ARTICLE - Blood Transfusion

Anti-HLA class I IgG subclasses skew platelet activation mechanisms in transfusion refractoriness

Adèle Couvidou,[1*] Mathieu Wald,[2*] Catherine Angénieux,[1] Juliana Pires-Marafon,[1] Marie-Joëlle Apithy,[3] Catherine Humbrecht,[3] Luc-Matthieu Fornecker,[2] Nicolas Congy-Jolivet,[4] Arnaud Dupuis,[1] Jérôme Rollin[5,6] and Blandine Maître[1]

[1]Université de Strasbourg, INSERM, Etablissement Français du Sang (EFS) Grand Est, BPPS UMR_S 1255, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Strasbourg; [2]Institut de Cancérologie de Strasbourg (ICANS), Service d'Hématologie, Strasbourg; [3]Etablissement Français du Sang (EFS) Grand Est, Strasbourg; [4]U1297 INSERM I2MC Team «Cell signaling in Response to Arterial Stress: Impact on Cardiovascular Diseases», Université Toulouse 3, Laboratoire d'Histocompatibilité, CHU de Toulouse, Toulouse; [5]Université de Tours, Institut National de la Santé et de la Recherche Médicale (INSERM) UMR 1327 ISCHEMIA, Tours and [6]Centre Hospitalier Régional Universitaire de Tours, Service d'Hématologie-Hémostase, Tours, France

**AC and MW contributed equally as first authors.*

Correspondence: B. Maître
blandine.maitre@efs.sante.fr

Received: March 5, 2025.
Accepted: September 8, 2025.
Early view: September 18, 2025.

https://doi.org/10.3324/haematol.2025.287677

Supplemental Materials

APC anti-human CD62P (Clone AK4) antibodies were purchased from Biolegend. FITC-coupled Annexin V was purchased from Roche. As previously reported, CD9 antibody (ALMA-12) and Tyrode's albumin (TA) buffer (5mM Hepes, pH 7.35, 0.35% human serum albumin) were prepared in our laboratory. Anti-CD32a antibody (clone IV.3) was purchased from StemCell. Eculizumab was provided by the University Hospital of Strasbourg. Subclass-specific secondary antibodies coupled to phycoerythrin were purchased from LifeSpan Biosciences: murine anti-hIgG1 (clone HP6001), murine anti-hIgG2 (clone 31-7-4), murine anti-hIgG3 (clone HP6050) and murine anti-hIgG4 (clone HP6025).

Supplemental Methods

Clinical study

This monocentric study was called the ACERTP trial ("Anticorps associés aux états réfractaires aux transfusions plaquettaires") and was performed in the hematology department of the "Institut de Cancérologie Strasbourg Europe" (ICANS), a comprehensive cancer center situated in Strasbourg, France. To be eligible for the trial, the patients had to be adults and had to sign the informed consent form before starting the trial. Biological and clinical data were prospectively collected from March 2022 to September 2023. All patients received chemotherapy-based treatment for allogenic or autologous hematopoietic stem cell transplantation (HSCT) or for induction and consolidation of acute leukemia treatment (Table 1).

Platelet concentrates production and issuing

Platelet concentrates (PC) were produced by the Etablissement Français du Sang Grand Est, and were either apheresis platelets collected on Trima (Terumo BCT), or buffycoat platelets made from 8 whole blood donations, and later divided in 2 twin subunits. All PC were stored in 60-65% additive solution (Intersol, Terumo BCT) and pathogen reduced with amotosalen + UVA (Intercept Blood System, Cerus, USA). Each PC underwent a platelet count, i.e. the platelet count was known for each PC (routine procedure in France). Shelf life was 7 days at +20-24°C, with agitation. Patients' weight and height were specified by the haematologist on the prescription form, and registered in the laboratory information system (LIS: Edgeblood, InLog, France). French guidelines recommend a posology interval between 0,5 and $0{,}7.10^{11}$ platelets/10Kg body weight, and are respected as often as possible, depending of the inventory at issuing.

Anti-HLA-I IgG subclass assay

A high-definition LABScreen single-antigen Class I (One Lambda, Canoga Park, CA) was performed on the LABScan200 flow cytometer (Luminex® Corporation, Austin, TX) to determine the specificity of anti-HLA-I IgG antibodies. A positive result was defined as a mean fluorescence intensity (MFI) greater than 1,000 for total IgG Luminex®, a positivity threshold determined by the HLA-I laboratory based on negative controls. IgG subclasses detection was performed using the same protocol, except for the use of a secondary antibody specific for each subclass. The subclass of an anti-HLA-I antibody, already detected in total IgG Luminex®, was determined to be positive when the MFI was twice that of the corresponding negative control in subclass detection.

Aggregation test

Blood was drawn into a hirudinated tube. PRP or washed platelets were obtained by centrifugation as previously described[2]. Platelet aggregation induced by anti-HLA-I antibodies was measured turbidimetrically as described previously[2].

Supplemental legends

Supplemental Table 1: Pre-and post-transfusion platelet counts and CCI value in immunized patients

Platelet count pre- and 24h post-transfusion (TS) (G/L) and the CCI value at 24h was reported for each allo-immunized patient without non-immune factors.

Supplemental Figure 1: Anti-HLA-I IgG analysis in transfused patients enrolled in a clinical study

A. Anti-HLA-I total IgG were analyzed by Luminex® and represented on a heat map. The MFI values of the anti-HLA-I total IgG are plotted against their specificities in the group of transfusions with a CCI value < 7 (left part) or ≥ 7 (right part). **B**. Anti-HLA-I IgG subclasses were analyzed by Luminex® and represented on a heat map per IgG subclass (IgG1, IgG2 and IgG3). The MFI values of HLA-I IgG subclasses are plotted against their specificities in the group of transfusions with a CCI value < 7 (left part) or ≥ 7 (right part).

Supplemental Figure 2 : Effect on IgG4-W632 on platelet activation

Hirudinated PRP was incubated with buffer, CRP (5 µg/mL) or human chimeric W632-IgG4 (10 µg/mL). P-selectin exposure was measured by flow cytometry.

Supplemental Figure 3: Effect on IV.3 on platelet activation mediated by anti-CD9 antibodies

Hirudinated PRP was incubated with anti-CD9 antibodies +/- IV.3, a CD32a inhibitor at 20µg/ml. Effect of IV.3 was evaluated by flow cytometry using activation markers such as P-selectin exposure (Psel) and Annexin V binding **(A)** or by aggregation test **(B)**.

Supplemental Figure 4 : Platelet aggregation tests induced by hIgG1, hIgG2 or hIgG3-W632

(A) Aggregation test with hirudinated PRP incubated with hIgG1 (left panel), hIgG2 (middle panel) or hIgG3 (right panel) with IV.3 (+IV.3) or without (Ctrl). Representative trace of n=3 experiments is shown. **(B)** Aggregation test with hirudinated PRP incubated with hIgG1 (left panel), hIgG2 (middle panel) or hIgG3 (right panel) with Eculizumab (+Ecu) or without (Ctrl). Representative trace of n=3 experiments is shown. **(C)** Time-lags (time between the agonist addition and the beginning of aggregation are presented as the mean ± SEM (n=3); Time =600 sec was considered as the maximal time lag (i.e. no aggregation observed in this time frame).

References

1. Congy-Jolivet N, Drocourt D, Portet S, Tiraby G, Blancher A. Production and characterization of chimeric anti-HLA monoclonal antibodies targeting public epitopes as tools for standardizations of the anti-HLA antibody detection. Journal of immunological methods. 2013;390(1-2):41-51.
2. Cazenave JP, Ohlmann P, Cassel D, Eckly A, Hechler B, Gachet C. Preparation of washed platelet suspensions from human and rodent blood. Methods in molecular biology. 2004;272(13-28.

A

CCI ≥ 7

Platelet count pre-TS (G/L)	Platelet count post-TS 24h (G/L)	CCI value 24h
16	36	**9.4**
59	81	**12.4**
4	20	**7.6**
11	27	**8.9**

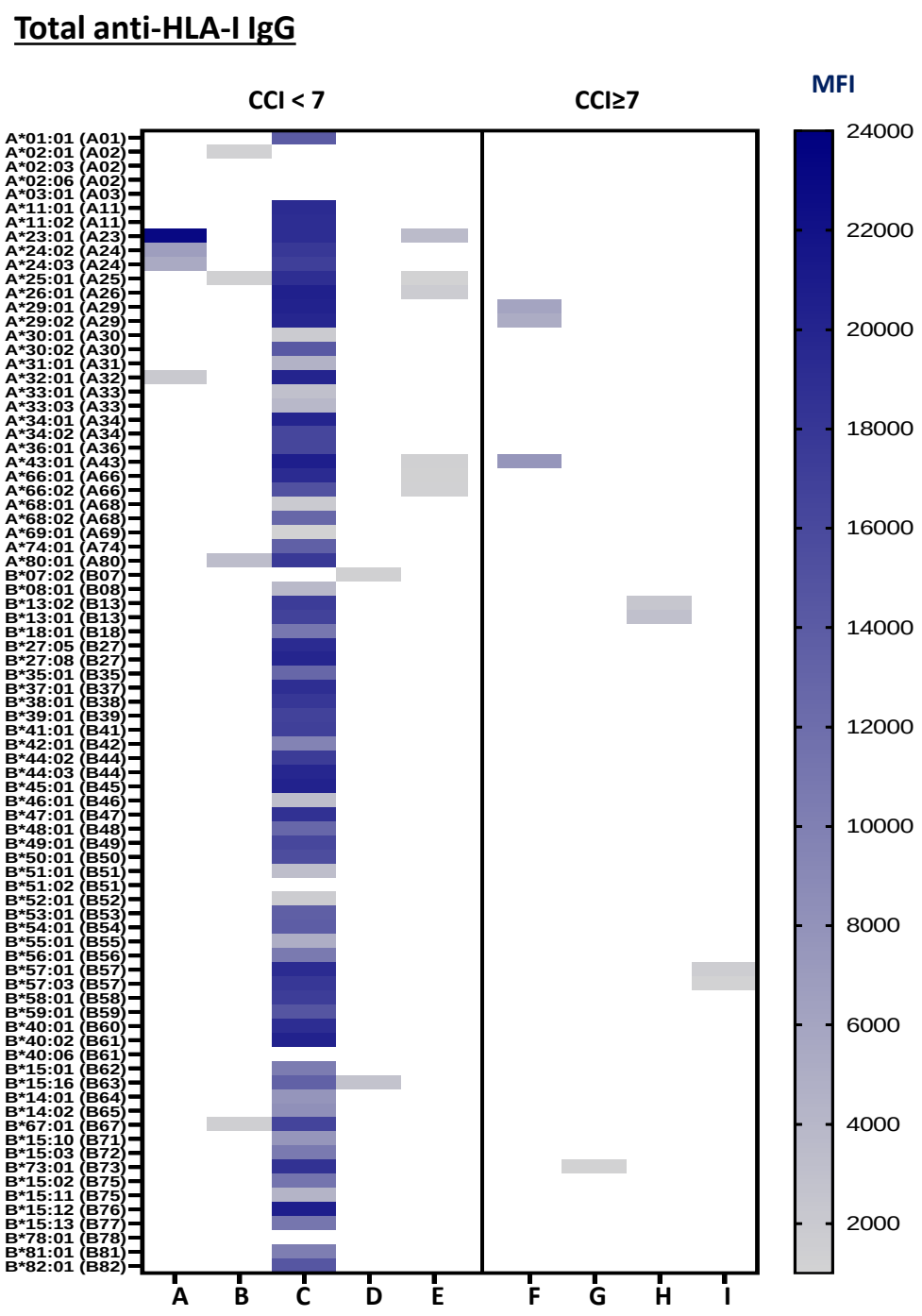
B

CCI < 7

Platelet count pre-TS (G/L)	Platelet count post-TS 24h (G/L)	CCI value 24h
42	50	**5.2**
4	10	**2.9**
10	9	**0**
22	33	**5.2**
10	21	**6.9**

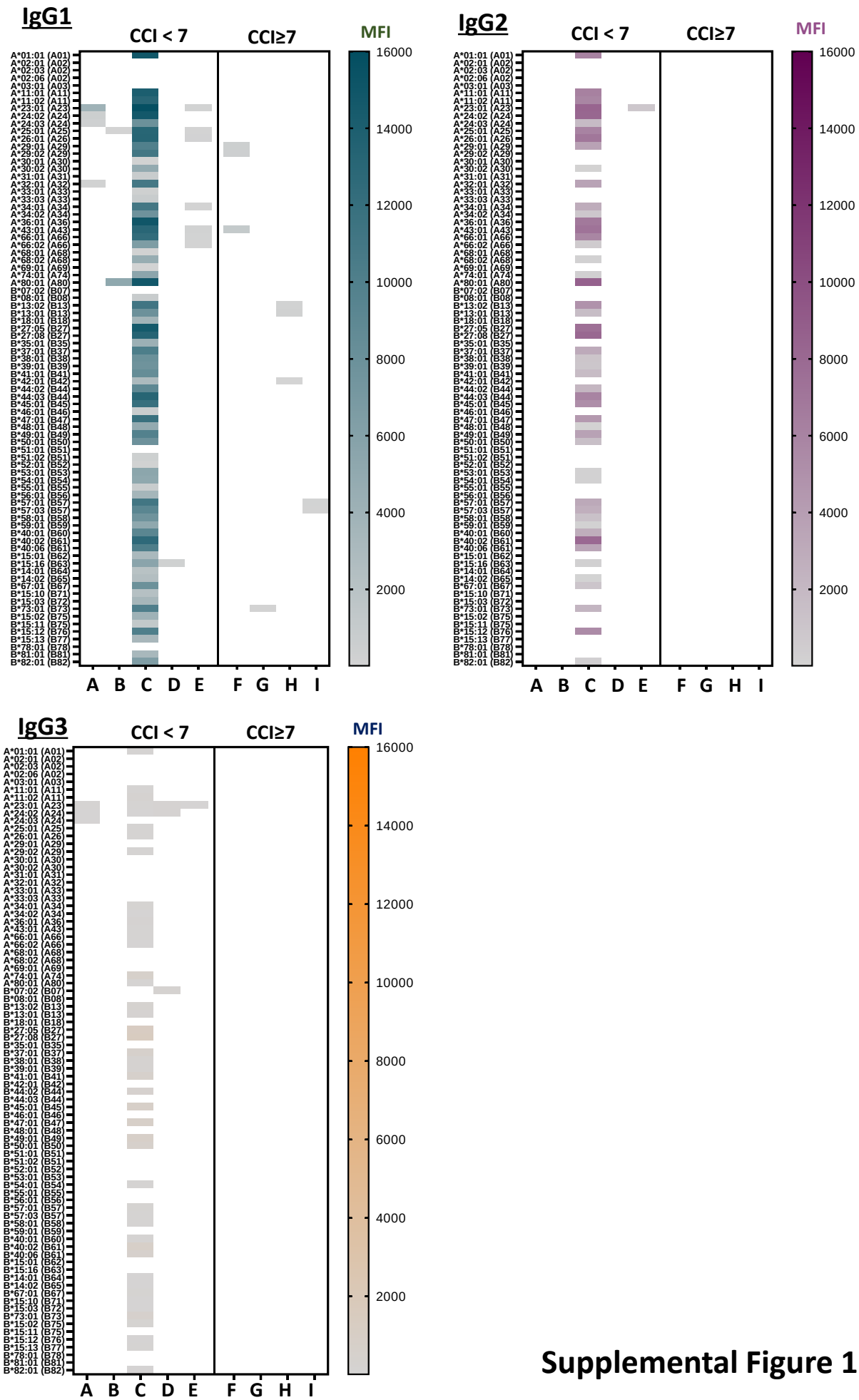
Supplemental table 1

A

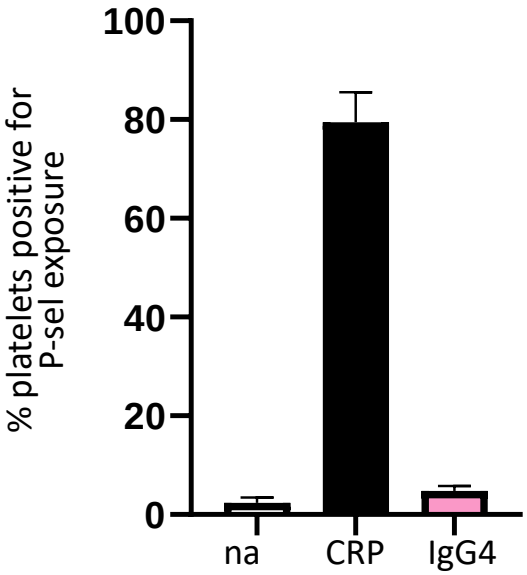


Supplemental Figure 1

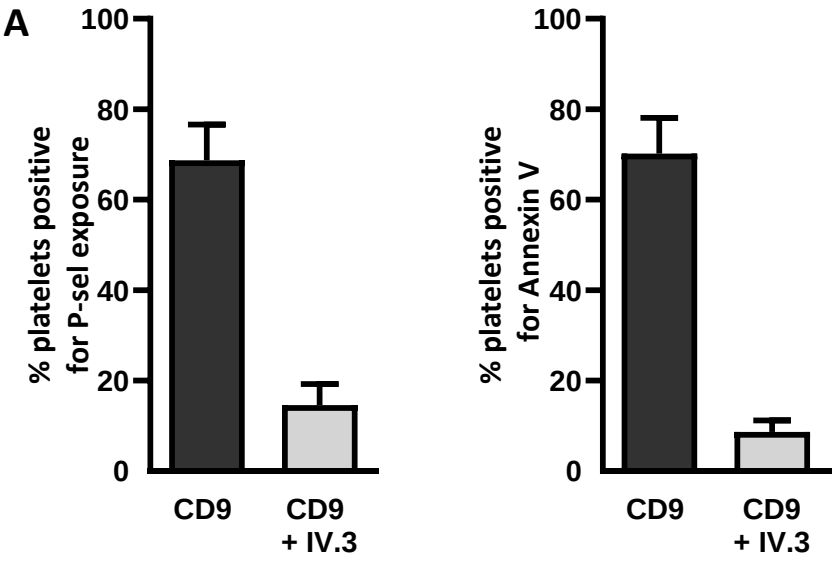
B

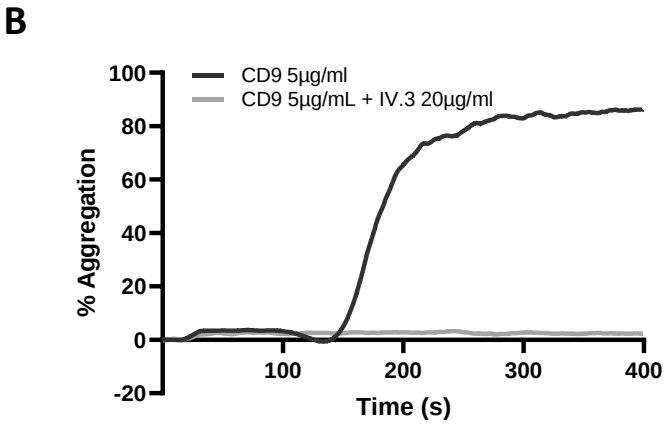


Supplemental Figure 1

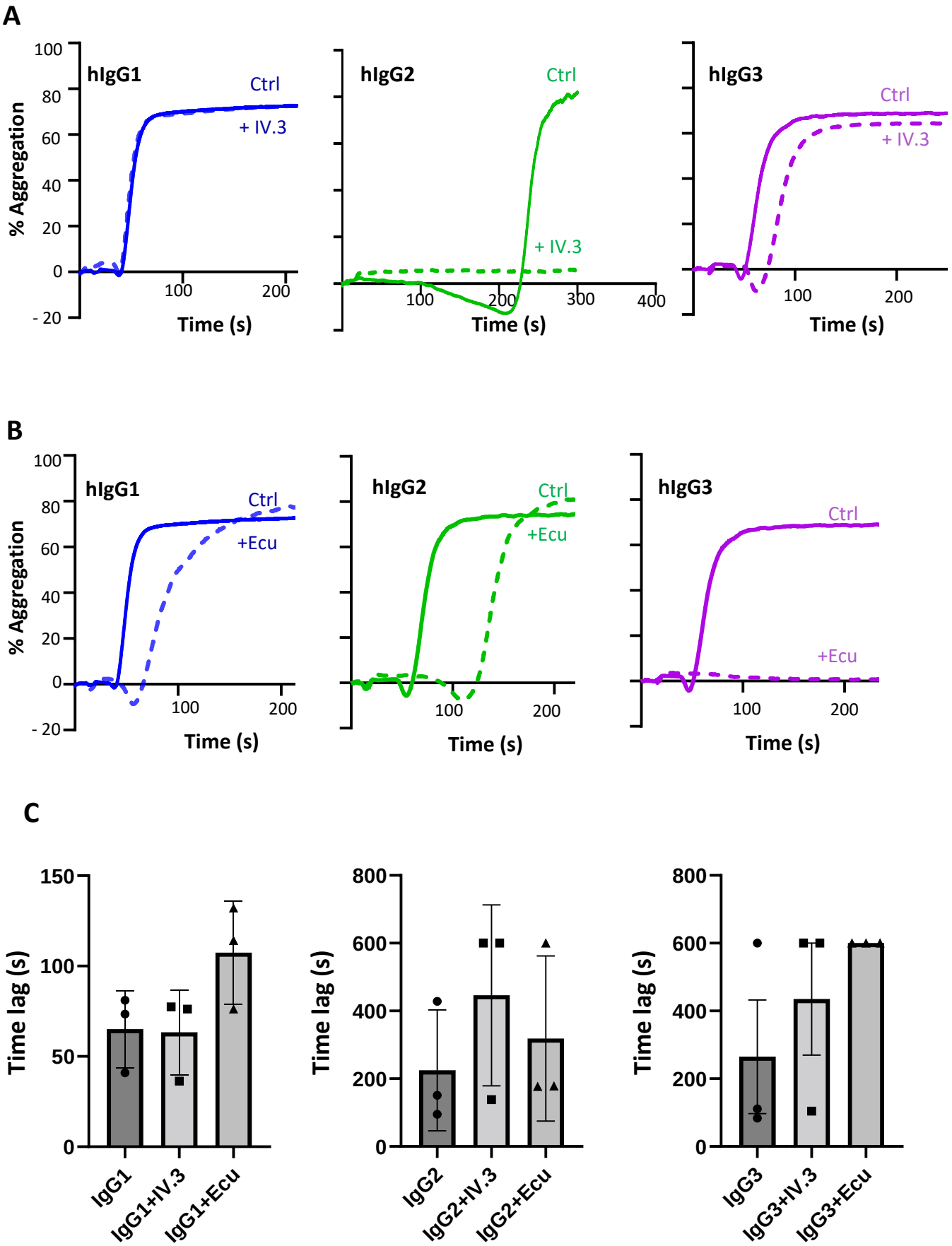


Supplemental Figure 2





Supplemental Figure 3



Supplemental Figure 4