SUPPLEMENTARY MATERIAL

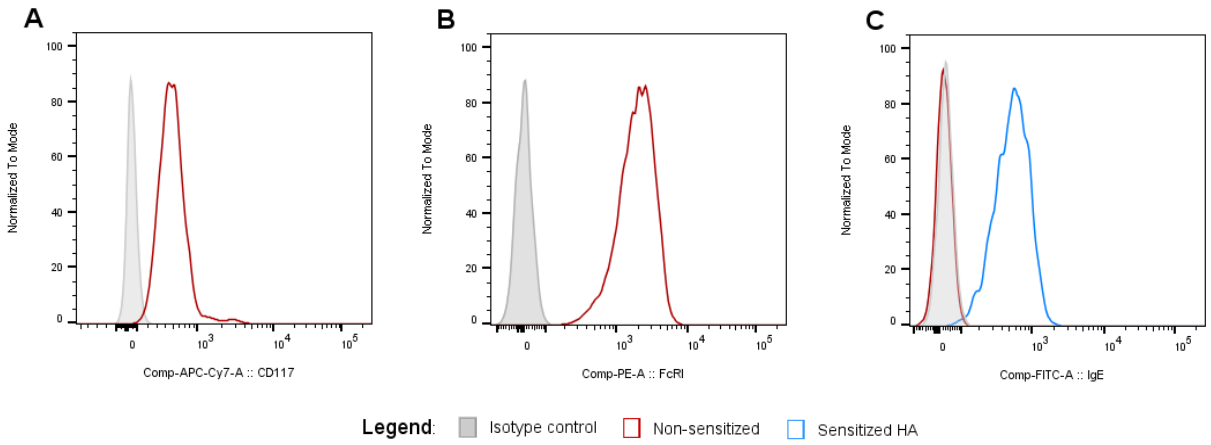

Figure S1. LAD2 immunophenotyping. Expression of A) CD117, B) FcεRI, and C) IgE on the LAD2 cells. IgEs were detectable on the LAD2 surface after sensitization with the plasma of *Hymentoptera* venom allergic patient. APC-Cy7, Allophycocyanin/Cyanine7; PE, phycoerythrin; FITC, fluorescein isothiocyanate.

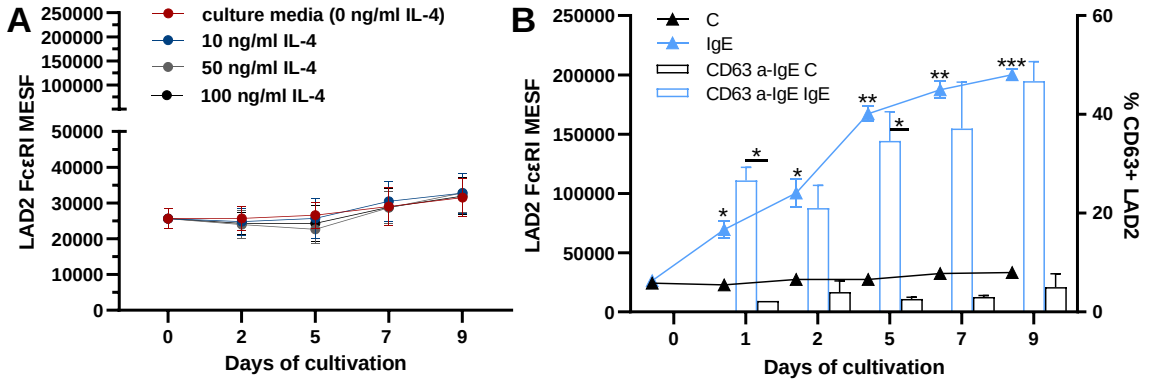

Figure S2. Effects of IL-4 and IgE treatment on FcεRI cell surface expression and activation potential of LAD2 cells. A) IL-4 stimulation does not increase FcεRI expression on the surface of LAD2 cells, whereas B) IgE treatment (1 µg/ml) significantly increases FcεRI expression and potentiates LAD2 cell degranulation. MESF: Molecules of Equivalent Soluble Fluorochrome. C: control media; IgE: IgE treatment; a-IgE: anti-IgE 10 µg/ml. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ for comparing groups using the paired t-test.

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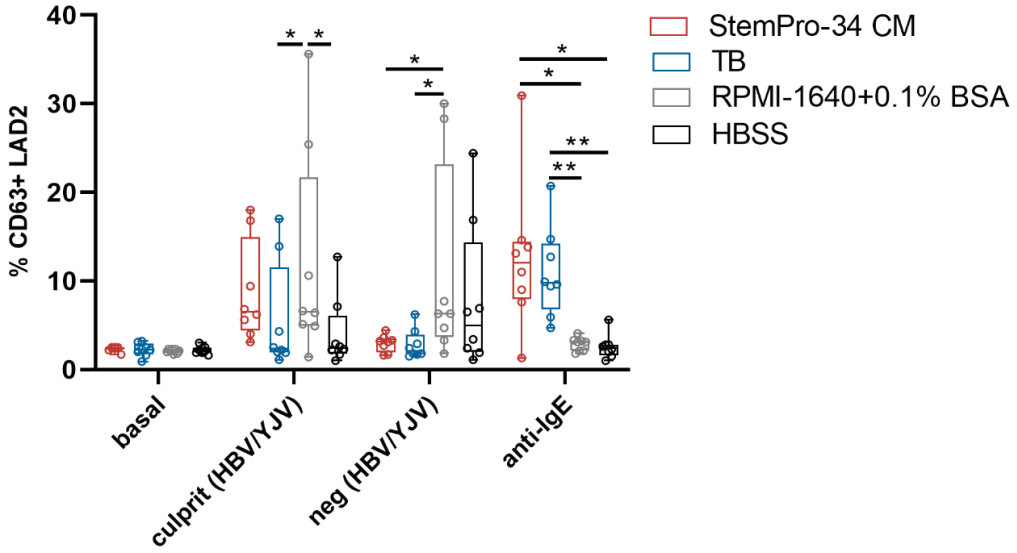

Figure S3. Comparison of different stimulation media/buffers on degranulation of LAD2 after basal stimulation, positive stimulation with anti-IgE, culprit allergen stimulation and negative allergen stimulation in 8 patients with *Hymenoptera* venom allergy (4 out of 8 were allergic to honey bee venom (HBV), and 4 out of 8 were allergic to yellow jacket venom (YJV)).

CM: Complete media; TB: tyrode buffer; BSA: bovine serum albumin. * *P*< .05, ** *P*< .01 for comparing groups using the Wilcoxon matched-pairs signed rank test.

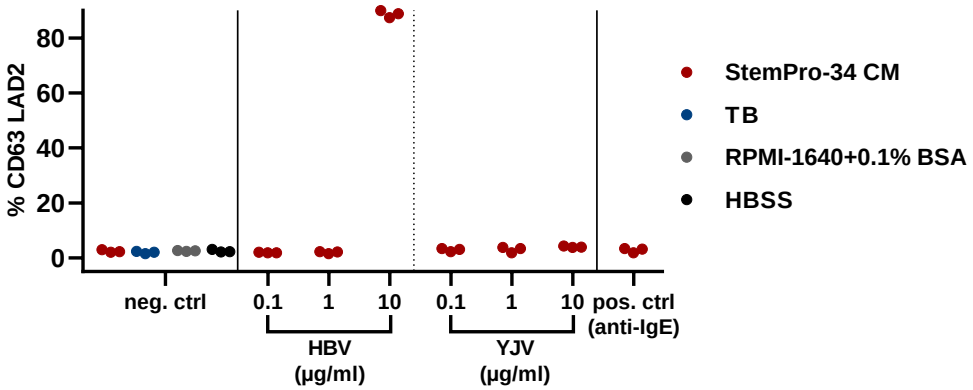

Figure S4. Comparison of different stimulation media/buffers on degranulation of non-sensetized LAD2 after basal stimulation (neg. ctrl), stimulation with positive control for LAD2 MAT anti-IgE (pos ctrl), and stimulation with allergen (HBV/YJV).

CM: Complete media; TB: tyrode buffer; BSA: bovine serum albumin; HBV: Honey bee venom; YJV: Yellow jacket venom

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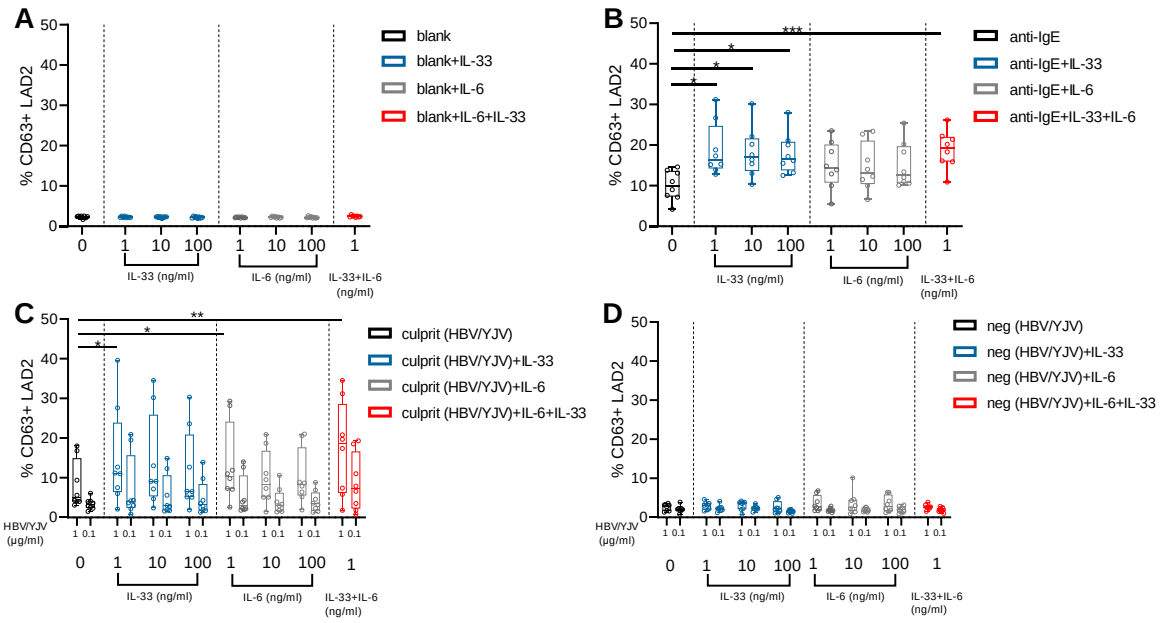

Figure S5. Effect of IL-33 and IL-6 treatment on LAD2 degranulation capacity. Sensitized LAD2 cells were treated with IL-33 (1-100 ng/ml) and IL-6 (1-100 ng/ml) and investigated for CD63 activation after A) StemPro-34 complete media (blank), B) positive control anti-IgE, C) culprit allergen and D) negative allergen stimulation. The combined effect of IL-33 and IL-6 was evaluated at 1 ng of IL-33 and 1 ng/ml of IL-6. Experiments were performed on 8 *Hymenoptera* venom allergic patients (4 were allergic to honey bee venom (HBV) and 4 were allergic to yellow jacket venom (YJV), respectively).

***P*< .01 and **P*< .05 for comparing groups using the paired t-test.

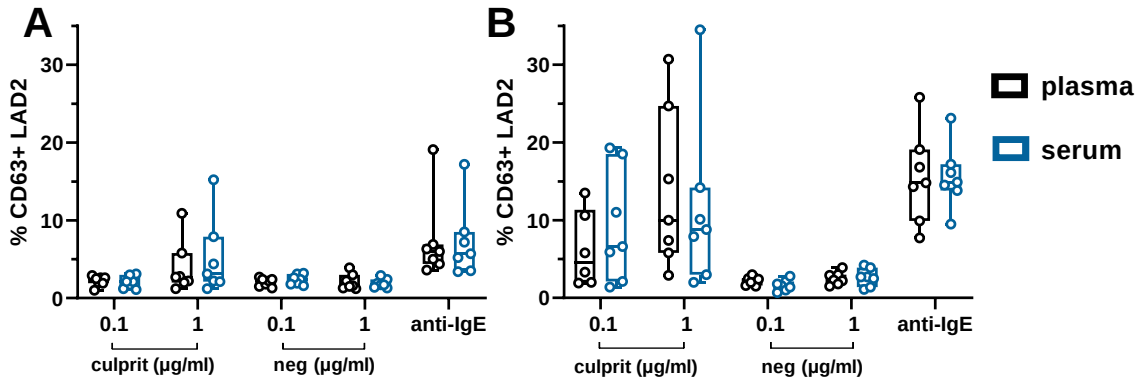

Figure S6. The comparison of the use of plasma and serum in LAD2 MAT without (A) or with (B)IL-33 and IL-6 prestimulation. LAD2 cells were sensitized with plasma (black) or serum (blue) of 7 patients overnight and then stimulated with the allergen (culprit, negative) or the positive control anti-IgE mAb.

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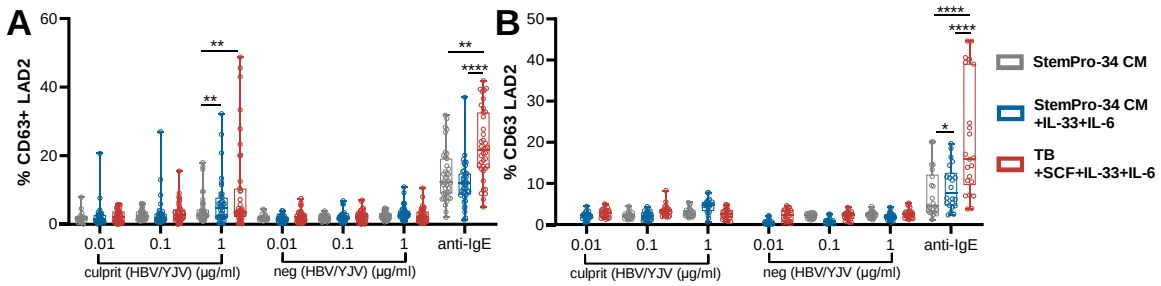

Figure S7. Comparison of different LAD2 MAT protocols in A) 39 *Hymenoptera* venom allergic patients and B) 22 controls. Grey: StemPro-34 complete media (CM); Blue: StemPro-34 CM with IL-33 and IL-6 prestimulation; Red: Tyrode buffer (TB) with added SCF (100 ng/ml) with IL-33 and IL-6 prestimulation.

* *P*< .05, ** *P*< .01, **** *P*< .0001 for comparing groups by using the Mann-Whitney U test.

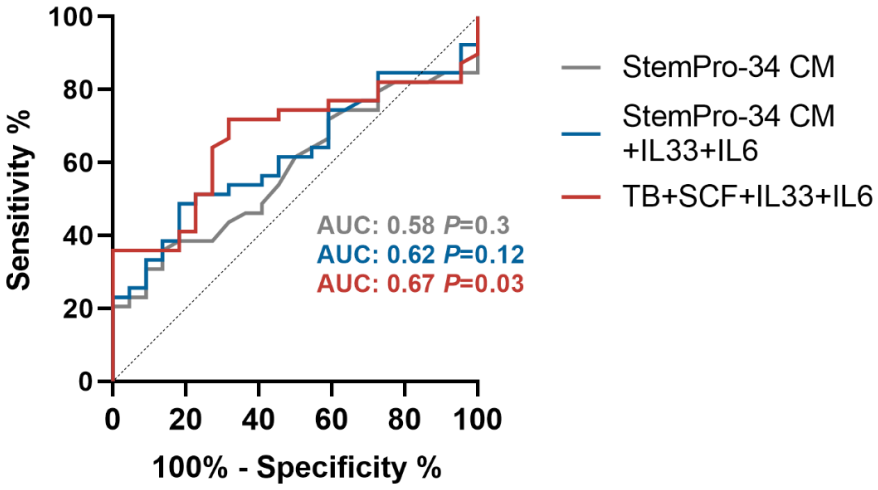

Figure S8. Receiver operating characteristic curve for the LAD2 MAT to diagnose *Hymenoptera* venom allergy using different stimulation media and prestimulation with IL-33 and IL-6. Grey: StemPro-34 complete media (CM); Blue: StemPro-34 CM with IL-33 and IL-6 prestimulation; Red: Tyrode buffer (TB) and SCF with IL-33 and IL-6 prestimulation.

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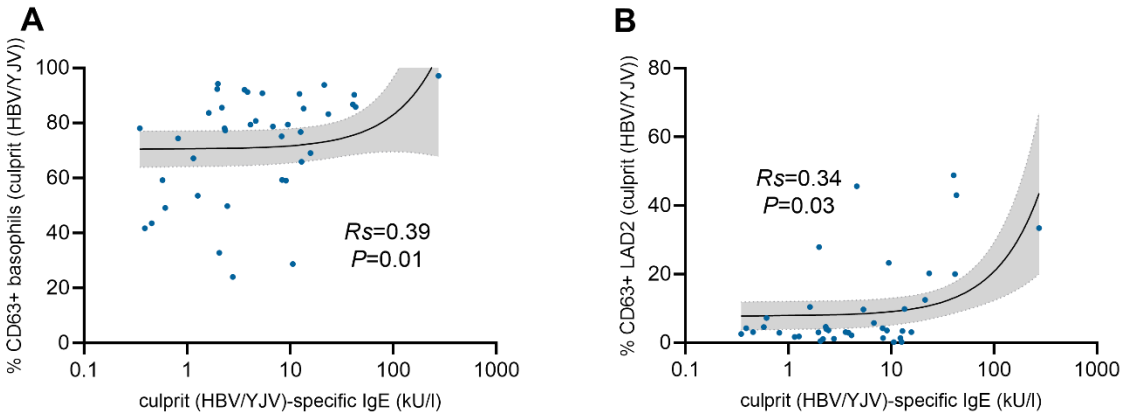

Figure S9. Spearman's coefficient correlation analysis between A) BAT results at the concentration of 1 µg/ml of culprit venom and culprit venom-specific IgE results and B) LAD2 MAT result at the concentration of 1 µg/ml of culprit venom and culprit venom-specific IgE results in 39 *Hymenoptera* venom allergic patients.

HBV: honey bee venom; YJV: yellow jacket venom.

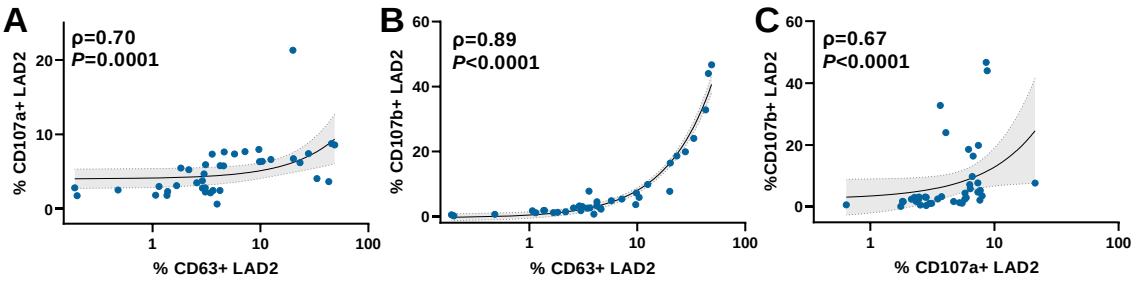

Figure S10. Spearman's coefficient correlation analysis between expressions of lysosomal-associated membrane proteins (LAMPs) on the surface of LAD2 cells after stimulation with the culprit allergen in 39 patients with *Hymenoptera* venom allergy. Correlations between A) LAMP1 (CD107a) and LAMP3 (CD63), B) LAMP2 (CD107b) and CD63 and C) CD107b and CD107a

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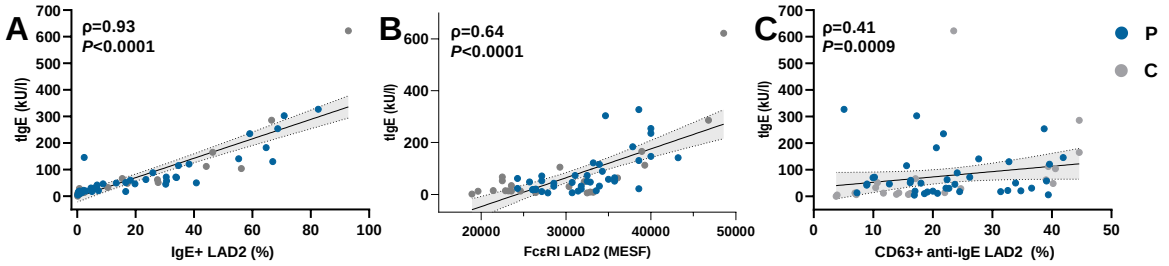

Figure S11. Spearman's coefficient correlation analysis between serum total IgE (tIgE) levels and IgE LAD2 positivity (A), FcεRI expression (B), and CD63-response to anti-IgE stimulation (C) after passive overnight sensitization of LAD2 cells with patients` plasma. Data are presented for 39 *Hymenoptera* allergic patients (P) and 22 controls (C).

MESF: Molecules of the equivalent soluble fluorochrome.

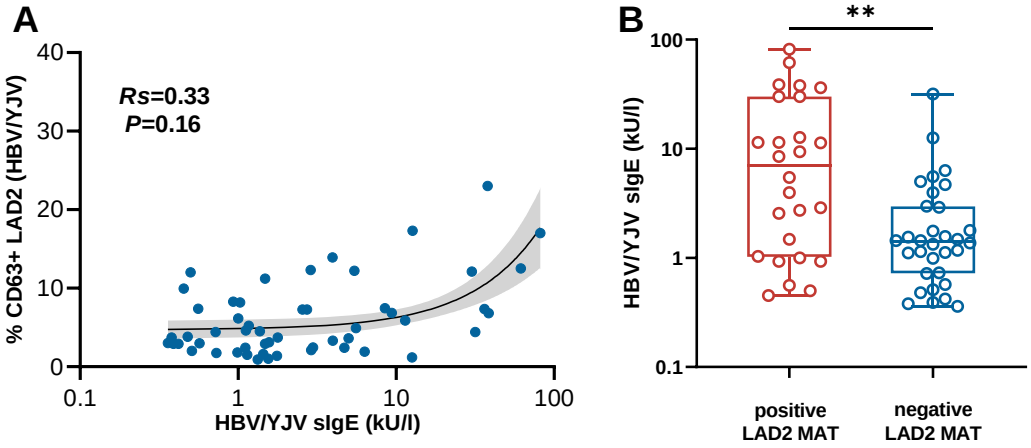

Figure S12. A) Spearman's coefficient correlation analysis between LAD2 MAT results at the stimulation with 1 µg/ml honey bee venom (HBV) or yellow jacket venom (YJV) and HBV/YJV-specific sIgE levels in 37 patients with nonresponding basophils (noninterpretable BAT). B) Comparison of HBV/YJV-specific IgE levels between patients with noninterpretable BAT but positive LAD2 MAT result (n=20) and patients with noninterpretable BAT and negative LAD2 MAT result (n=17).

** *P*< .01 for comparing groups by using the Mann-Whitney U test.

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