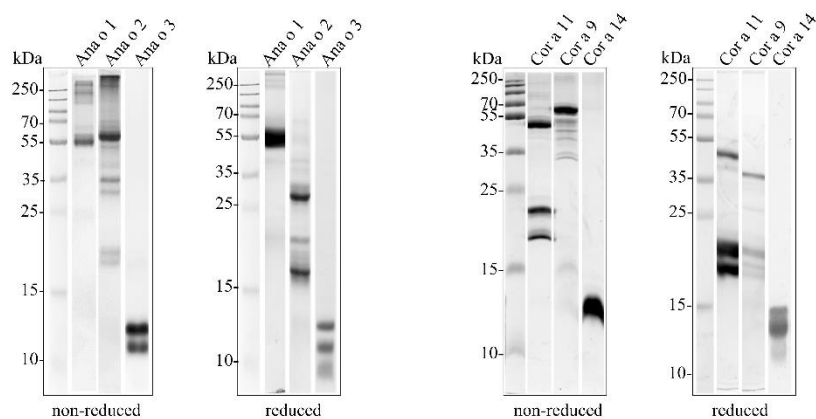


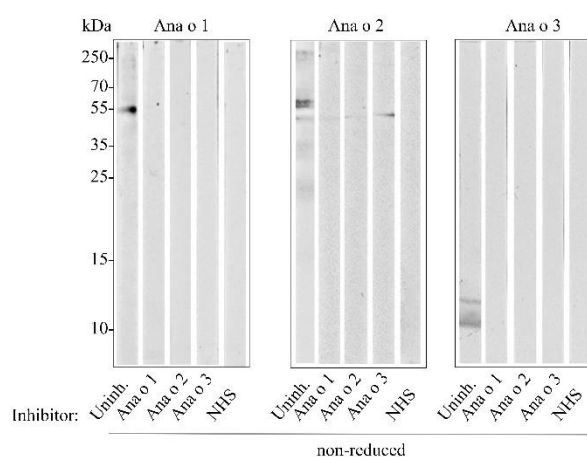
## SUPPLEMENTARY MATERIAL

**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.

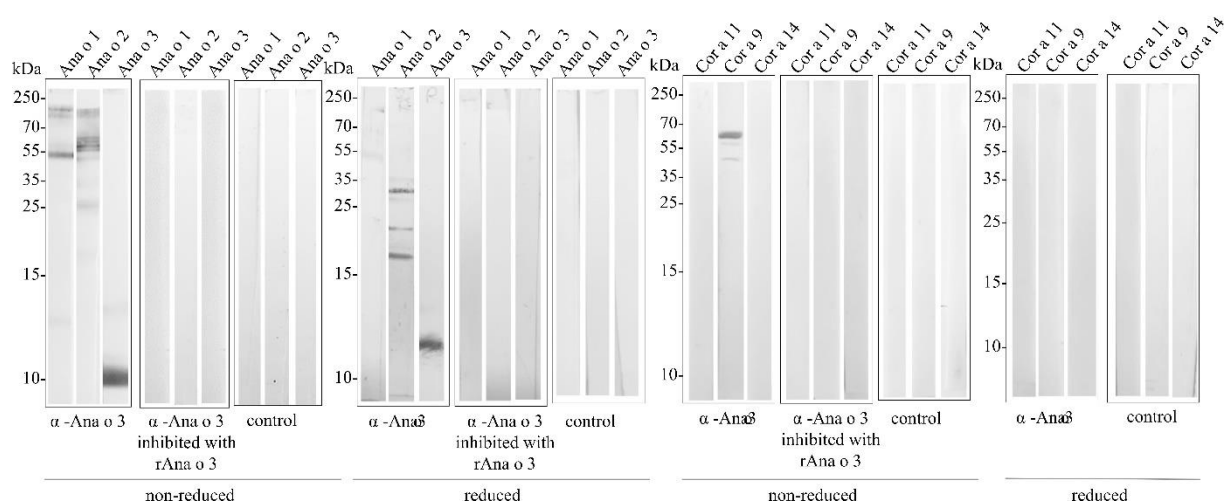
### A) Coomassie stained 15% SDS-PAGE



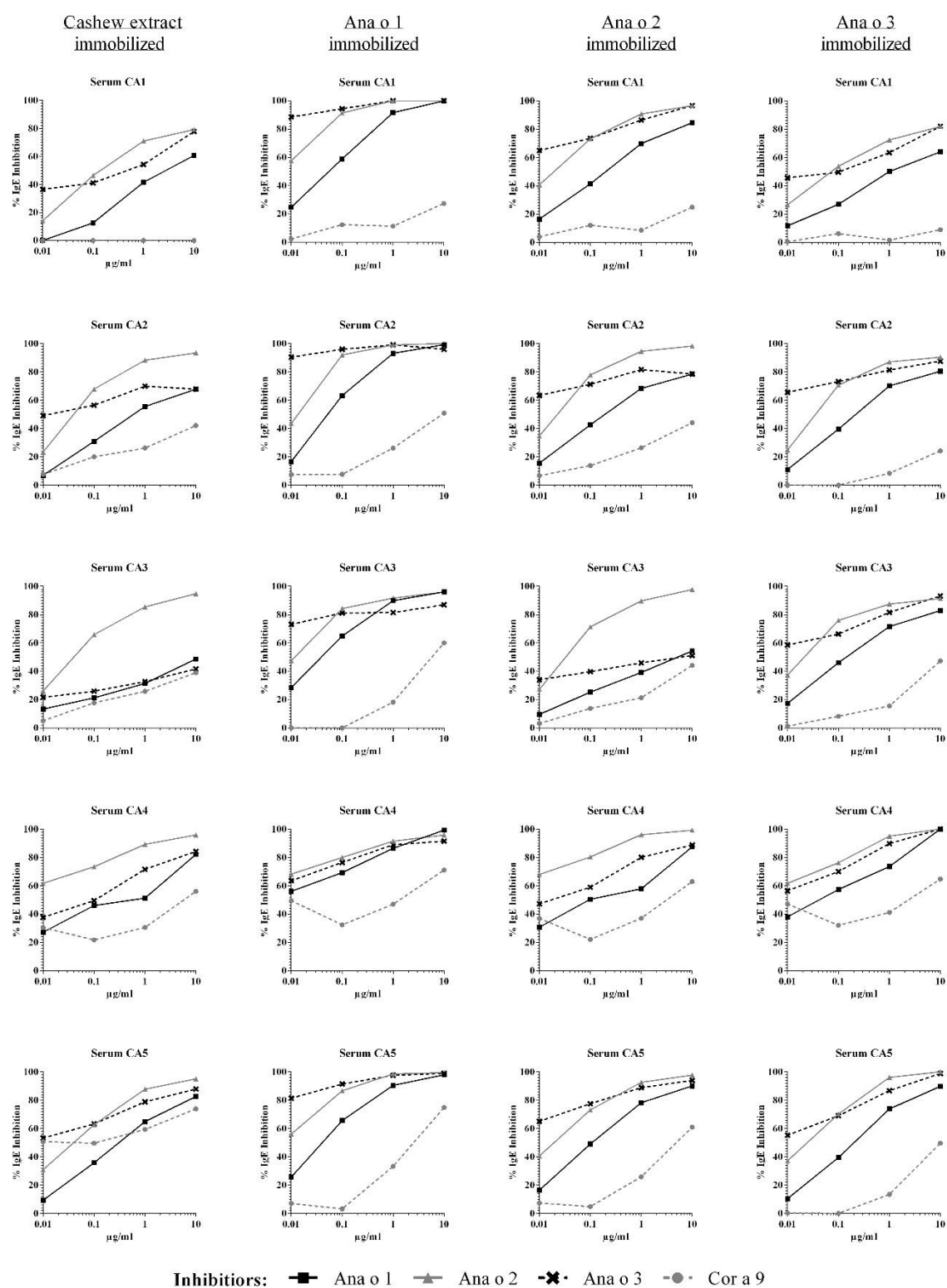
### B) IgE Immunoblots and immunoblot inhibitions with a pool of sera from cashew allergic patients



### C) Immunoblots with a human monoclonal anti-Ana o 3 antibody



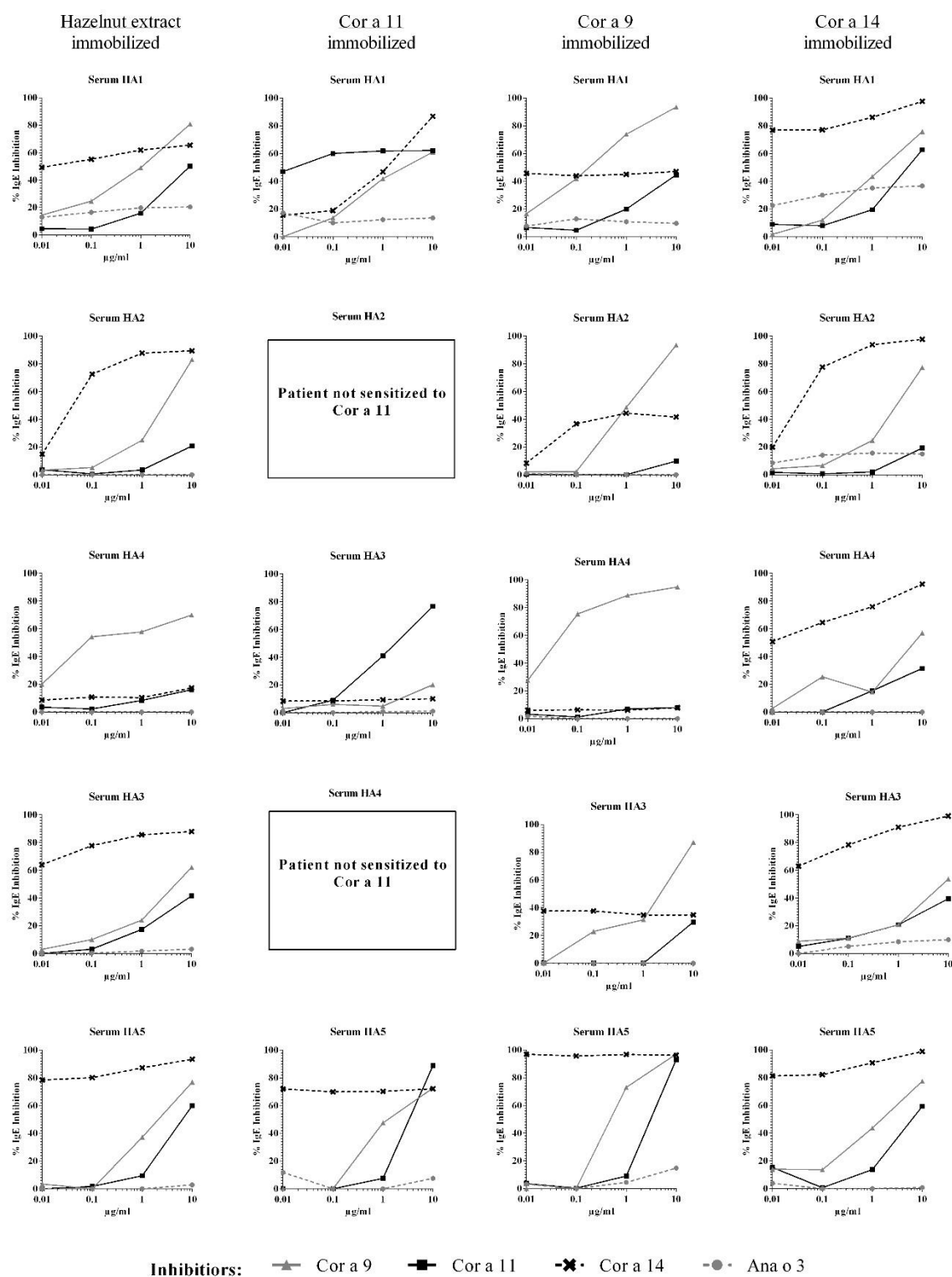
**Figure S2.** IgE cross-reactivity between Ana o 1, Ana o 2, and Ana o 3 tested using an IgE ELISA inhibition assay. Cross-inhibitions were performed in a dose-dependent manner (0.01 - 10  $\mu\text{g/ml}$ ) with 10 sera from patients with cashew nut allergy. Values of 5 representative sera (CA 1-5)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



**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.

	Inhibitor 10µg/ml	Ana o 2		Ana o 3		Cor a 9	
		Ana o 2	Alcy. Ana o 2	Ana o 3	AlkyAna o 3	Cor a 9	AlkyCor a 9
% IgE inhibition to Ana o 1	CA1	100	40	100	78	27	21
	CA3	96	9	87	83	60	35
	CA4	100	41	100	72	75	20
	CA6	100	14	99	72	70	21
	CA7	99	21	98	73	100	20
% IgE inhibition to Ana o 2	CA1	97	14	97	37	25	3
	CA3	98	8	51	38	44	29
	CA4	99	18	79	22	53	26
	CA6	100	13	100	56	60	3
	CA7	99	4	75	49	50	26
% IgE inhibition to Ana o 3	CA1	82	4	82	23	9	4
	CA3	91	21	93	38	47	0
	CA4	100	0	100	32	71	0
	CA6	86	4	79	54	46	11
	CA7	98	17	94	51	45	24

**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  $\mu\text{g/ml}$ ) with 10 sera from patients with hazelnut allergy. Values of 5 representative sera (HA 1-5) are shown.



**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	<b>QRQFEEQQRFRNCQRYVKQ</b>	EVQRGGRYNQREQESLRECCQEL	80
Ana o 1.0101			42	<b>QRQYDEQ</b>	50
Ana o 1.0101			99	<b>CMRQCERQ</b>	106
Ana o 1.0101				116	<b>RFRCQERY</b> 123
Ana o 1.0101				57	<b>CEKYYKEK</b> 64

Ana o 3.0101	81	<u>QEVDRRCRCQNLEQMVRQL</u>	<b>QQQE</b>	<u>QIKGEEVRELYETASELPRICSISPSQGCQFQSSY</u>	138
Ana o 2.0101			189	<b>QQQH</b> QSRG	196

---

**B**

