

SUPPLEMENTARY MATERIAL

TABLES

Table S1. CD34⁺-derived MCs characterization.

Subject ID	Number of cells	Receptor expression		Viability
		KIT+	FcεRI+	
1	540000	77.00%	63.50%	70.00%
2	830000	95.70%	59.80%	72.30%
3	2000000	64.70%	71.80%	84.50%
4	500000	95.70%	53.50%	73.30%
5	300000	64.30%	69.10%	85.00%
6	1500000	91.50%	86.10%	80.00%
	945000	81.48%	67.30%	77.52%
7	690000	75.80%	80.80%	74.40%
8	500000	70.20%	64.20%	80.00%
9	5000000	75.20%	47.20%	80.00%
10	2000000	84.80%	86.90%	80.50%
11	500000	81.30%	57.90%	71.00%
	1738000	77.46%	67.40%	77.18%
12	2000000	95.80%	75.30%	78.00%
13	2000000	64.60%	52.90%	83.00%
14	1000000	94.80%	67.50%	70.00%
15	500000	64.50%	74.90%	85.00%
	1375000	79.93%	67.65%	79.00%

Characteristics of CD34⁺-derived MCs from each group of patients (anaphylaxis, sensitized and healthy volunteers). A= Anaphylaxis; S= Sensitized; H= Healthy volunteers.

Table S2. Raw data of the mast cell activation test.

GROUP	Subject ID	Stimuli	Pooled sera		
			Healthy volunteers	Anaphylaxis	Sensitized
Anaphylaxis	1	Sera	4.0%	5.6%	0.0%
		Sera + Pru p 3	4.3%	73.7%	6.7%
	2	Sera	4.5%	5.2%	0.0%
		Sera + Pru p 3	5.0%	75.3%	22.1%
	3	Sera	2.0%	5.3%	0.0%
		Sera + Pru p 3	4.7%	37.1%	14.9%
	4	Sera	6.2%	7.6%	0.0%
		Sera + Pru p 3	7.6%	66.6%	9.7%
	5	Sera	7.0%	5.9%	0.0%
		Sera + Pru p 3	7.4%	64.9%	7.8%
	6	Sera	7.8%	7.1%	0.0%
		Sera + Pru p 3	10.1%	77.7%	32.9%
Sensitized	7	Sera	0.0%	4.1%	0.1%
		Sera + Pru p 3	3.8%	72.9%	23.6%
	8	Sera	6.6%	4.8%	0.0%
		Sera + Pru p 3	6.9%	90.7%	21.1%
	9	Sera	0.0%	4.8%	0.1%
		Sera + Pru p 3	6.1%	81.9%	17.6%
	10	Sera	7.3%	5.0%	0.0%
		Sera + Pru p 3	11.6%	63.8%	24.0%
	11	Sera	3.1%	4.2%	0.0%
		Sera + Pru p 3	5.3%	68.1%	8.6%
Healthy volunteers	12	Sera	5.0%	5.5%	0.0%
		Sera + Pru p 3	5.6%	62.6%	14.9%
	13	Sera	4.8%	7.4%	0.1%
		Sera + Pru p 3	6.8%	64.5%	29.1%
	14	Sera	3.0%	4.9%	0.0%
		Sera + Pru p 3	5.7%	70.8%	18.0%
	15	Sera	2.0%	5.7%	0.0%
		Sera + Pru p 3	4.1%	64.1%	18.4%

MCs incubated overnight with different pooled sera (Anaphylaxis, Sensitized and Healthy volunteers), washed and activated with Pru p 3.

Figures

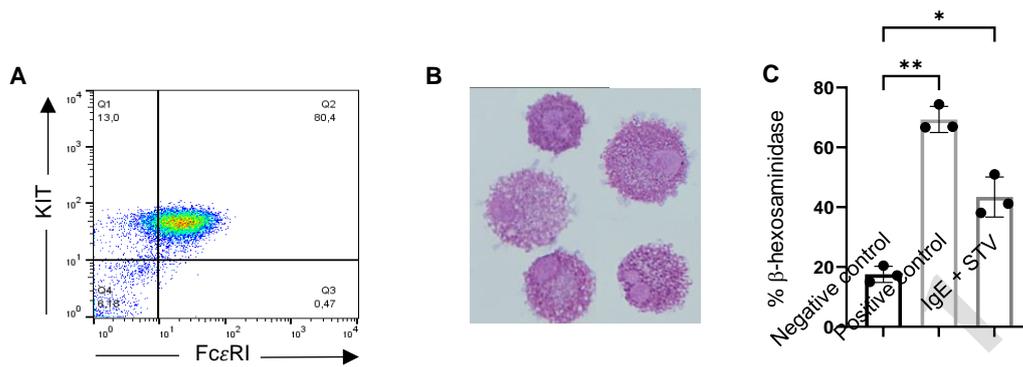


Figure S1. CD34⁺-derived mast cells characterization. A) FcεRI and KIT expression of MCs after 7 weeks. **B)** May Grünwald Giemsa staining of huMCs. **C)** Degranulation measured by β-hexosaminidase assay. PMA and Ionomycin were used as a positive control (n=3). Results are expressed as mean ± SD. Significance was determined using 1-way ANOVA with Dunkey's multiple comparison analysis. $P < 0.05$ was considered significant. * $P < 0.05$; ** $P < 0.01$. Figure shows a representative example. STV= Streptavidin.

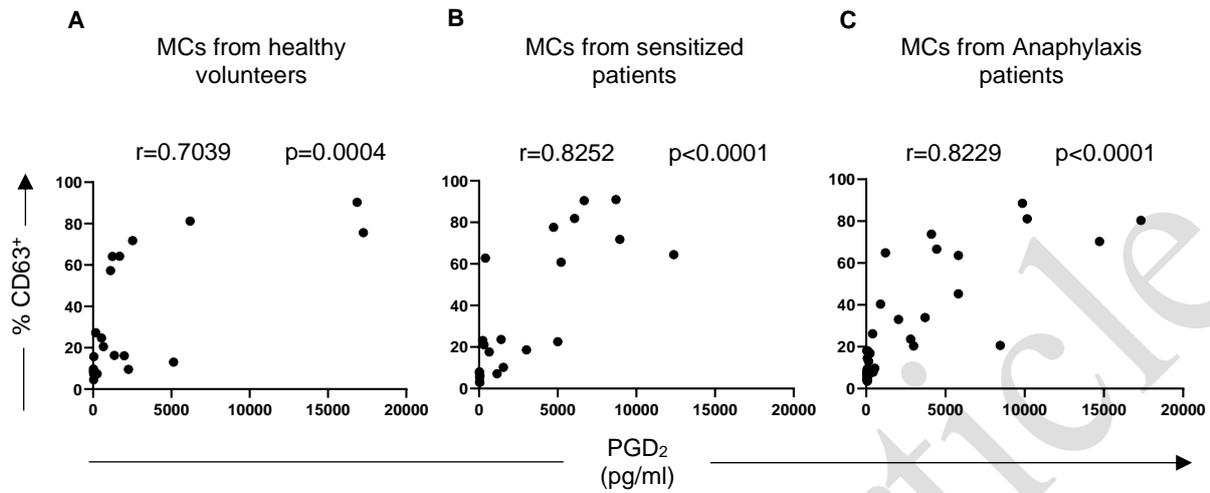


Figure S2. Correlation between degranulation by CD63⁺ and PGD₂ secretion. A) MCs from healthy volunteers. **B)** MCs from sensitized patients. **C)** MCs from anaphylaxis patients. Correlations were calculated by using Pearson R values. $P<0.05$ was considered significant.

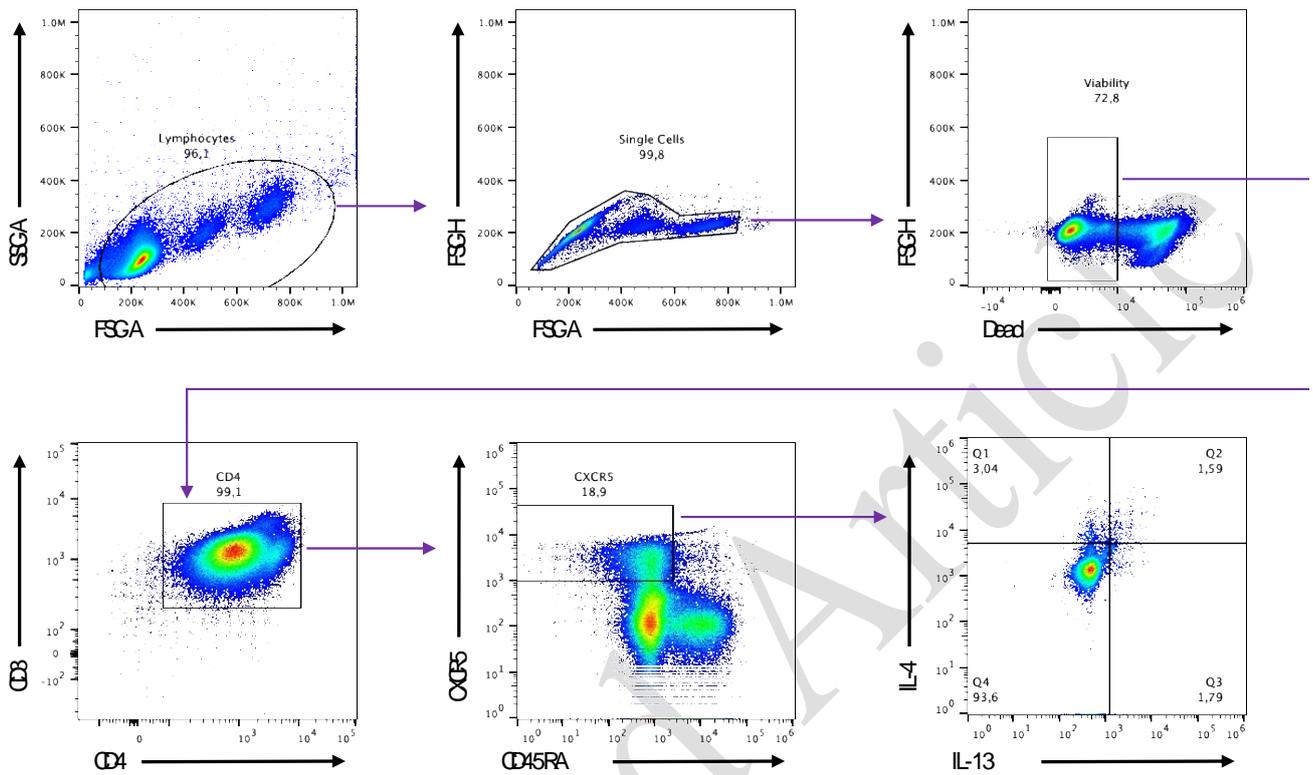


Figure S3. Gating strategy for T_{FH13} cells. Representative flow pot of PBMCs from an LTP-allergic patient stimulated with PMA and Ionomycin.

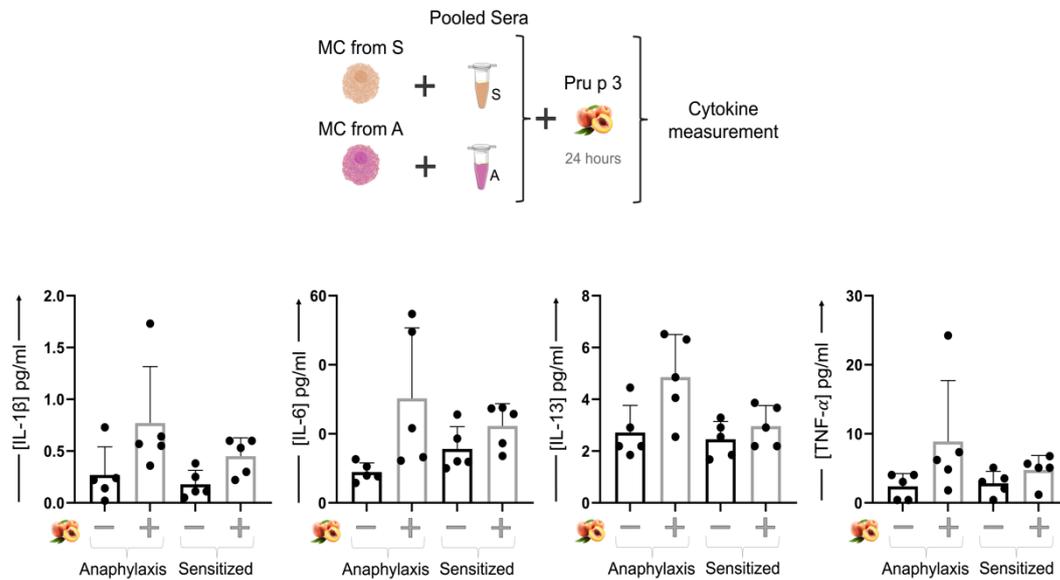


Figure S4. Sera from anaphylaxis patients induce a higher cytokine secretion.

Cytokine multiplex assay was performed in CD34⁺-derived mast cells from anaphylaxis (n=5) and sensitized patients (n=5). Results are expressed as mean \pm SD. Significance was determined using a T-test with Welch's correction. $P < 0.05$ was considered significant. MC=Mast cells; A=Anaphylaxis; S=Sensitized.