

Supplemental Material

Understanding the treatment benefit of hyperimmune anti-influenza intravenous immunoglobulin (Flu-IVIG) for severe human influenza

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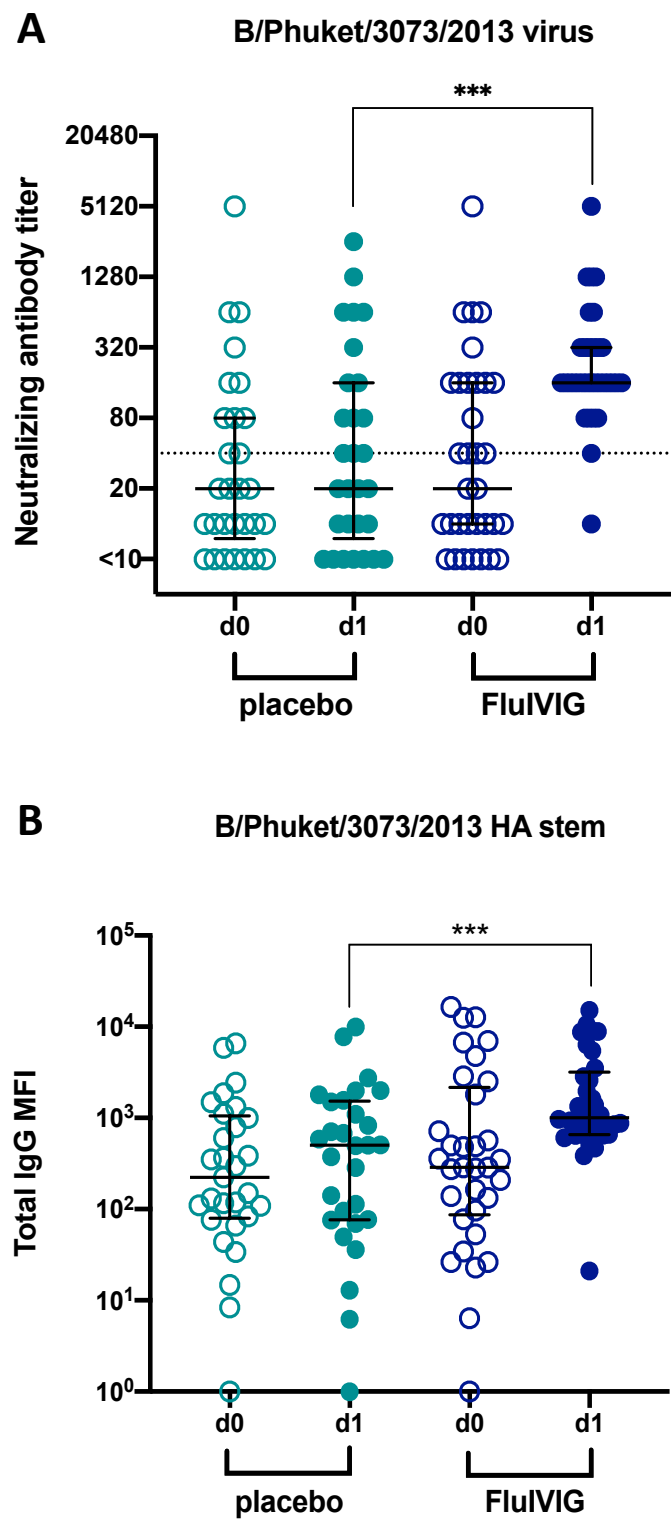
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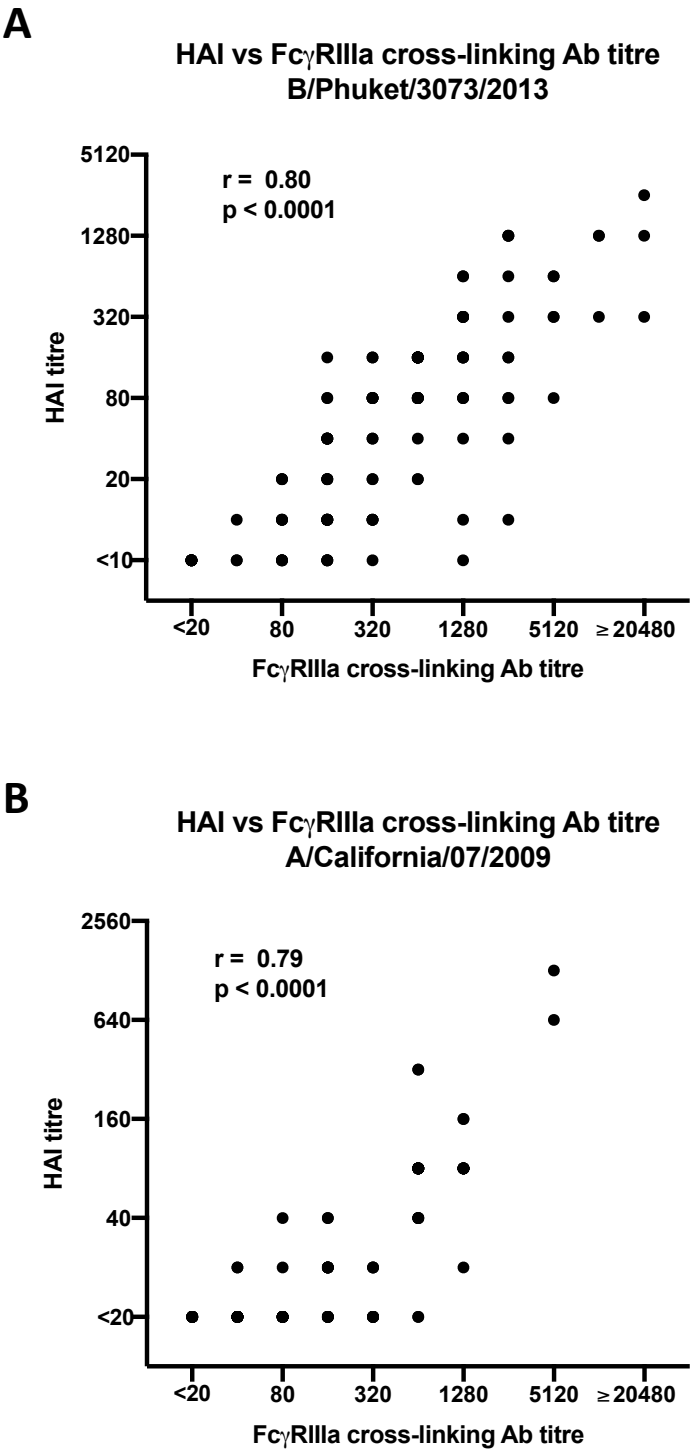
An independent DSMB had complete access to unblinded data during the trial’s conduct and was responsible for periodic review of safety and efficacy. Members of the DSMB were: William Blackwelder (chair), David Parenti, Mary Young, Wilbur Chen, Larry Moulton, and Nikhil Hirani.

Supplemental Figure 1



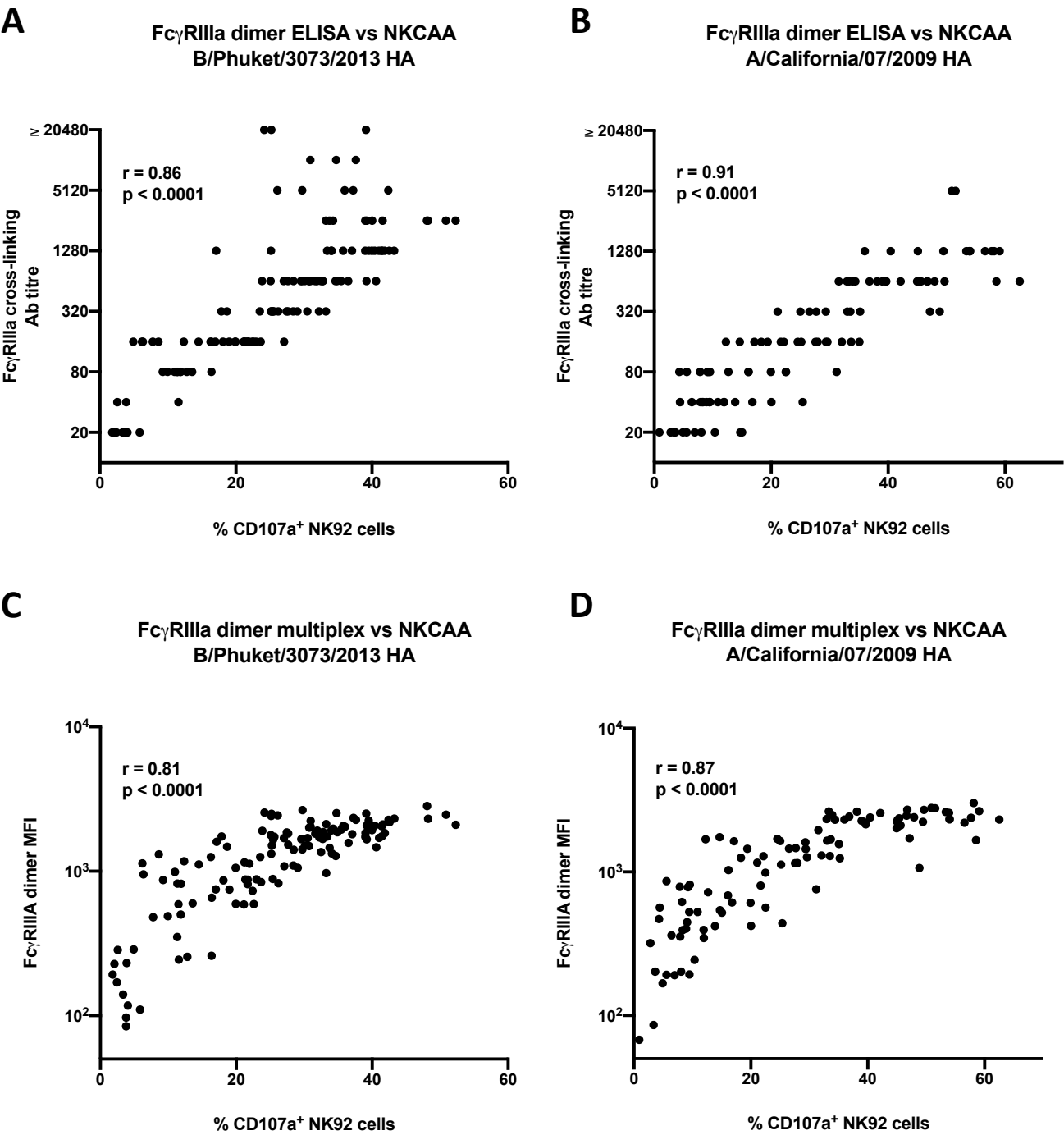
Supplemental Figure 1. Neutralizing antibody titers and HA stem-specific IgG day 1 post-infusion with influenza-specific hyperimmune immunoglobulin (Flu-IVIG) in patients hospitalised with influenza B. Pre-infusion (d0; open symbols) and post-infusion (d1; closed symbols) median neutralizing antibody titer (with interquartile range) by microneutralization assay (MNA) against the B/Phuket/3073/2013 virus (A) and median fluorescence intensity (MFI; with interquartile range) of total IgG against the B/Phuket/3073/2013 HA stem (B) are shown for the placebo (n = 29) or Flu-IVIG (n = 33) infused B/Yamagata patients. Analysis of covariance, with the pre-infusion or d0 level as a covariate, was used to compare differences between the placebo and Flu-IVIG treatment groups at d1 post-infusion. The p-value represents the difference between treatment groups for log₂ d1 post-infusion titer or MFI controlling for pre-infusion (d0) titer or MFI. *** P < 0.001

Supplemental Figure 2



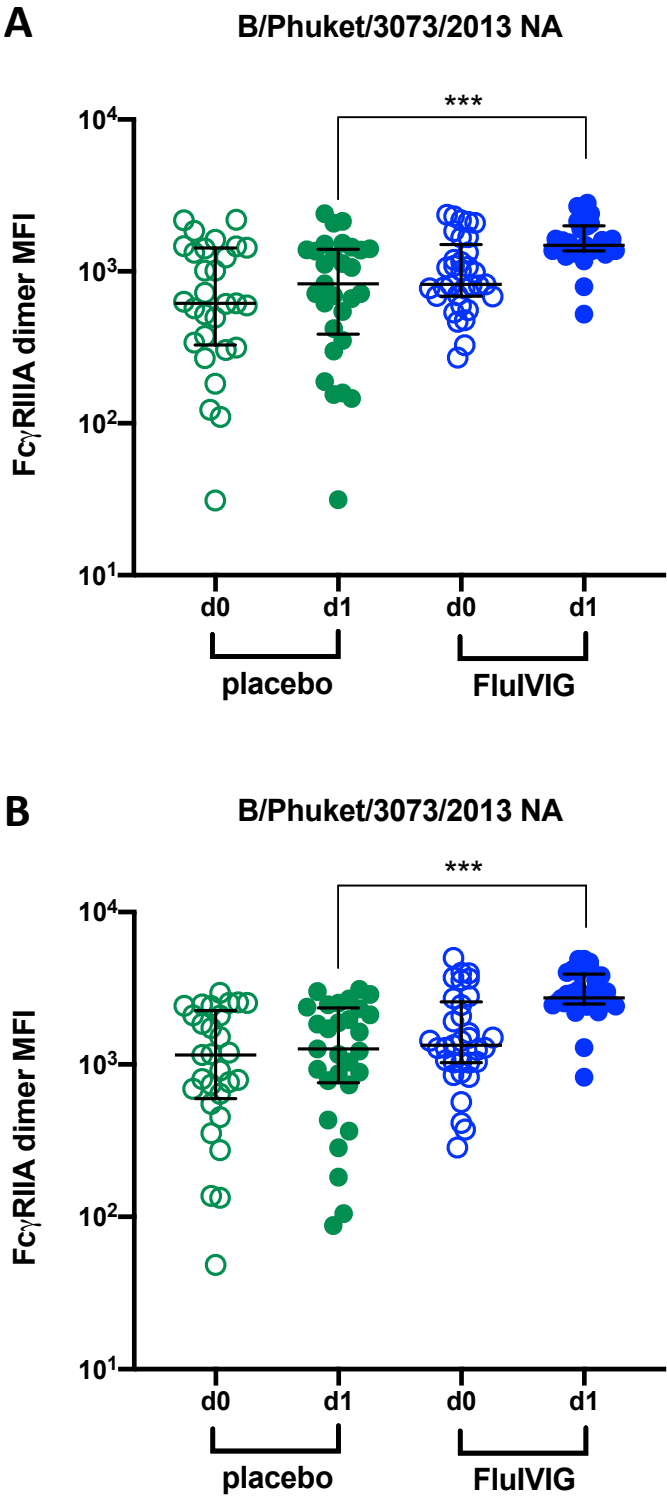
Supplemental Figure 2. Correlation between HAI and HA-specific FcγRIIIa cross-linking antibody titer in B/Yamagata and A/H1N1 infected patients treated with Flu-IVIG or placebo. Spearman correlations between HAI and HA-specific FcγRIIIa cross-linking antibody titers in pre-infusion (d0) and day 1 (d1) post-infusion serum samples following treatment with Flu-IVIG or placebo in B/Yamagata (n = 62; A) and A/H1N1 (n = 50; B) infected patients are shown. Some points represent multiple superimposed patient samples, who have identical HAI and FcγRIIIa cross-linking antibody titers.

Supplemental Figure 3



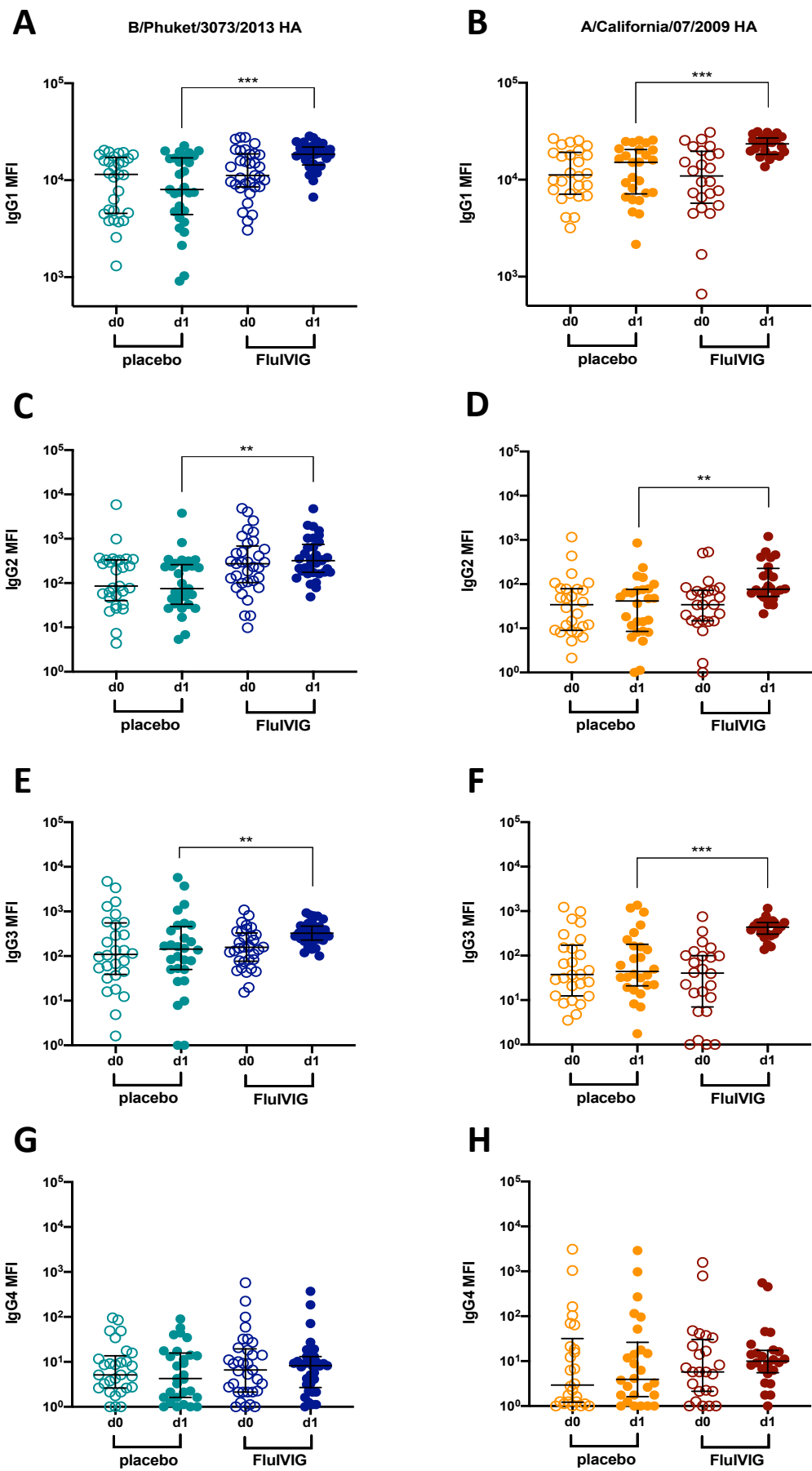
Supplemental Figure 3. Correlation between HA-specific Fc γ RIIIa cross-linking antibody and NK cell activation in B/Yamagata and A/H1N1 infected patients treated with Flu-IVIG or placebo. Spearman correlations are shown between NK cell activation (%CD107a⁺ NK-92-Fc γ RIIIa-GFP cells) and HA-specific Fc γ RIIIa cross-linking antibody titers detected by ELISA in pre- and day 1 (d1) post-infusion serum samples following treatment with Flu-IVIG or placebo in B/Yamagata (n = 62; A) and A/H1N1 (n = 50; B) infected patients. Spearman correlations between NK cell activation and median fluorescence intensity (MFI) of HA-specific Fc γ RIIIa cross-linking antibody detected by bead-based multiplex pre- and day 1 (d1) post-infusion serum samples following treatment with Flu-IVIG or placebo in B/Yamagata (n = 62; C) and A/H1N1 (n = 50; D) infected patients are also shown.

Supplemental Figure 4



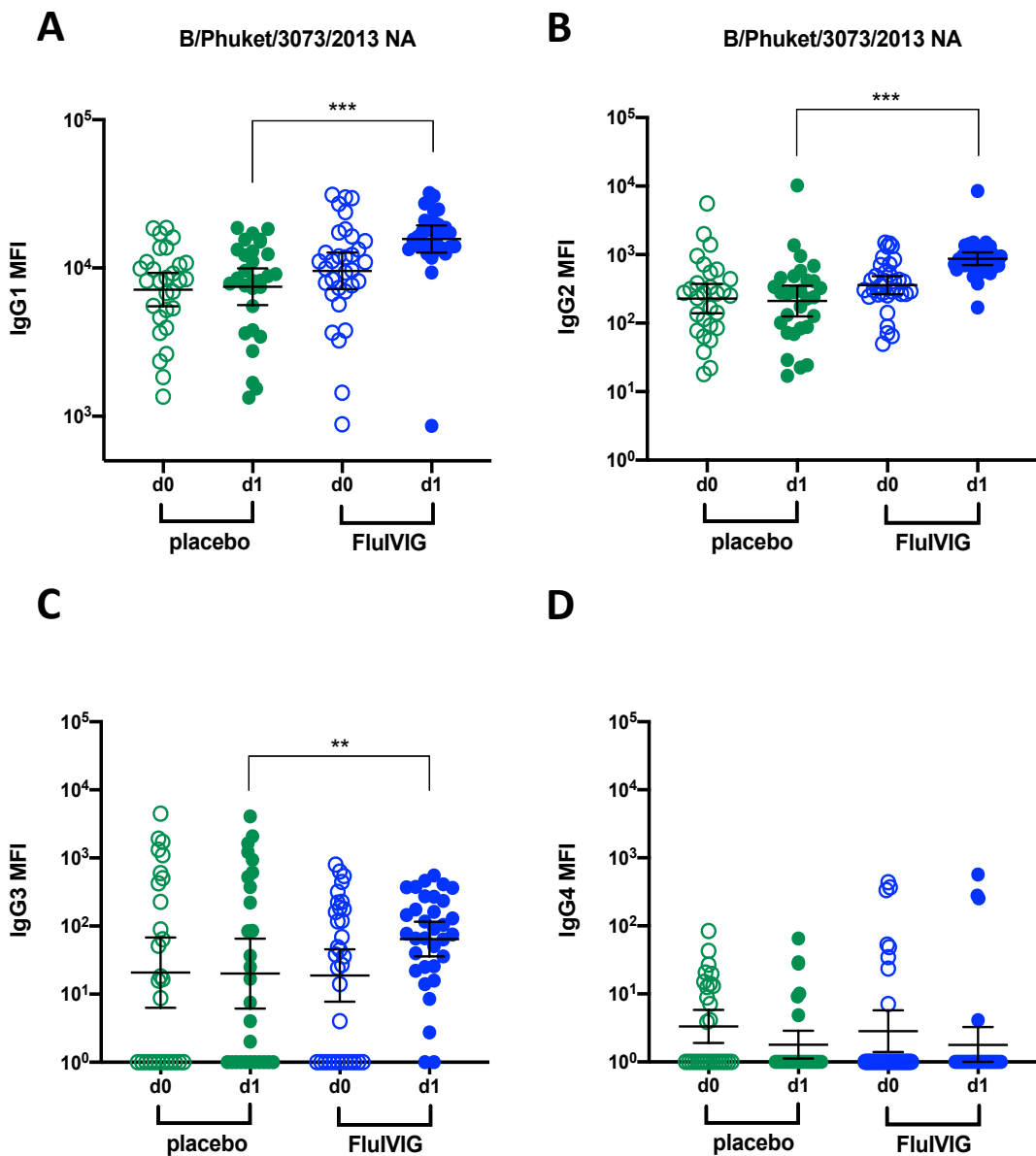
Supplemental Figure 4. NA-specific FcγR binding day 1 post-infusion with Flu-IVIG in patients hospitalised with severe influenza B. A bead-based FcγR dimer multiplex was used to examine the median fluorescence intensity (MFI) of NA-specific FcγRIIIa and FcγRIIa binding antibody in patient sera. Pre-infusion (d0; open symbols) and post-infusion (d1; closed symbols) FcγRIIIa dimer binding (A) and FcγRIIa dimer binding (B) median MFI with interquartile range against the B/Phuket/3073/2013 NA were measured for the placebo (n = 29) or Flu-IVIG (n = 33) treated B/Yamagata infected patients. Analysis of covariance, with the pre-infusion or d0 level as a covariate, was used to compare differences between the placebo and Flu-IVIG treatment groups at d1 post-infusion. The p-value represents the difference between treatment groups for log₂ d1 post-infusion controlling for pre-infusion (d) MFI. *** P < 0.001

Supplemental Figure 5



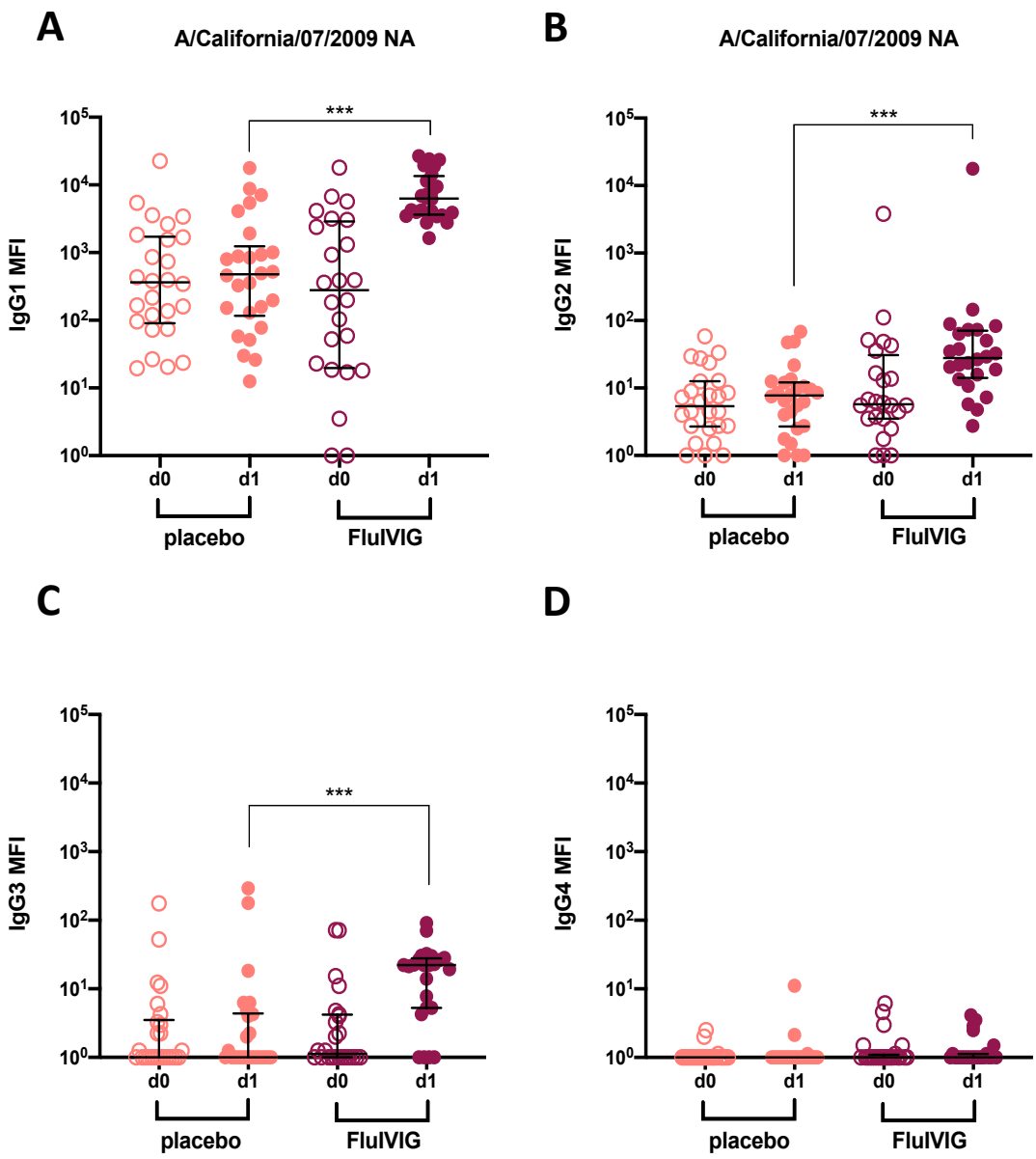
Supplemental Figure 5. HA-specific IgG subclasses following treatment with Flu-IVIG in patients hospitalised with severe influenza. A bead-based IgG subclass multiplex was used to determine the median fluorescence intensity (MFI) of HA-specific IgG subclasses in patient sera. Pre-infusion (d0; open symbols) and post-infusion (d1; closed symbols) median MFI with interquartile range for IgG1 (A & B), IgG2 (C & D), IgG3 (E & F) and IgG4 (G & H) against the B/Phuket/3073/2013 HA (left figure panels) or the A/California/07/2009(H1N1) HA (right figure panels) were measured for the placebo or Flu-IVIG treated B/Yamagata (Flu-IVIG n = 33 and placebo n = 29) and A/H1N1 (Flu-IVIG n = 24 and placebo n = 26) infected patients, respectively. Analysis of covariance, with the pre-infusion or d0 level as a covariate, was used to compare differences between the placebo and Flu-IVIG treatment groups at d1 post-infusion. The p-value represents the difference between treatment groups for log₂ d1 post-infusion controlling for pre-infusion (d1) MFI. ** P <0.01, *** P < 0.001

Supplemental Figure 6



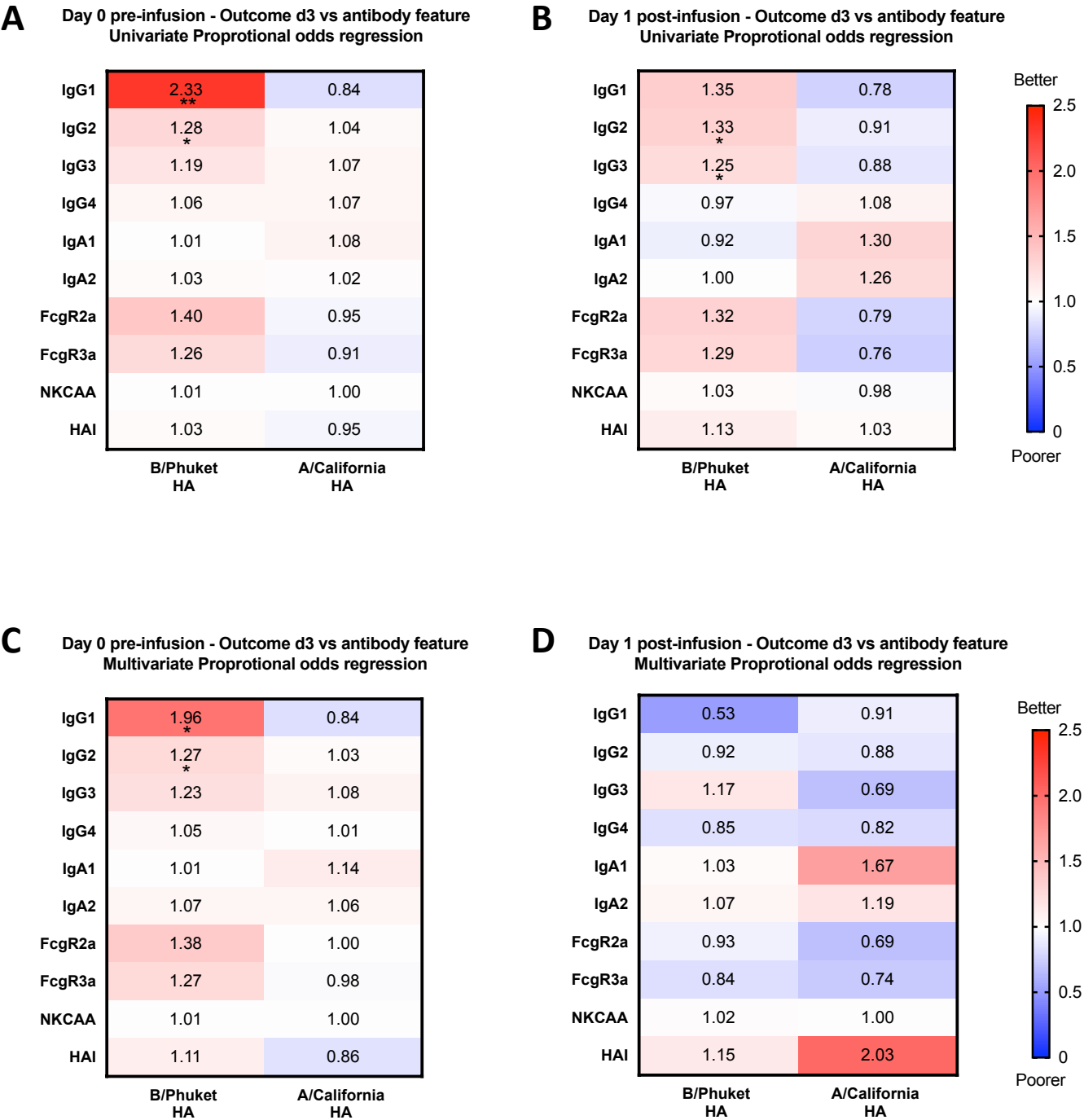
Supplemental Figure 6. NA-specific IgG subclasses day 1 post-infusion with Flu-IVIG in patients hospitalised with severe influenza B. A bead-based IgG subclass multiplex was used to examine the median fluorescence intensity (MFI) of NA-specific IgG subclasses in patient sera. Pre-infusion (d0; open symbols) and post-infusion (d1; closed symbols) median MFI with interquartile range for IgG1 (A), IgG2 (B), IgG3 (C) and IgG4 (D) against the B/Phuket/3073/2013 NA were measured for the placebo (n = 29) or Flu-IVIG (n = 33) treated B/Yamagata infected patients. Analysis of covariance, with the pre-infusion or d0 level as a covariate, was used to compare differences between the placebo and Flu-IVIG treatment groups at d1 post-infusion. The p-value represents the difference between treatment groups for log₂ d1 post-infusion controlling for pre-infusion (d0) MFI. ** P < 0.01, *** P < 0.001

Supplemental Figure 7



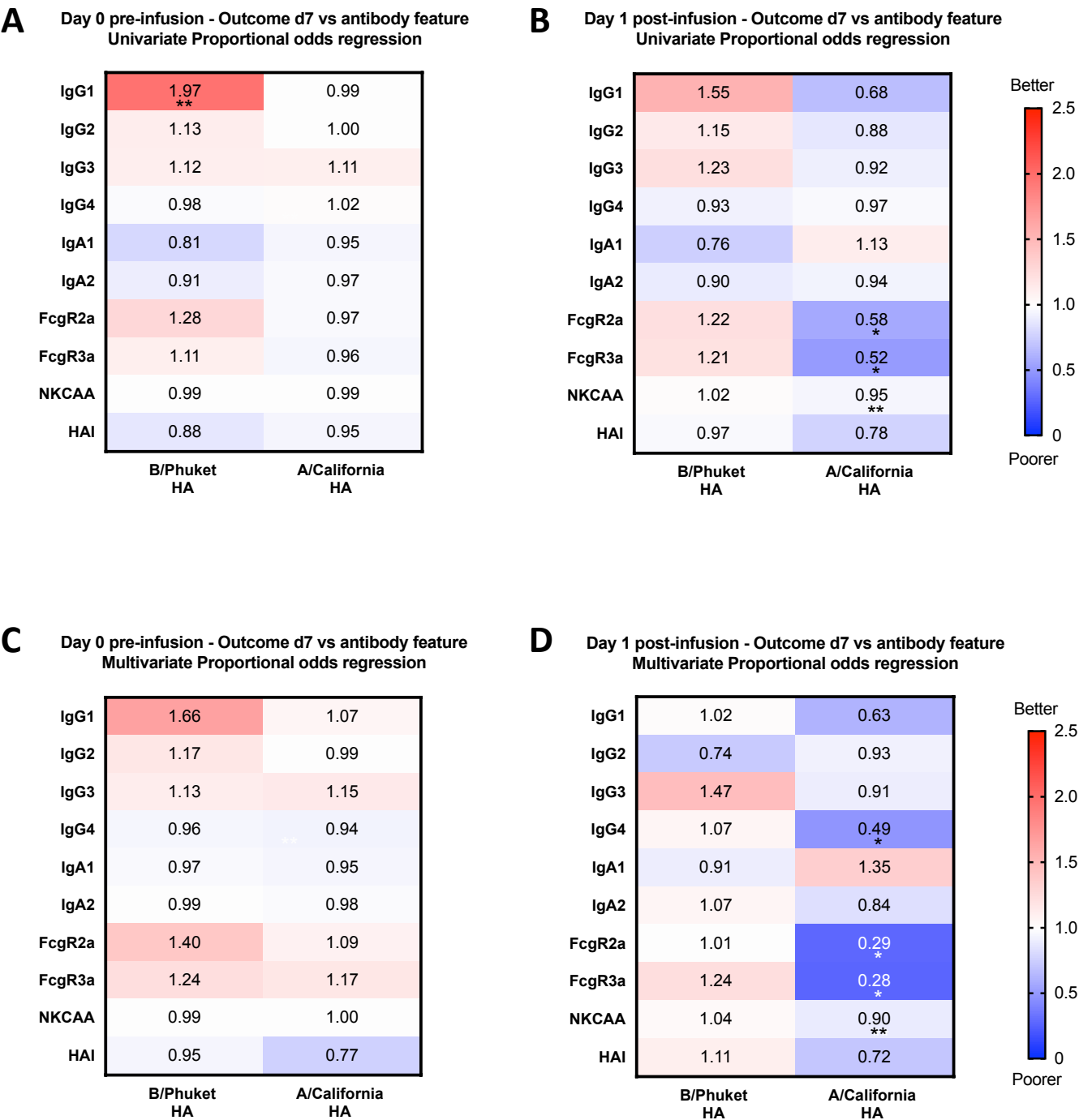
Supplemental Figure 7. NA-specific IgG subclasses day 1 post-infusion with Flu-IVIG in patients hospitalised with severe influenza A. A bead-based IgG subclass multiplex was used to examine the median fluorescence intensity (MFI) of NA-specific IgG subclasses in patient sera. Pre-infusion (d0; open symbols) and post-infusion (d1; closed symbols) median MFI with interquartile range for IgG1 (A), IgG2 (B), IgG3 (C) and IgG4 (D) against the A/California/07/2009(H1N1) NA were measured for the placebo (n = 26) or Flu-IVIG (n = 24) treated A/H1N1 infected patients. Analysis of covariance, with the pre-infusion or d0 level as a covariate, was used to compare differences between the placebo and Flu-IVIG treatment groups at d1 post-infusion. The p-value represents the difference between treatment groups for $\log_2$ d1 post-infusion controlling for pre-infusion (d0) MFI. *** P < 0.001

Supplemental Figure 8



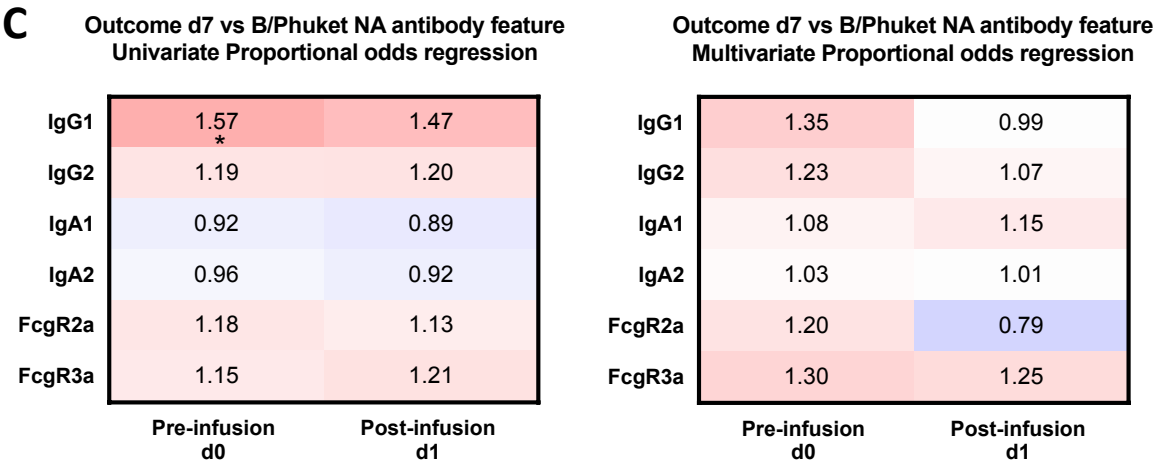
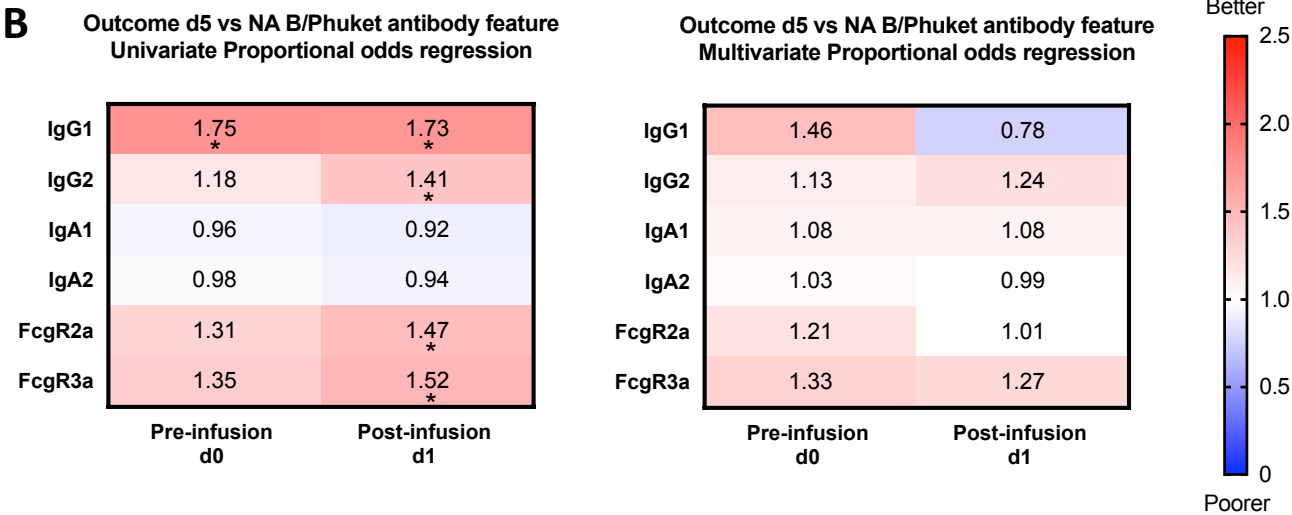
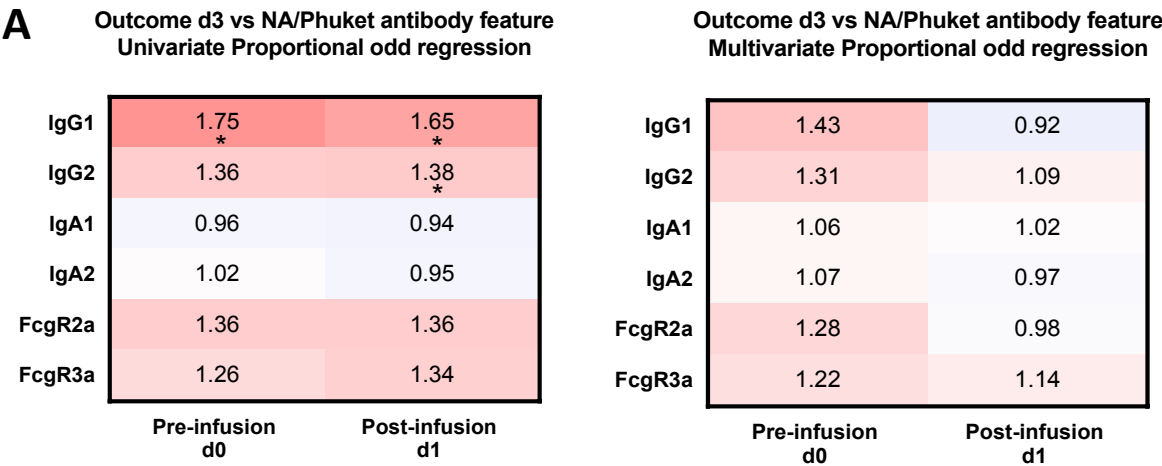
Supplemental Figure 8. Associations between influenza antibody features and clinical outcomes at day 3 post-infusion in patients hospitalised with severe B/Yamagata and A/H1N1 influenza. The association between antibody features and day 3 (d3) post-infusion ordinal outcomes were investigated using univariate and multivariate proportional odds regression models, with the multivariate model adjusting for baseline antibody level, treatment group (Flu-IVIG/placebo) and risk score at day 7. Heat maps show summary odds ratios (ORs) for patients hospitalised with B/Yamagata (n = 62; left side of heat map) and A/H1N1 (n = 50; right side of heat map) influenza at pre-infusion (A and C) and d1 post-infusion (B and D) timepoints generated using univariate (A and B) and multivariate (C and D) proportional odds regression models. ORs greater than one indicate that patients with higher antibody levels have improved odds of being in a better outcome category at d3 post-infusion and ORs less than one indicate that patients with lower antibody levels have improved odds of being in a better outcome category. * P < 0.05, ** P < 0.01

Supplemental Figure 9



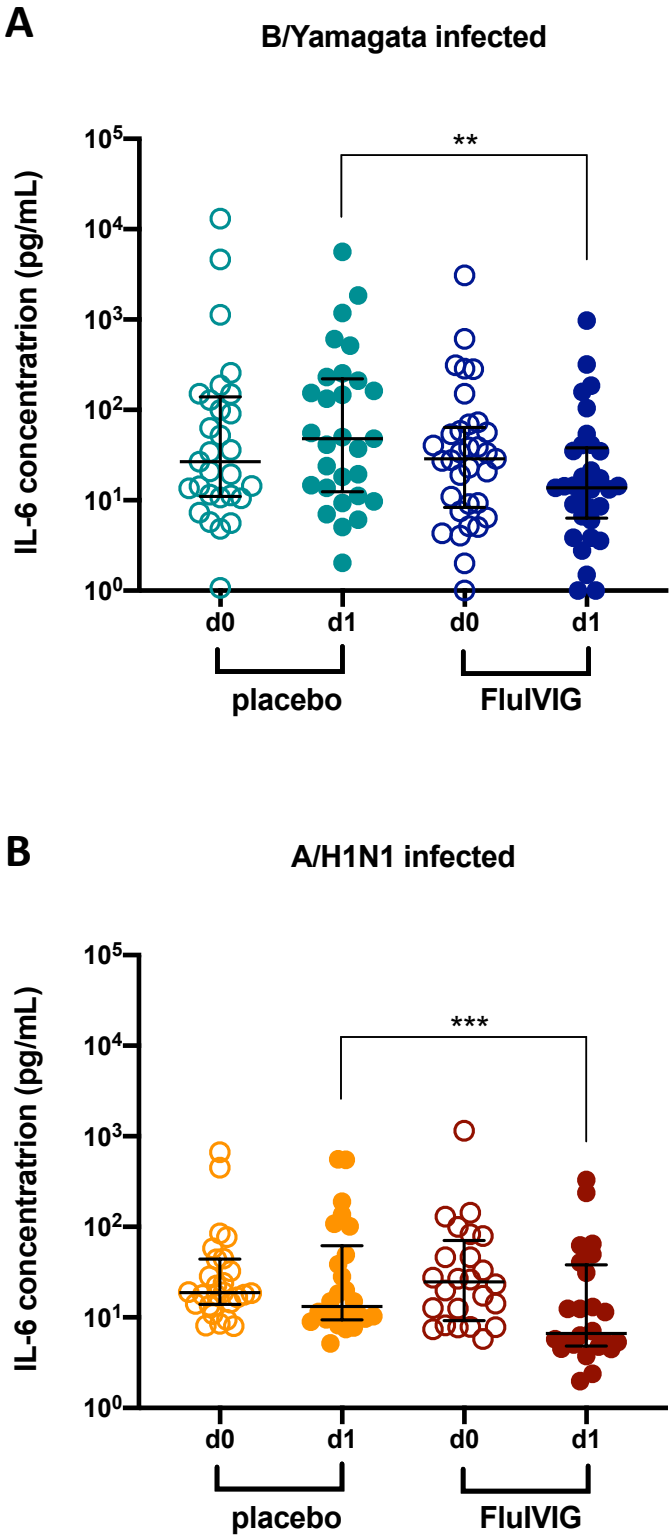
Supplemental Figure 9. Associations between influenza antibody features and clinical outcomes at day 7 post-infusion in patients hospitalised with severe B/Yamagata and A/H1N1 influenza. The association between antibody features and day 7 (d7) post-infusion ordinal outcomes were investigated using univariate and multivariate proportional odds regression models, with the multivariate model adjusting for baseline antibody level, treatment group (Flu-IVIG/placebo) and risk score at day 7. Heat maps show summary odds ratios (ORs) for patients hospitalised with B/Yamagata (n = 62; left side of heat map) and A/H1N1 (n = 50; right side of heat map) influenza at pre-infusion (A and C) and d1 post-infusion (B and D) timepoints generated using univariate (A and B) and multivariate (C and D) proportional odds regression models. ORs greater than one indicate that patients with higher antibody levels have improved odds of being in a better outcome category at d7 post-infusion and ORs less than one indicate that patients with lower antibody levels have improved odds of being in a better outcome category. * P < 0.05, ** P < 0.01

Supplemental Figure 10



Supplemental Figure 10. Associations between NA-specific influenza antibody features and clinical outcomes in patients hospitalised with severe influenza B. The association between antibody features and (A) day 3 (d3), (B) day 5 (d5) and (C) day 7 (d7) post-infusion ordinal outcomes were investigated using univariate and multivariate proportional odds regression models, with the multivariate model adjusting for baseline antibody level, treatment group (Flu-IVIG/placebo) and risk score at day 7. Heat maps show summary odds ratios (ORs) for patients hospitalised with B/Yamagata influenza (n = 62) at pre-infusion (left side of heat map) and d1 post-infusion (right side of heat map) timepoints generated using univariate (right figure panel) and multivariate (left figure panel) proportional odds regression models. ORs greater than one indicate that patients with higher antibody levels have improved odds of being in a better outcome category at the post-infusion timepoint indicated, whereas ORs less than one indicate that patients with lower antibody levels have improved odds of being in a better outcome category. * P <0.05

Supplemental Figure 11



Supplemental Figure 11. Serum concentration of interleukin-6 (IL-6) day 1 post-infusion with Flu-IVIG in patients hospitalised with severe influenza. A bead-based multiplex was used to determine the concentration of IL-6 in patient sera. Pre-infusion (d0; open symbols) and post-infusion (d1; closed symbols) median concentrations of IL-6 with interquartile ranges were measured for the placebo or Flu-IVIG treated (A) B/Yamagata (Flu-IVIG n = 33 and placebo n = 29) and (B) A/H1N1 (Flu-IVIG n = 24 and placebo n = 26) infected patients. Analysis of covariance, with the pre-infusion or d0 concentration as a covariate, was used to compare differences between the placebo and Flu-IVIG treatment groups at d1 post-infusion. The p-value represents the difference between treatment groups for log₂ d1 post-infusion controlling for pre-infusion (d1) IL-6 concentration. *** P < 0.001