
Heme induces human and mouse platelet activation through C-type-lectin-like receptor-2

Joshua H. Bourne,^{1*} Martina Colicchia,^{1*} Ying Di,¹ Eleyna Martin,¹ Alexander Slater,¹ Lubka T. Roumenina,² Jordan D. Dimitrov,² Steve P. Watson,^{1,3} and Julie Rayes^{1,3}

¹Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK; ²Centre de Recherche des Cordeliers, INSERM, Sorbonne Université, USPC, Université Paris Descartes, Université Paris Diderot, Paris, France and ³Center of Membrane Proteins and Receptors (COMPARE), Universities of Birmingham and Nottingham, The Midlands, UK

*JHB and MC contributed equally as co-first authors

Correspondence: JULIE RAYES - j.rayes@bham.ac.uk

doi:10.3324/haematol.2020.246488

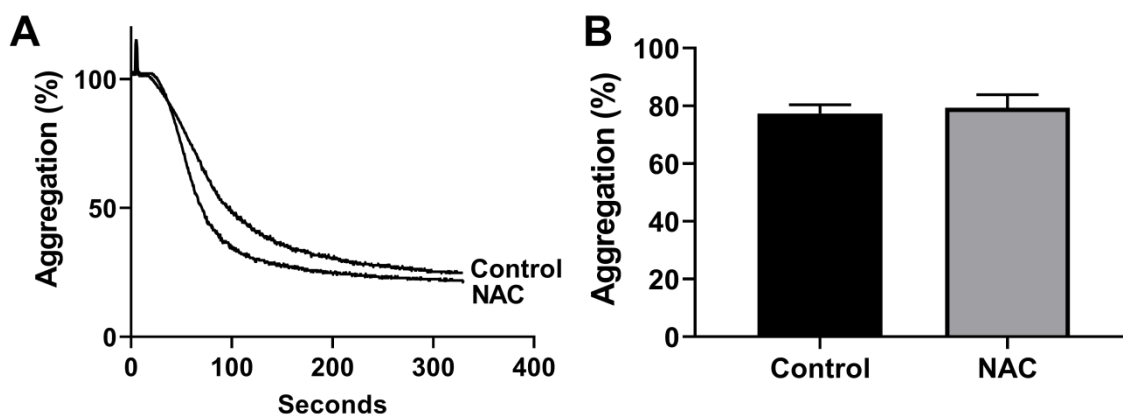


Figure S1: Hemin-mediated human platelet activation does not depend on oxidative stress. (A, B) Human washed platelets ($2 \times 10^8/\text{ml}$) were incubated with hemin ($6.25 \mu\text{M}$) in the presence of Ca^{2+} (2mM). N-acetyl cysteine (NAC, $100 \mu\text{M}$) was incubated with platelets for 5 minutes prior to addition of hemin. Platelet aggregation was assessed for 6 minutes using light transmission aggregometry ($n=3$).

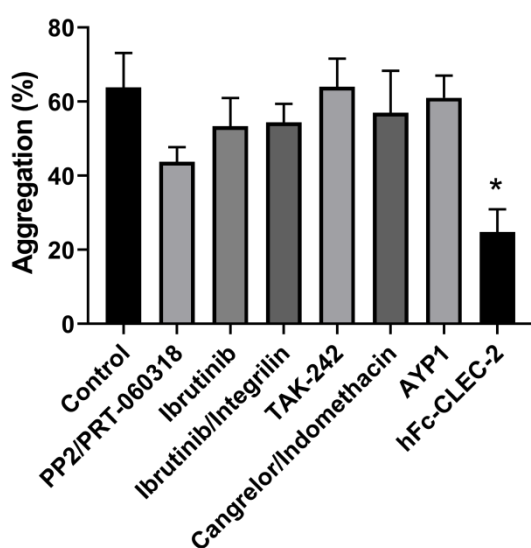


Figure S2: Recombinant human CLEC-2 (hFc-CLEC-2) inhibits platelet agglutination by high concentrations of hemin. Human washed platelets ($2 \times 10^8/\text{ml}$) were incubated with hemin ($50 \mu\text{M}$). Platelet aggregation was assessed using light transmission aggregometry. Ibrutinib (500nM), PP2 ($20 \mu\text{M}$), PRT-060318 ($20 \mu\text{M}$), TAK-242 ($10 \mu\text{M}$), Cangrelor ($10 \mu\text{M}$), Indomethacin ($10 \mu\text{M}$) were preincubated for 5 min with platelets prior hemin addition. Recombinant hFc-CLEC-2 ($50 \mu\text{g}/\text{ml}$) was preincubated with hemin for 15 minutes at 37°C prior addition to platelets. Histogram data are shown as mean \pm SD. The statistical significance was analyzed using a one-way ANOVA with

Tukey's multiple comparisons test using Prism 8 (GraphPad Software Inc, USA).
Significance is shown compared to control * $p < 0.05$.