

Table S1: Sensitization data of the eight walnut allergic subjects selected for the initial SDS–PAGE and immunoblot experiments.

Subject	SPT walnut wheal size (mm)	CAP walnut extract (kU/l)	CAP Jug r 1 (kU/l)	ISAC Jug r 2 (ISU)	CAP Jug r 3 (kU/l)	ISAC Bet v 1 (ISU)
8	n.t.	11	6,0	0.7	0,1	49
9	9	0.5	0,5	0	0	0
20	7	9.7	6,0	0	0	0
25	11	51	45	0.5	0	7.0
26	8	10.9	6,6	0	0,2	91
27	8	51	58	0.4	0,2	0
40	9	8.3	0	0	1,0	51
51	3	0	0	0	0	1.6

SPT: skin prick test, CAP: ImmunoCAP, ISAC: ImmunoCAP ISAC. n.t. not tested

Table S2: LC-MS identification of protein bands cut out of the SDS-PAGE gel with the three walnut extracts

Band	Accession	protein name	family	Allergen	species	Score	Coverage	# Peptides	# PSM	MW [kDa]
1	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	53,81	17,03	12	21	69,9
2	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	410,07	44,86	32	130	69,9
3	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	474,69	53,46	36	179	69,9
4	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	11,99	9,44	5	5	69,9
5	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	9,00	8,77	5	5	69,9
	Q2TPW5	11S globulin	Cupin	Jug r 4	<i>Juglans regia (English walnut)</i>	6,43	7,89	3	4	58,1
6	P93198	2S albumin fragment	seed storage	Jug r 1	<i>Juglans regia (English walnut)</i>	1094,45	66,19	15	324	16,4
	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	28,50	17,03	11	15	69,9
	Q2TPW5	11S globulin	Cupin	Jug r 4	<i>Juglans regia (English walnut)</i>	19,67	12,43	6	7	58,1
	C5H617	Non-specific lipid-transfer protein	nSLTP	Jug r 3	<i>Juglans regia (English walnut)</i>	15,39	43,70	5	7	11,8
7	Q9SEW4	Viciline fragment	cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	218,78	55,48	34	69	69,9
	Q2TPW5	11S globulin	seed storage	Jug r 4	<i>Juglans regia (English walnut)</i>	19,77	11,83	6	7	58,1
	P93198	2S albumin fragment	seed storage	Jug r 1	<i>Juglans regia (English walnut)</i>	9,18	23,74	3	3	16,4
8	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	85,56	21,08	15	32	69,9
	Q2TPW5	11S globulin	Cupin	Jug r 4	<i>Juglans regia (English walnut)</i>	6,87	6,51	3	3	58,1
9	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	14,55	8,26	5	6	69,9
10	Q7Y1C1	Vicilin fragment	seed storage	Jug n 2	<i>Juglans nigra (Black walnut)</i>	12,36	10,40	5	6	55,6
	Q2TPW5	11S globulin	Cupin	Jug r 4	<i>Juglans regia (English walnut)</i>	5,74	4,34	2	2	58,1
11	Q2TPW5	11S globulin	Cupin	Jug r 4	<i>Juglans regia (English walnut)</i>	105,46	32,15	15	28	58,1
	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	29,51	16,02	8	9	69,9
12	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	73,92	44,86	8	26	25,4
13	Q7Y1C1	Vicilin fragment	seed storage	Jug n 2	<i>Juglans nigra (Black walnut)</i>	4,08	3,74	2	2	55,6
14	Q7Y1C1	Vicilin fragment	seed storage	Jug n 2	<i>Juglans nigra (Black walnut)</i>	6,79	6,86	3	4	55,6
15	Q9SEW4	Viciline fragment	Cupin	Jug r 2	<i>Juglans regia (English walnut)</i>	14,15	12,14	6	6	69,9
	Q2TPW5	11S globulin	Cupin	Jug r 4	<i>Juglans regia (English walnut)</i>	8,63	8,28	4	4	58,1
	G8H6H9	oleosin			<i>Juglans regia (English walnut)</i>	5,01	12,23	2	2	14,7
16	Q2TPW5	11S globulin (cupin)	Cupins	Jug r 4	<i>Juglans regia (English walnut)</i>	23,10	14,79	6	7	58,1
	Q7Y1C1	Vicilin	Cupins	Jug n 2	<i>Juglans nigra (Black walnut)</i>	12,61	8,52	4	4	55,6
17	P93198	2S albumin fragment	seed storage	Jug r 1	<i>Juglans regia (English walnut)</i>	43,66	37,41	5	15	16,4

Table S3: LC-MS/MS identification of proteins recognized by IgE from sera of subject 8 and 27. IgE was coupled with magnetic beads for immune precipitation.

Subject 8, walnut PBS extract							
Accession	Allergen	source	Score	Coverage	# Peptides	# PSM	MW [kDa]
P93198	Jug r 1	Juglans regia	223,01	56,83	9	68	16,4
Q9SEW4	Jug r 2	Juglans regia	128,12	43,00	26	39	69,9
Q7Y1C1	Jug n 2	Juglans nigra	114,57	46,57	21	33	55,6
B3STU7	7S vicilin	Carya illinoensis = pecan	67,02	16,71	12	21	94,2
Q2TPW5	jug r 4	Juglans regia	33,21	21,50	7	9	58,1

Subject 8, walnut urea extract							
Accession	Allergen	source	Score	Coverage	# Peptides	# PSM	MW [kDa]
P93198	Jug r 1	Juglans regia	153,01	72,66	14	47	16,4
Q2TPW5	Jug r 4	Juglans regia	129,32	32,35	17	37	58,1
Q9SEW4	Jug r 2	Juglans regia	75,52	38,62	20	27	69,9
B3STU7	7S vicilin	Carya illinoensis = pecan	29,82	14,92	9	12	94,2

Subject 27, walnut PBS extract							
Accession	Allergen	source	Score	Coverage	# Peptides	# PSM	MW [kDa]
P93198	Jug r 1	Juglans regia	191,92	57,55	10	62	16,4
Q9SEW4	Jug r 2	Juglans regia	96,75	36,93	23	31	69,9
B3STU7	7S vicilin	Carya illinoensis = pecan	57,74	20,15	15	19	94,2
Q2TPW5	Jug r 4	Juglans regia	31,54	16,96	6	9	58,1

Subject 27, walnut urea extract							
Accession	Allergen	source	Score	Coverage	# Peptides	# PSM	MW [kDa]
P93198	jug r 1	Juglans regia	142,43	66,19	13	42	16,4
Q2TPW5	jug r 4	Juglans regia	125,90	38,66	20	35	58,1

Table S4: LC-MS analysis of the walnut extract on ImmunoCAP

Accession	Protein	Score	Coverage	# Peptides	MW [kDa]
P93198	Albumin seed storage protein (Jug r 1)	57,73	39,57	4	16,4
Q9SEW4	Vicilin-like protein (Jug r 2)	51,02	9,78	6	69,9
Q43321	Enolase	24,21	26,82	7	47,6
Q2PWC3	Glyceraldehyde-3-phosphate dehydrogenase (Fragment)	12,13	24,29	2	14,9
W9QV79	Heat shock 70 kDa protein	11,09	6,25	3	71,7
Q9FSY7	luminal binding protein	10,49	7,63	4	73,5
A0A072UID0	Cobalamin-independent methionine synthase	10,29	5,75	3	84,4
A0A067JM51	Phosphoglycerate kinase	9,29	10,22	3	42,6
W9RLB1	5-methyltetrahydropteroyltriglutamate	8,90	4,54	3	83,0
A0A0B2S856	4-hydroxy-4-methyl-2-oxoglutarate aldolase	8,56	24,10	3	17,8
H9M3M2	Histone H4 (Fragment)	7,94	46,88	3	7,1
A5C524	Leucine aminopeptidase 3	7,80	5,83	2	60,7
A0A059BU15	UTP--glucose-1-phosphate uridylyltransferase	7,44	6,82	2	48,3
E9KNA6	SGF14a	7,31	10,51	2	29,0
K3XIT0	Fructose-bisphosphate aldolase	6,86	6,98	2	41,6
A0A0D3L730	Betaine-aldehyde dehydrogenase	6,12	6,96	3	54,5
A0A0D6QT83	Actin like	6,05	7,73	2	41,6
A5BDU8	Dihydrolipoyl dehydrogenase	5,36	5,33	2	49,5
A0A059A5I7	Uncharacterized protein	5,32	5,95	2	38,0
B9GZ15	Transaldolase family protein	5,27	5,96	2	47,5
V4UKW2	Glyoxalase_1	2,99	9,09	2	24,7
U5D569	HSP20-like_chaperone	1,65	12,26	2	17,5