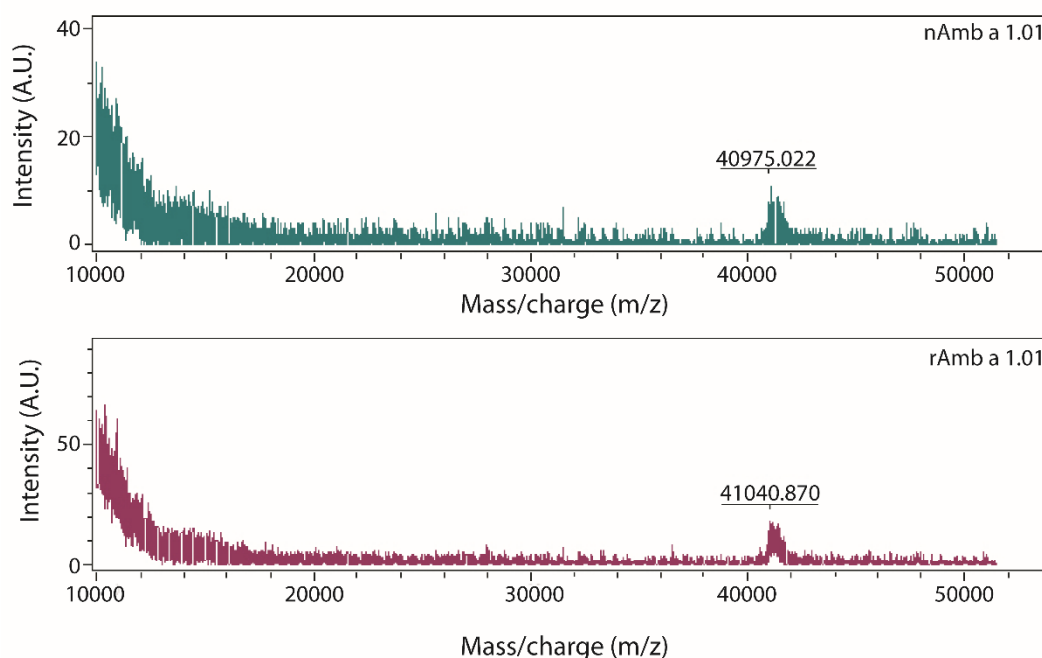


**Figure S1.** Sequence similarity of Amb a 1.01 with other Amb a 1 isoforms. **(a)** Sequence identity matrix of Amb a 1 isoforms. The identity percentage was determined by Clustal Omega. **(b)** Sequence alignment of Amb a 1.01 with other Amb a 1 isoforms. The

isoform sequences were ordered according to the percentage of homology with Amb a 1.01 isoform. The identical sequences were highlighted in grey, the N-glycosylation site was highlighted in yellow, the cysteine residues reported to form the disulphide bonds in Amb a 1.01 were highlighted in pink and the signal peptide was highlighted in blue. The sequence alignment was performed in GeneDoc.



**Figure S2.** MALDI-TOF analysis of nAmb a 1.01 and rAmb a 1.01. The mass/charge ratio is indicated on the x-axis, and intensity is shown as absorption units (A.U.) of the most intensive signals obtained in the mass range (y-axis)

**Table S1.** IgE reactivity of rAmb a 1.01 and nAmb a 1.01 in ELISA and other sensitization of the patients included in the study.

Patient number	rAmb a 1.01* (cut off = 0.09)	nAmb a 1.01* (cut off = 0.06)	Other sensitization than ragweed pollen
156	1.177	1.175	-
157	0.752	0.663	Hazel, Birch, Wheat, Grass pollen mix, Peanuts, Hazelnuts
158	0.109	0.127	Grass pollen mix, HDM, Cat, <i>Blatella</i>
159	0.292	0.359	Grass pollen mix, HDM, Cat, Dog
160	0.905	0.666	-
161	0.208	0.293	-
162	0.386	0.404	Hazel, Birch, Beech, Wheat, Grass pollen mix, Mugwort
163	0.511	0.562	Hazel, Birch
164	0.908	0.928	Wheat, Grass pollen mix, Mugwort, HDM, Cat, Dog
165	0.347	0.411	-
166	0.445	0.576	-
167	0.292	0.358	Birch, Lawn grass
168	0.641	0.950	-
169	0.643	0.849	Wheat, HDM, <i>Aspergillus</i> , <i>Alternaria</i>
170	1.098	1.205	-
171	0.061	0.047	-

172	0.510	0.491	Wheat, Timothy grass, Barley, Orchard grass
173	0.132	0.108	-
174	1.407	1.487	Birch, Lawn grass, Timothy grass, Barley, Orchard grass, HDM
175	0.546	0.514	-
176	0.903	0.906	Hazel, Wheat, Barley, Mugwort, HDM
177	0.499	0.425	HDM
178	0.169	0.159	Birch, Lawn grass, Barley, Orchard grass
179	0.430	0.485	Birch, Wheat, Grass pollen mix, Mugwort
180	0.583	0.813	-
181	0.250	0.380	Hazel, Lawn grass
182	0.603	0.864	Mugwort, HDM, <i>Alternaria</i> , <i>Candida</i>
183	0.872	1.021	Timothy grass, Dog
184	0.426	0.477	-
185	0.345	0.454	-
186	0.211	0.294	-
187	0.241	0.276	Wheat, Grass pollen mix, Cat
188	0.551	0.462	Beech
189	0.141	0.115	Birch, Wheat, HDM
190	1.354	1.865	Dactylis glomerata, Barley, Mugwort, Blatella, <i>Candida</i>
191	0.610	0.607	HDM
192	0.245	0.250	HDM
193	0.466	0.479	Mugwort, HDM
194	0.800	0.927	HDM
195	1.971	2.208	-
196	0.338	0.555	Timothy grass, Lawn grass, Barley, HDM
197	0.103	0.144	HDM
198	1.327	1.217	-
199	2.581	2.527	Hazel, Wheat, Timothy grass, Lawn grass, Dactylis glomerata, Barley, Mugwort
200	0.180	0.175	HDM
201	0.494	0.495	Grass pollen mix, Barley,
202	0.650	0.629	Wheat, Lown grass, Mugwort
203	0.836	1.032	Mugwort
204	1.646	1.500	Mugwort
205	0.818	0.615	Grass pollen mix, Lawn grass, Timothy grass, Dactylis glomerata, Mugwort
206	0.414	0.198	-
207	1.167	1.293	Mugwort, HDM
208	0.326	0.381	Mugwort
209	0.344	0.336	HDM

210	1.331	1.445	Hazel, Birch, Wheat, Grass pollen mix, Rye, Mugwort, HDM
211	0.494	0.711	Grass pollen mix
212	0.123	0.145	Hazel, Birch, Wheat, Grass pollen mix, Lawn grass, Timothy grass, Barley, Cat
213	2.811	2.643	-
214	0.554	0.607	-
215	0.486	0.469	Grass pollen mix, HDM
216	0.560	0.188	-
217	0.930	1.237	HDM
218	1.088	1.261	-
219	2.419	2.598	Wheat, Lawn grass, Timothy grass, Barley, Dactylis glomerata, Mugwort
220	2.394	2.305	HDM
221	1.197	1.365	-
222	1.250	1.995	Hazel, Birch, Wheat, Grass pollen mix, Lawn grass, Timothy grass, Barley, Dactylis glomerata, Mugwort, HDM
223	1.112	1.321	-
224	0.611	1.056	-
225	0.144	0.200	Hazel, Birch
226	0.569	0.495	-
227	0.661	0.724	Lawn grass
228	1.161	1.232	Wheat, Grass pollen mix, Lawn grass, Timothy grass, Barley, Dactylis glomerata, Blatella
229	1.230	1.285	Wheat, Grass pollen mix, Lawn grass, Timothy grass, Barley, Dactylis glomerata,
230	0.144	0.180	-
231	0.738	1.049	-
232	0.612	0.564	-
233	1.041	1.102	-
234	0.374	0.469	Barley, Mugwort
235	0.947	1.063	Hazel, Birch, Barley, HDM
236	2.592	2.706	Grass pollen mix, Barley, HDM
237	1.235	1.573	-
238	1.885	2.293	Hazel, Birch, Grass pollen mix, HDM, Cat
239	1.281	1.294	Timothy grass, Dactylis glomerata, Mugwort
240	1.413	1.092	-
241	0.851	0.882	-
242	0.907	0.936	-
243	0.823	0.950	Dog
244	0.222	0.234	-
245	0.119	0.094	-

	246	<b>0.996</b>	<b>1.335</b>	Hazel, Birch, Wheat, Grass pollen mix, Barley
	247	<b>1.719</b>	<b>1.993</b>	Wheat, Lawn grass, Barley
	248	<b>0.347</b>	<b>0.489</b>	Grass pollen mix, Barley
	249	<b>1.436</b>	<b>1.411</b>	Wheat, Grass pollen mix, Barley, Mugwort, Cat
	250	<b>1.578</b>	<b>1.610</b>	Hazel, Birch, Grass pollen mix, HDM, Alternaria
	251	<b>0.601</b>	<b>0.877</b>	-
	252	<b>1.009</b>	<b>1.367</b>	-
	253	<b>1.079</b>	<b>1.241</b>	HDM, Alternaria
	254	<b>0.333</b>	<b>0.518</b>	HDM
	255	<b>0.367</b>	<b>0.368</b>	Hazel, Birch, Grass pollen mix, Barley
Total	100	99	99	
Sensitization profile				
Monosensitized			37%	
Polysensitized			63%	
Mugwort pollen			20%	
Tree pollen			21%	
Grass pollen			42%	
HDM			29%	
Fungi			5%	
Cat			7%	
Dog			4%	
Blatella			3%	

ELISA positive values were marked with the bold and red color font \*mean OD values of the duplicates. HDM - house dust mite; Grass pollen mix: Orchard grass, Lawn grass, Red fescue, Rye, Timothy grass, Meadow soft grass

**Table S2.** Mediator release assay results after stimulation with nAmb a 1.01 and rAmb a 1.01

Allergen concentration	Patient 199		Patient 204		Patient 207		Patient 213		Patient 226		Patient 241		Patient 248		Patient 255	
	nAmb a 1.01	rAmb a 1.01	nAmb a 1.01	rAmb a 1.01	nAmb a 1.01	rAmb a 1.01	nAmb a 1.01	rAmb a 1.01	nAmb a 1.01	rAmb a 1.01	nAmb a 1.01	rAmb a 1.01	nAmb a 1.01	rAmb a 1.01	nAmb a 1.01	rAmb a 1.01
0	6.03	6.03	2.94	2.94	4.67	4.67	2.74	2.74	5.1	5.1	3.87	3.87	3.71	3.71	4.84	4.84
0.01	12.05	6.91	5.6	4.86	6.31	4.96	9.39	6.34	7.42	5.37	4.02	3.23	3.67	3.42	4.72	3.28
0.1	27.19	8.55	15.24	6.61	9.62	5.43	40.33	18.65	5.83	4.93	5.87	3.36	5.61	3.79	4.38	4.68
1	44.31	29.55	45.34	20.41	31.25	10.26	59.95	48	12.31	5.55	17.16	4.9	3.93	4.53	4	3.32
10	59.57	57.99	63.63	64.28	52.3	41.17	60.47	69.39	36.42	20.17	43.87	25.9	13.56	8.06	14.02	6.71
100	58.23	69.44	62.28	80.46	51.09	67.32	55.6	72.1	40.19	41.85	58.88	60.55	21.72	22.26	20.53	21.59
1000	58.69	75.25	63.61	86.14	60.93	73.45	50.43	71.45	44.99	55.32	62.49	71.46	17.82	26.74	18.66	24.11

The green color marks an RBL degranulation above 20%.

**Table S3.** ImmunoCAP IgE inhibition results after sera incubation with buffer, rAmb a 1.01 and ragweed pollen extract

<b>Patient</b>	<b>Buffer</b> (IgE level - kUA/L)	<b>rAmb a 1.01</b> (IgE level - kUA/L)	<b>Ragweed pollen</b> <b>extract</b> (IgE level - kUA/L)	<b>rAmb a 1.01</b> (Inhibition %)	<b>Ragweed pollen</b> <b>extract</b> (Inhibition %)
198	11.093	0.657	0.934	94.07	91.58
199	52.807	7.43	8.047	85.93	84.76
204	17.708	2.052	2.292	88.41	87.05
207	20.14	1.191	1.757	94.08	91.27
213	102.769	24.742	25.447	75.92	75.23
226	9.239	1.016	1.509	89.00	83.66
232	5.241	0.881	0.885	83.19	83.11
241	9.969	0.865	0.726	91.32	92.71
243	10.541	0.817	1.326	92.24	87.42
245	0.383	0.006	0.035	98.43	90.86
248	4.335	0.256	0.656	94.09	84.86
255	4.466	0.397	0.458	91.11	89.74
NC	0	0.004	0	-	-
<b>Mean inhibition (%)</b>				<b>89.82</b>	<b>86.86</b>

NC – negative control