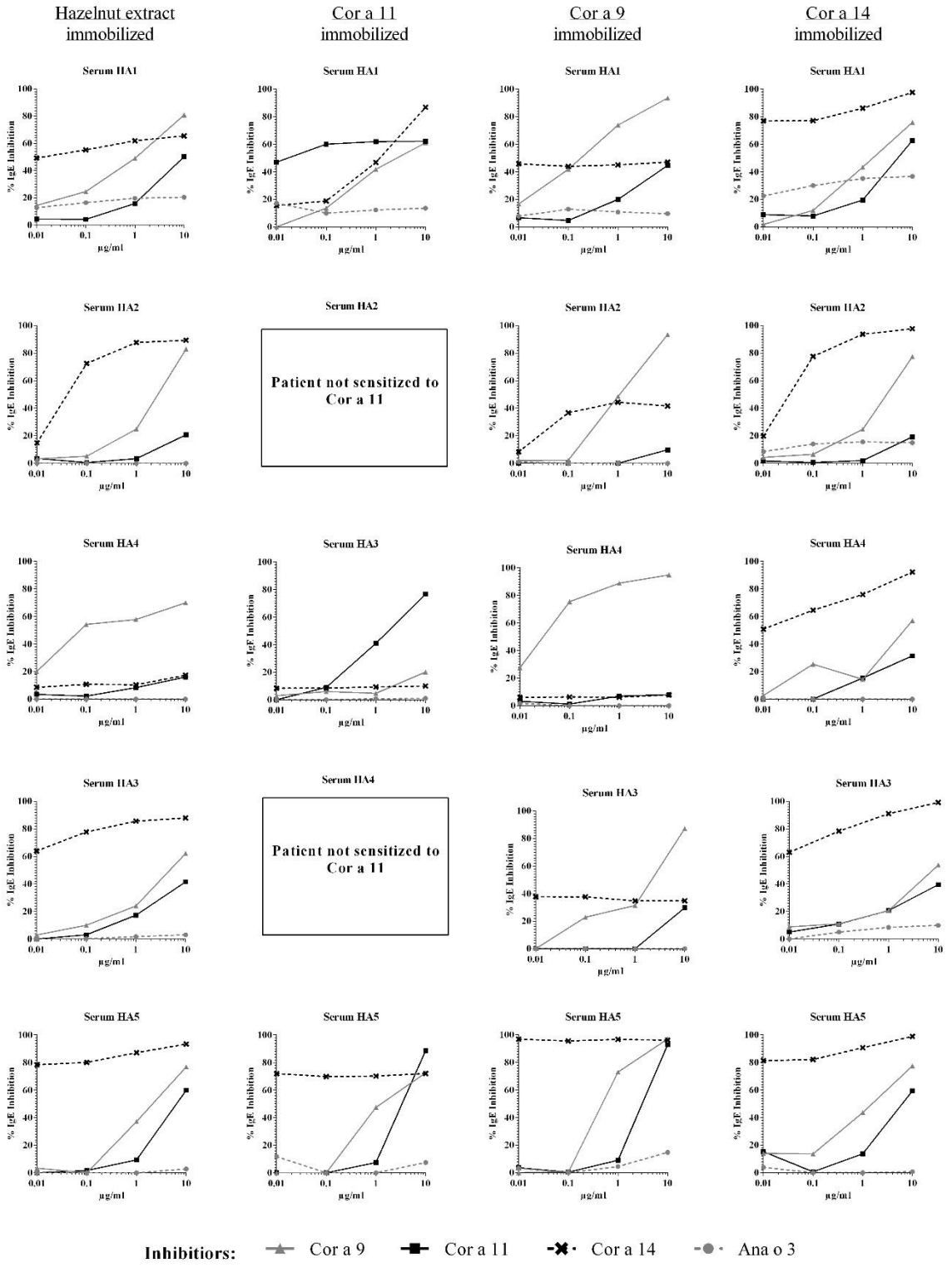
Figure S3. Summary heat map of inhibition ELISA results of five representative cashew allergic patients. For the inhibition ELISA, cashew allergens Ana o 1, Ana o 2, and Ana o 3 were immobilized, and residual IgE binding after preincubating sera with 10 µg/ml reduced and alkylated (RA) Ana o 3, Ana o 2 or Cor a 9 was measured.



doi: 10.18176/jiaci.0867

Figure S4 IgE cross-reactivity between Cor a 11, Cor a 9, and Cor a 14 tested using an IgE ELISA inhibition assay. Cross-inhibitions were performed in a dose-dependent manner (0.01 - 10 µg/ml) with 10 sera from patients with hazelnut allergy. Values of 5 representative sera (HA 1-5) are shown.



doi: 10.18176/jiaci.0867

Figure S5 A. Alignment of peptide sequences obtained by comparing Ana o 3.0101 with Ana o 1.0101, and Ana o 2.0101. Residues identical between Ana o 3.0101, Ana o 1.0101 and Ana o 2.0101 are shown in bold. UniProt accession numbers: Q8L5L5 (Ana o 1.0101), Q8GZP6 (Ana o 2.0101), Q8H2B8 (Ana o 3.0101). All residue numbers refer to numberings of the original UniProt entries that include signal peptides. Previously identified IgE binding peptides of Ana o 3 are underlined in blue [6]. **B.** Model of Ana o 3 showing localization of potentially cross-reactive peptides (red for Ana o 1 and blue for Ana o 2) on the structure of Ana o 3 generated by homology modelling. Ana o 3 was modelled with SWISS-MODEL using the structure of *Moringa oleifera* 2S albumin (PDB accession no.: 5dom). Graphics were generated with UCSF Chimera.

A

Ana o 3.0101 21 SIYRAIVEVEEDSGREQSC**QRQ**F**EEQQRFR**N**C**Q**RY**V**K**QEVQRGGRYNQRQESLRECCQEL 80

Ana o 1.0101 42 **QRQ**YD**EQQ**K 50
Ana o 1.0101 99 CM**RQ**C**E**R**Q** 106
Ana o 1.0101 116 **RFR**CQE**RY** 123
Ana o 1.0101 57 **C**EK**Y**Y**K**EK 64

Ana o 3.0101 81 QEVDRRCRCQNLEQMVRQL**QQQ**E**Q**IK**G**EEVRELYETASELPRICSISPSQGCQFQSSY 138

Ana o 2.0101 189 **QQQ**H**Q**SR**G** 196

B