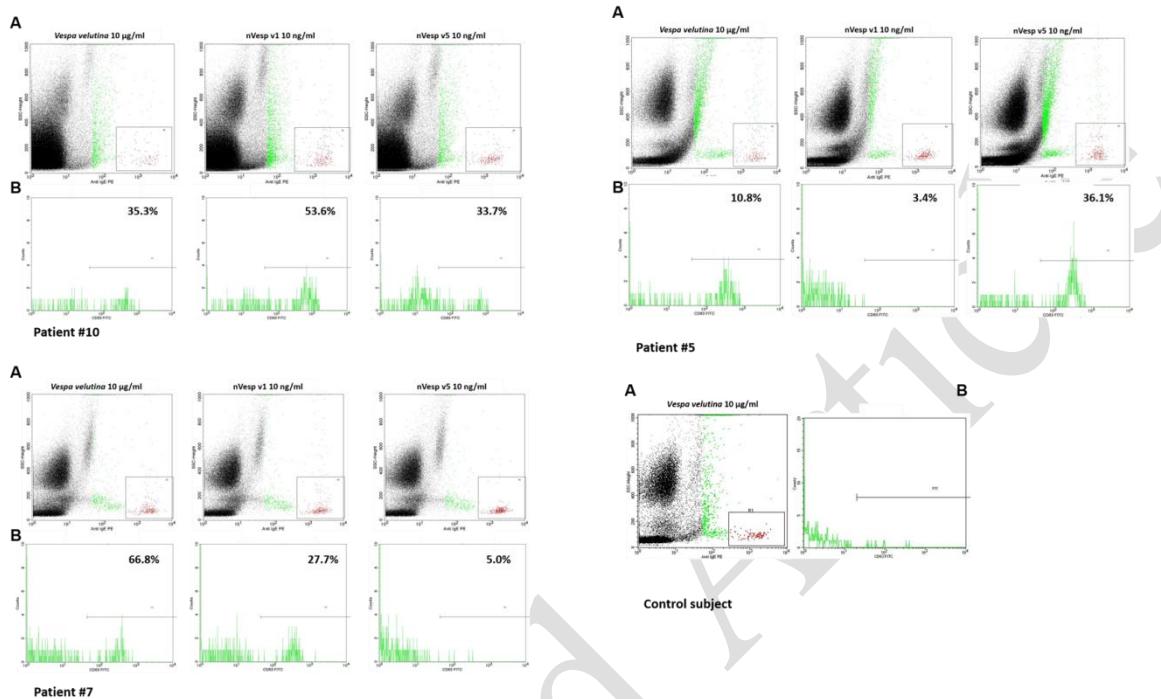


Supplementary material

Supplementary Figure 1. Example of BAT in three selected patients and one atopic control



A) Basophils were defined as cells expressing IgE of high intensity in dot plots (red dots); low intensity anti-IgE cells appear in green; B) Activated basophils were defined as cells expressing CD63+ in histograms. Results are expressed as % of activated CD63+ basophils over total basophil population acquired. A minimum of 500 basophils were acquired for all samples. Patient#10 shows activation against *Vespa velutina* whole venom and both molecules (nVesp v 1 and nVesp v5); Patient#5 shows activation against *Vespa velutina* whole venom and nVesp v 5; Patient#7 shows activation against *Vespa velutina* whole venom and nVesp v 1; Control subject shows no activation.

Supplementary Table 1. Patient characteristics

Parameter	Patient									
	#1 #9		#2 #10		#3		#4		#5	
	#6	#7	#8							
Age (years)	26	48	59	64	79	74	81	36	45	55
Grade of anaphylaxis	1	1	3	3	3	3	2	2	2	2
REMA score	1	0	1	0	1	-2	1	-2	-2	-2
Total serum IgE (UI/mL)	172	356	496	1134	91	488	88	167	119	70
Baseline tryptase (ng/mL)	14.5	4.8	11.1	4.7	4.2	6.7	4.9	5.7	5.2	2.1
slgE MUXF3 (kU _A /L)	0.15	0.32	0.02	0.13	0.07	8.03	0.01	0.01	0.10	0.07
slgE <i>Apis mellifera</i> (kU _A /L)	0.44	0.63	4.64	1.63	0.60	60.90	0.36	0.03	0.11	1.48
slgE rApi m 1 (kU _A /L)	0.04	0.02	0.65	0.03	0.04	22.20	0.29	0.00	0.00	0.00
slgE rApi m 5 (kU _A /L)	0.03	2.20	0.18	10.40	0.99	30.60	0.01	0.02	0.37	2.70
slgE rApi m 10 (kU _A /L)	0.00	0.10	12.30	0.02	0.15	6.89	0.00	0.04	0.00	0.00
slgE <i>Vespula spp</i> (kU _A /L)	3.35	16.70	>100	>100	8.77	35.30	6.44	9.82	6.84	6.37
slgE rVes v 1 (kU _A /L)	2.77	19.50	0.40	1.42	0.02	0.78	0.03	12.50	0.02	6.89
slgE rVes v 5 (kU _A /L)	0.13	1.21	>100	>100	12.30	20.40	4.13	0.60	7.25	0.11
slgE <i>Polistes dominula</i> (kU _A /L)	0.14	36.00	48.80	>100	1.56	28.60	3.30	10.30	3.47	19.9
slgE rPol d 5 (kU _A /L)	0.19	14.80	83.20	>100	2.00	23.30	1.42	1.44	5.17	0.77
slgE <i>Vespa crabro</i> (kU _A /L)	0.87	5.82	2.94	7.79	0.30	24.10	3.39	8.02	0.87	1.87
slgE <i>Vespa velutina</i> (kU _A /L)	1.11	4.93	8.29	2.42	0.41	32.60	2.80	7.66	2.25	1.63
slgE nVesp v 1 (kU _A /L)	0.70	1.12	0.56	0.53	0.07	9.44	0.12	1.17	0.05	0.91
slgE nVesp v 5 (kU _A /L)	0.03	1.71	26.90	62.40	1.51	14.20	1.36	0.16	5.41	0.07

MUXF3, N-glycan from bromelain; r, recombinant; REMA score, measurement recommended by the European Competence Network on Mastocytosis to calculate the risk of suffering from a clonal mast cell disorder (increased risk with REMA score higher than 2)

Supplementary Table 2. Individual results of basophil activation test with whole *Vespa velutina nigrithorax* venom and with venom antigen components (nVesp v 1 and nVesp v 5) in the 10 patients studied.

Patient	Negative control	Positive control	<i>Vespa velutina nigrithorax</i> venom				nVesp v 1				nVesp v 5			
			10 µg/mL	1 µg/mL	0.1 µg/mL	0.01 µg/mL	10 ng/mL	1 ng/mL	0.1 ng/mL	0.01 ng/mL	10 ng/mL	1 ng/mL	0.1 ng/mL	0.01 ng/mL
#1	1.7	30.7	13.7	16.1	5.6	3.3	3.2	5.1	2.1	1.0	1.0	2.0	0.5	1.1
#2	5.8	36.2	31.3	38.2	35.2	27.6	19.2	20.9	13.9	9.7	36.0	37.5	36.9	36.1
#3	1.6	47.1	6.5	7.9	3.7	3.3	2.6	2.5	1.6	2.5	5.9	5.7	4.9	6.1
#4	3.6	30.4	15.5	14.9	10.9	5.1	4.8	4.3	8.4	4.1	21.3	28.3	27.4	23.0
#5	4.2	43.3	10.8	12.5	7.5	2.6	3.4	2.4	1.6	1.6	36.1	33.2	36.0	16.1
#6	1.2	25.0	31.7	33.7	29.1	22.2	1.8	5.1	0.8	1.5	32.8	31.1	32.2	31.3
#7	2.1	16.4	61.6	62.5	55.7	44.6	45.4	35.3	24.9	2.0	64.3	60.3	50.9	56.1
#8	4.8	27.3	66.8	32.2	20.0	14.4	27.7	27.6	21.6	14.8	5.0	5.3	4.6	2.9
#9	1.7	31.4	57.0	59.3	64.3	61.2	21.8	12.9	4.6	2.0	61.1	63.8	69.8	62.0
#10	4.0	45.4	35.3	59.0	60.8	41.4	53.6	60.0	58.1	50.3	33.7	35.5	39.3	30.6

Data are percentages of basophil activation at every antigen concentration with the CD63-based kit. Negative control, wash solution. Positive control, N-formyl-Met-Leu-Phe peptide.

Supplementary Table 3. Individual results of basophil activation test with whole *Vespa velutina nigrithorax* venom in 5 control patients (4, atopic and 1 (#5), non-atopic)

Control subject	Negative control	Positive control	<i>Vespa velutina nigrithorax</i> venom			
			0.01 µg/mL	0.1 µg/mL	1 µg/mL	10 µg/mL
#1	2.1	27.6	0.9	0.5	2.47	3.4
#2	3.3	24.0	2.4	5.2	2.8	6.5
#3	1.4	21.7	1.1	2.6	2.5	2.1
#4	1.4	37.2	1.2	2.0	2.7	1.8
#5	0.2	12.0	0.0	0.0	0.0	0.0

Data are percentages of basophil activation at every antigen concentration with the CD63-based kit. Negative control, wash solution. Positive control, N-formyl-Met-Leu-Phe peptide.