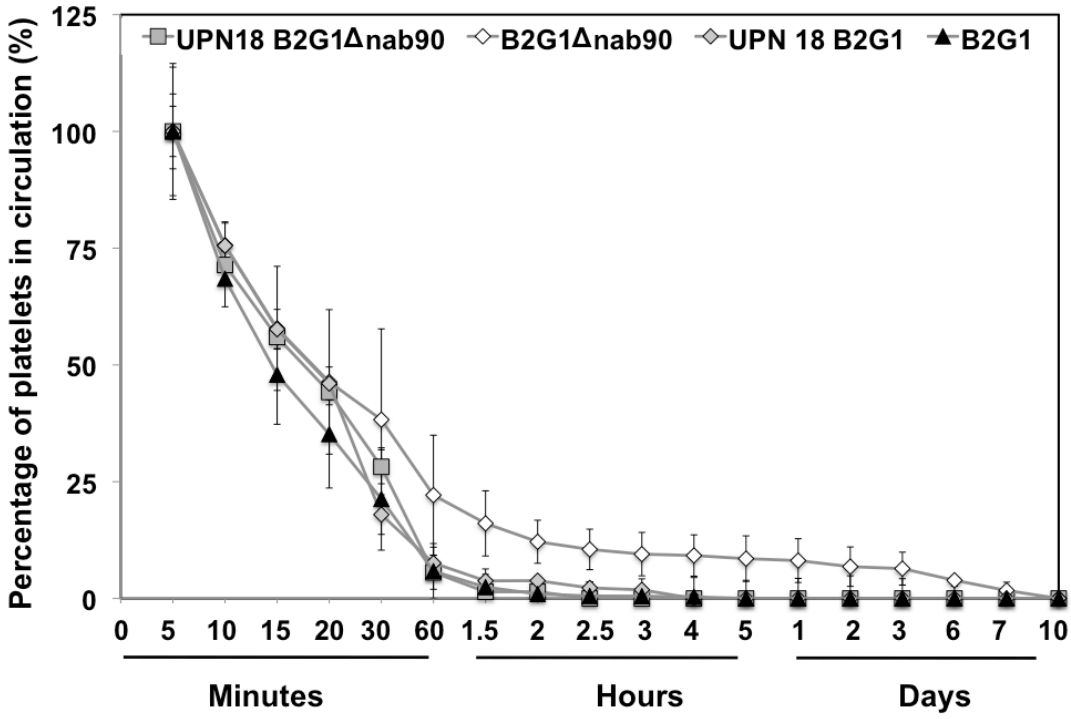
Supplementary Materials and Methods

Clinical-grade antibodies were produced under European Union good manufacturing practice conditions[31] in the NHSBT's Clinical Biotechnology Centre, Bristol, UK and tested in accordance with the European Union guidelines for the production and quality control of monoclonal antibodies [32]. Cell lines secreting B2G1 or B2G1Δnab were adapted into IS-MAB-V serum & animal-free medium supplemented with L-glutamine, cholesterol and iron chelate solution (Irvine Scientific, UK) before the establishment and testing of cGMP Master Cell banks. For routine production, MCB were expanded up to a 2 L inoculum of 5 x 10^5 cells/ml in a spinner flask prior to transfer into a 14 L Fermenter (Electrolab Ltd, UK). Cells were grown in the fermenter at 37 °C, 30% dissolved oxygen, 50 rpm agitation at pH 7.2 in batch mode culture over 2 – 3 weeks. The bulk harvest supernatant was harvested by clarification on a 0.2 µM ultrafiltration cartridge (GE Healthcare Sciences, UK) when the cell viability dropped below 70%. Sub-batches of bulk harvest were concentrated and diafiltered into PBS by crossflow ultrafiltration on a 30 kDa nominal MW cut-off hollow fibre device (GE Healthcare Sciences, UK). Antibodies were purified from the bulk harvest by a combination of affinity chromatography (rProtein A Sepharose Fast Flow resin) and cation exchange chromatography (SP-Sepharose Fast Flow resin) on an $AKTA_{FPLC}$ System (all materials and equipment from GE Healthcare Sciences, UK). Antibodies were eluted in PBS pH 7.2 and diluted to 0.2 mg/mL in PBS before viral filtration, sterile filtration and bottling. The Final Drug Product consisting of 80 vials of B2G1 Batch AB007 and 49 vials of B2G1Δnab Batch AB009 (each vial containing 4ml total volume) were stored at +2 to 8 °C until required. Both antibodies were quality control tested to confirm purity and integrity by SDS-PAGE and size exclusion chromatography along with the required safety and quality testing. Both antibodies have undergone a successful 5 year real time stability trial at the defined storage conditions.

HPA-1a specificity of the clinical grade antibodies was confirmed by the indirect platelet immunofluoresence test and the monoclonal antibody immobilization of platelet platelet antigen (MAIPA) assay. Antibody titre was shown to be similar to previous reports against the anti-HPA-1a international standard NIBSC 03/152 (NIBSC, Potters Bar, UK) in the MAIPA assay. A monocyte chemiluminescence assay confirmed previously reported activity and lack thereof of B2G1 and B2G1Δnab respectively.



Supplementary Figure 1: Platelet recovery for volunteer 18 (who had high expression levels of HPA-1a) and who had survival studies carried out with platelets sensitized with B2G1 and 90% B2G1Δnab/10% B2G1. Also presented is the average platelet recovery for all other volunteers after sensitization with either B2G1 n=7) or 90% B2G1Δnab/10% B2G1 (n=5). Error bars represent the standard deviation.