Supplemental data for manuscript:

Fc functional antibodies in humans with severe H7N9 and seasonal influenza

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Supplementary Table 1 – Infecting virus subtype/strain, HA proteins and influenza viruses studied for H7N9 and seasonal influenza infected cohorts

Cohort	Infecting virus subtype	Infecting virus strain	HA protein studied (FcγR dimer ELISA and NK cell activation assay)	Influenza virus studied (HI, FRA and LDH ADCC assay)
H7N9 (n=18)	H7N9 (n=18)	A/Shanghai/1/2013-like (n=18)	A/Shanghai/1/2013	**HI:** A/Shanghai/4664T/2013 **Pseudovirus Neutralisation assay:** non-replicative HIV expressing H7 and N9
Seasonal (n=16)	H1N1 (n=4)	A/California/07/2009-like (n=4)	A/California/04/2009	**HI:** A/California/07/2009 **FRA:** A/California/07/2009
	H3N2 (n=8)	A/Newcastle/22/2014-like (n=4) clade 3C.3 A/Switzerland/9715293/2013-like (n=2) clade 3C.3 A/South Australia/91/2014-like (n=1) clade 3C.3 A/New Caledonia/71/2014-like (n=1) clade 3C.2	A/Switzerland/9715293/ 2013	**HI:** A/Switzerland/9715293/2013 **FRA:** A/Switzerland/9715293/2013 **LDH ADCC assay:** X-31
	B (n=4)	B/Phuket/3073/2013-like (n=2) Yamagata lineage B/Brisbane/46/2015-like (n=1) Victoria lineage unknown (n=1)	B/Phuket/3073/2013	**HI:** B/Phuket/3073/2013 **FRA:** B/Phuket/3073/2013

Supplementary Figure 1

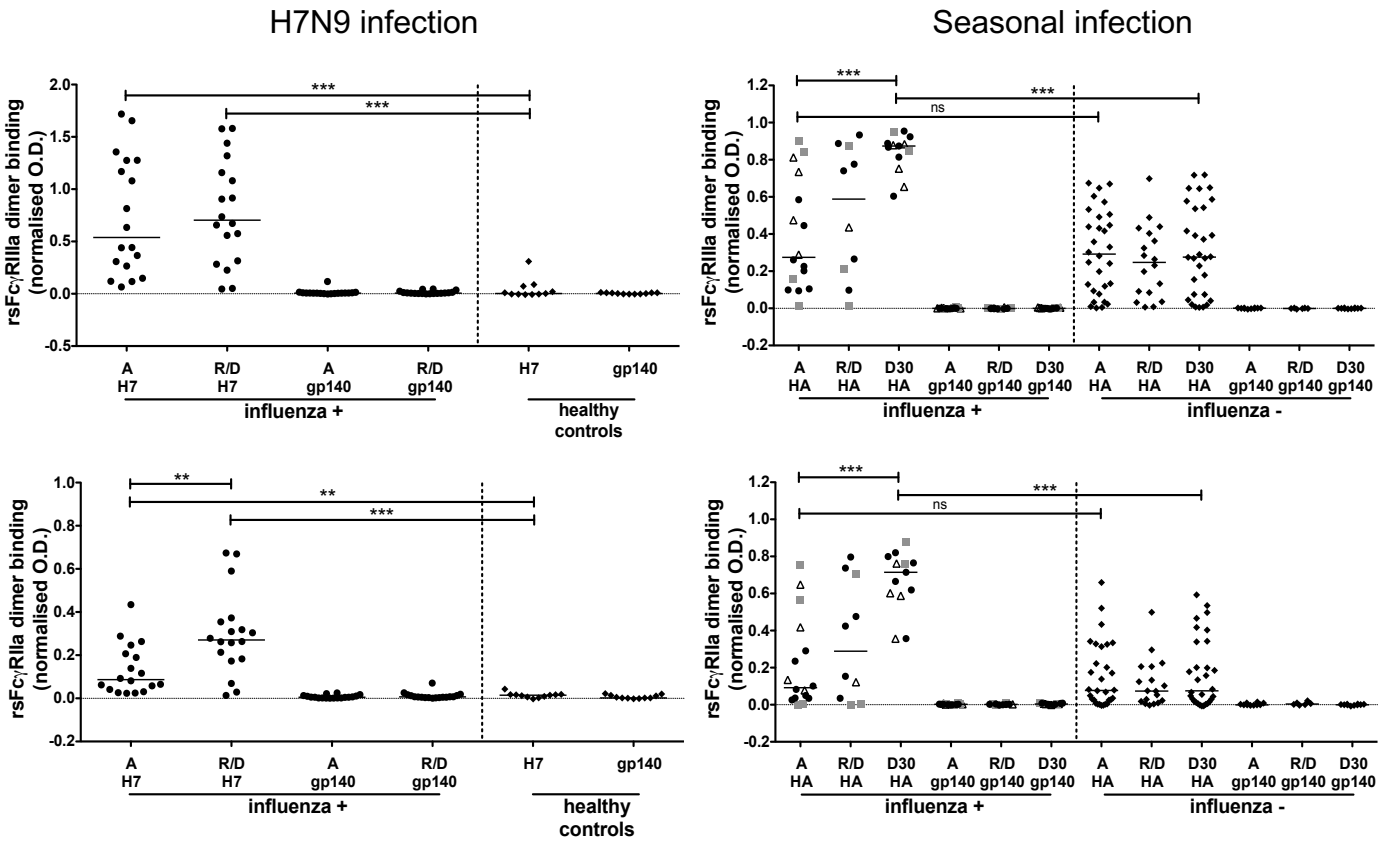


Figure S1. Increased HA-specific FcγR binding Abs after severe influenza infection. Data is shown as in Figure 1 with the addition of FcγR binding responses to a negative control HIV-1 gp140 protein for all time points. All subjects were HIV negative.

Supplementary Figure 2

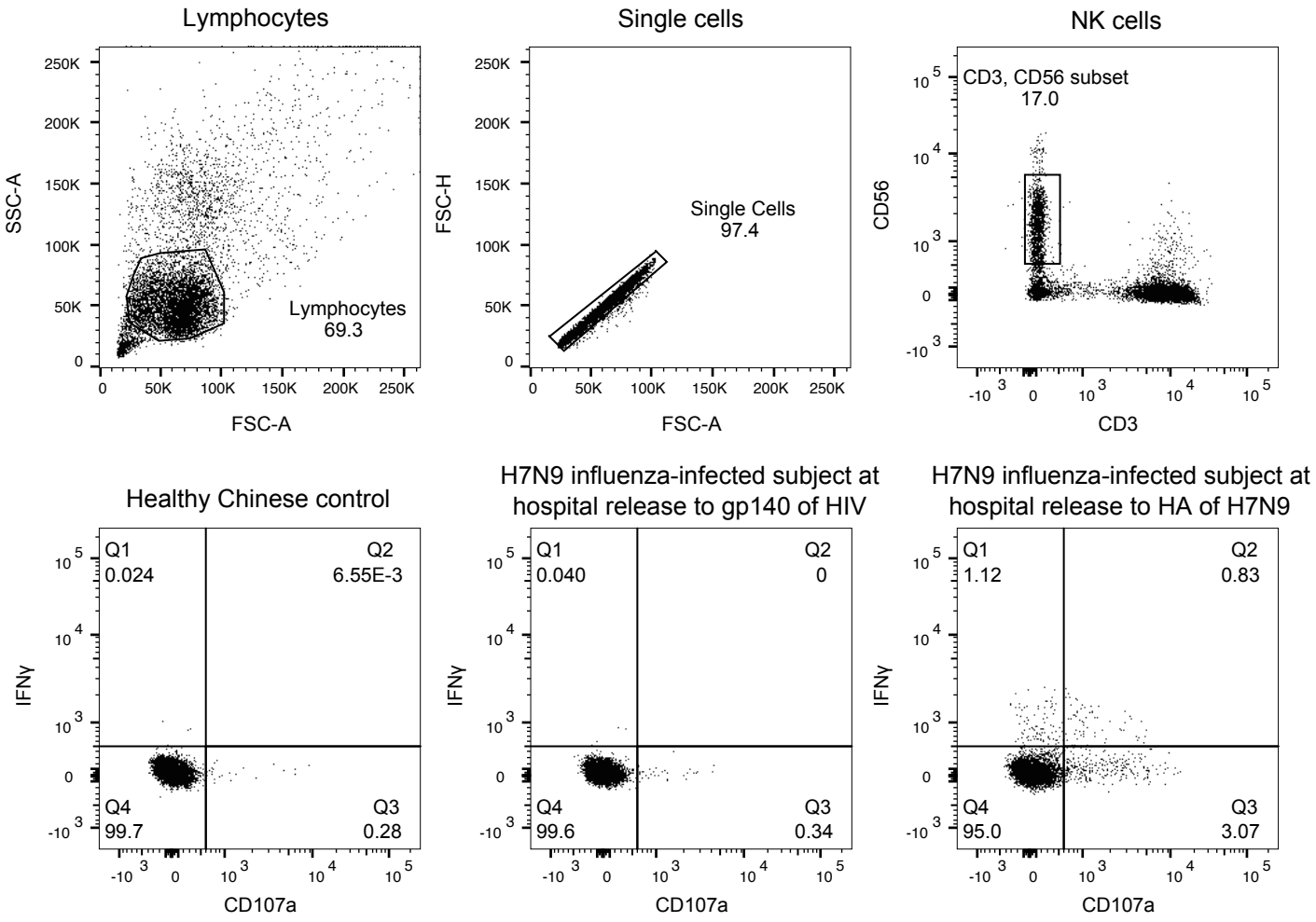


Figure S2. Gating strategy for primary NK cell activation assay. Freshly isolated PBMCs were gated for lymphocytes by size (FSC-A) and granularity (SSC-A) ensuring single cells (FSC-A vs. FSC-H). CD3-CD56+dim NK cells were selected for analysis using IFNγ and/or CD107a (Q1+Q2+Q3) as activation markers.

Supplementary Figure 3

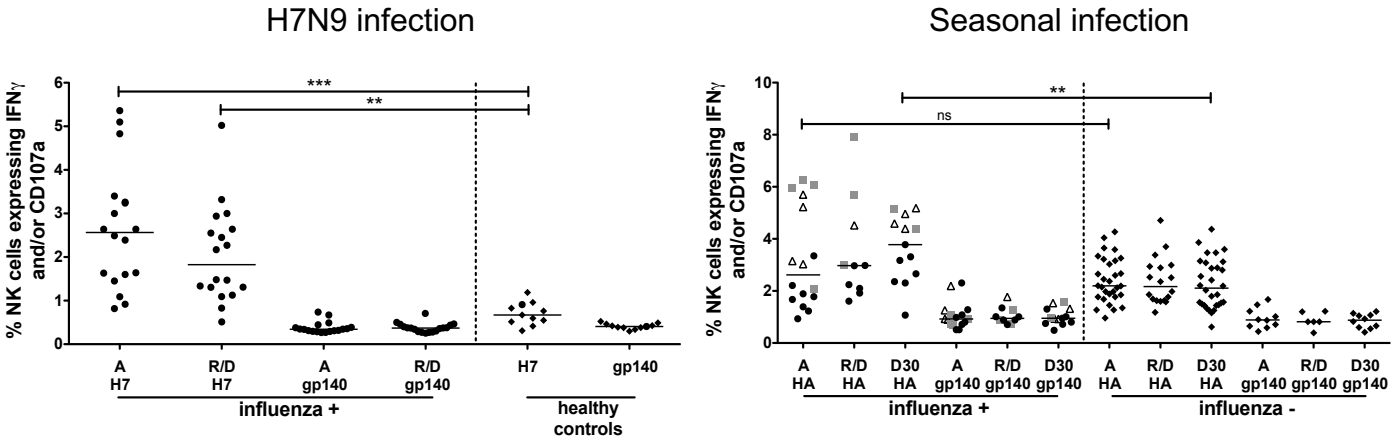


Figure S3. Increased NK cell activating HA-specific Abs after severe influenza infection. Data is shown as in Figure 2A and 2B with the addition of Ab-dependent NK cell activation to a negative control HIV-1 gp140 protein for all time points. All subjects were HIV negative.

Supplementary Figure 4

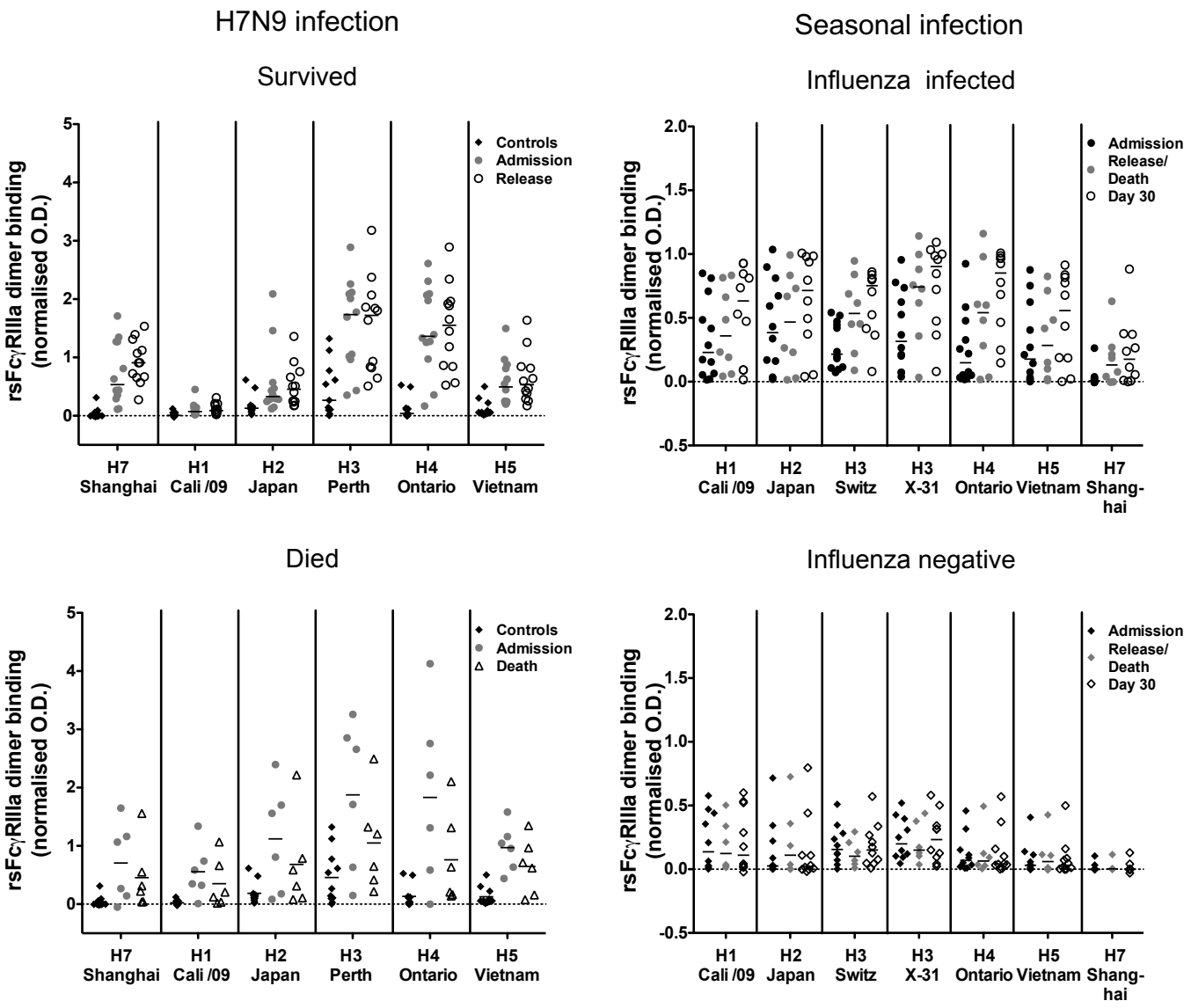


Figure S4. Breadth of rsFcγRIIIa binding Abs generated during severe influenza infection. Individual data points are shown for Figure 4A and 4B for each HA tested at the time points measured.

Supplementary Figure 5

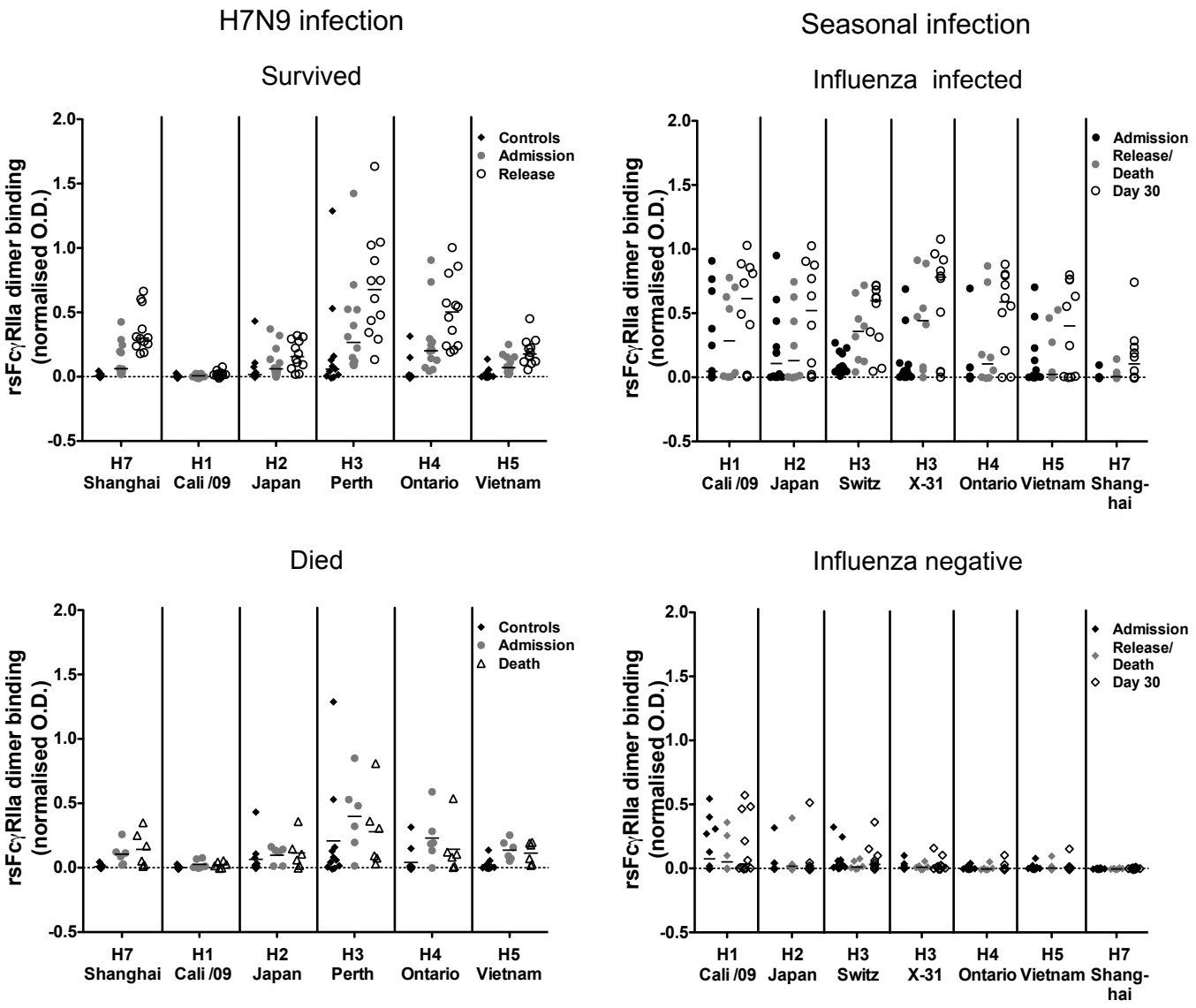


Figure S5. Breadth of rsFcγRIIa binding Abs generated during severe influenza infection. Individual data points are shown for Figure 4C and 4D for each HA tested at the time points measured.

Supplementary Figure 6

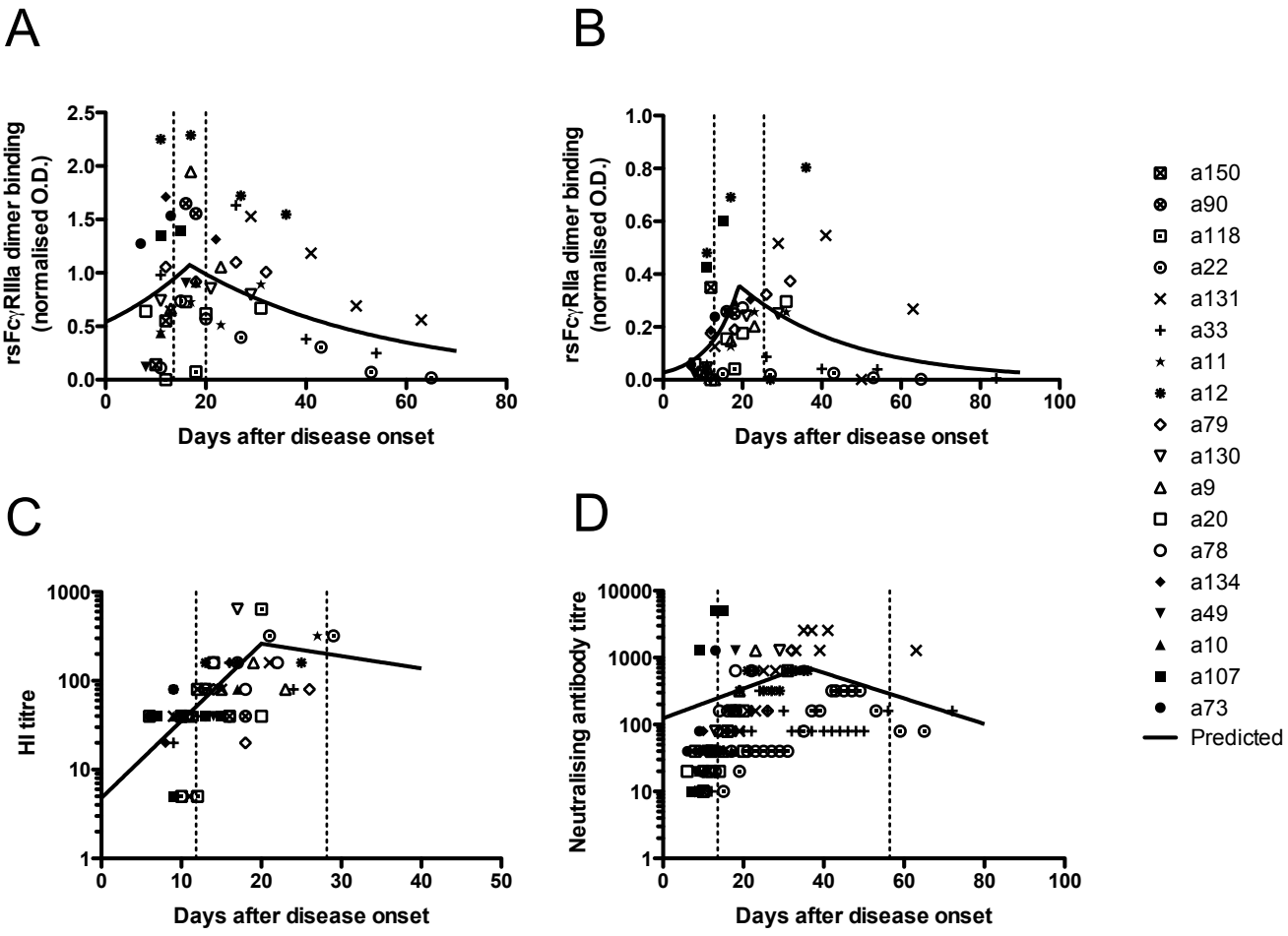


Figure S6. Kinetics of Fc functional and neutralising Ab responses in H7N9 influenza. Individual subjects and data points are shown for the data that was modelled in Figure 5A in the main text. Solid black line represents the population level model prediction, and dashed line indicates 95%CI for the time to peak.

Supplementary Figure 7

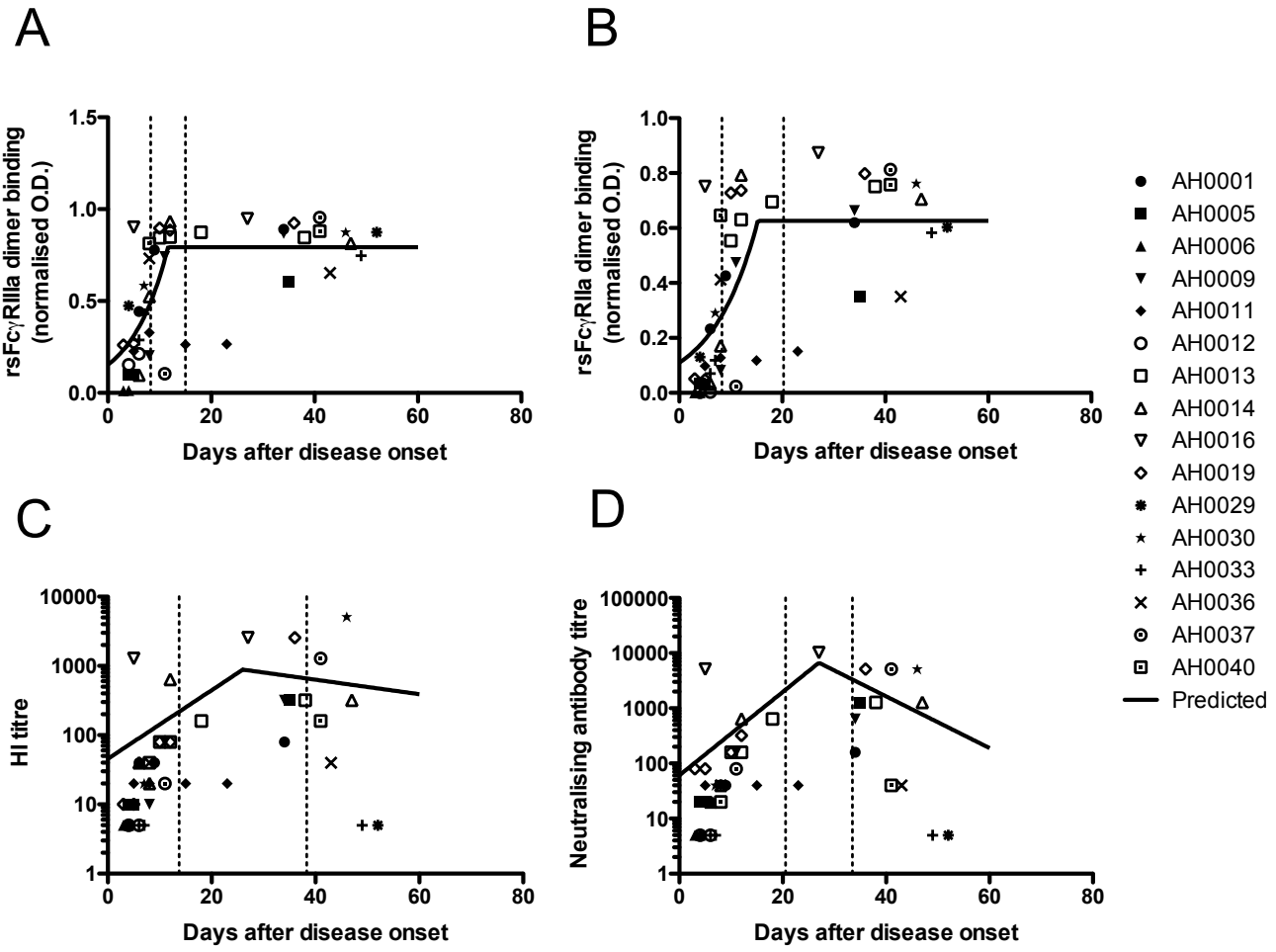


Figure S7. Kinetics of Fc functional and neutralising Ab responses in severe seasonal influenza. Individual subjects and data points are shown for the data that was modelled in Figure 5B in the main text. Solid black line represents the population level model prediction, and dashed line indicates 95%CI for the time to peak.

Supplementary Figure 8

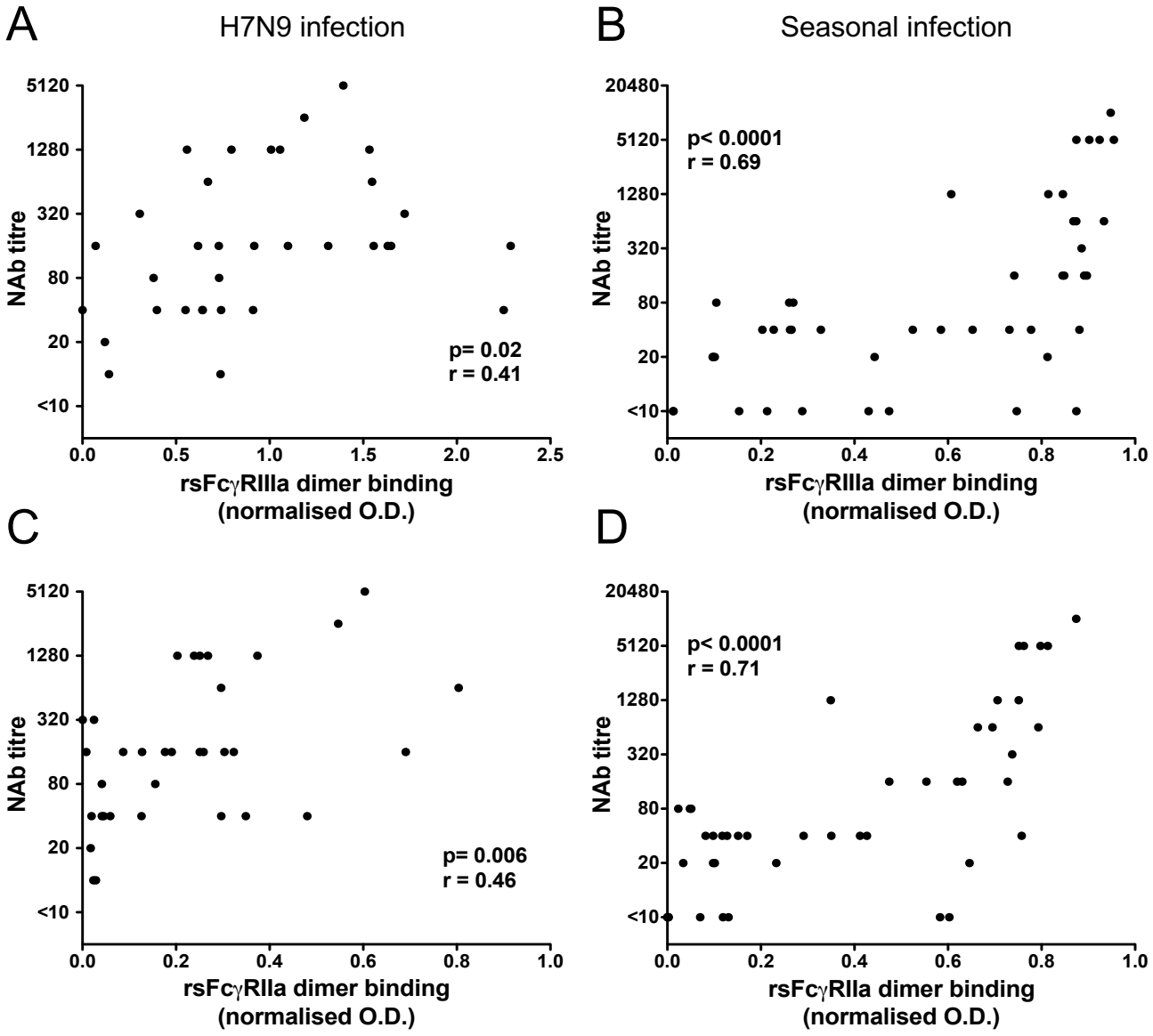


Figure S8. Correlation between neutralising and Fc functional Ab generation. NAb responses were plotted against rsFcγRIIIa binding (A, B) and rsFcγRIIa binding (C, D) Abs in severe H7N9 influenza using data from 18 subjects over multiple time points (A, C) and in severe seasonal influenza using data from 16 subjects over multiple time points (B, D). Viruses and HA proteins used for each cohort are described in the Methods and Table S1.

Supplementary Figure 9

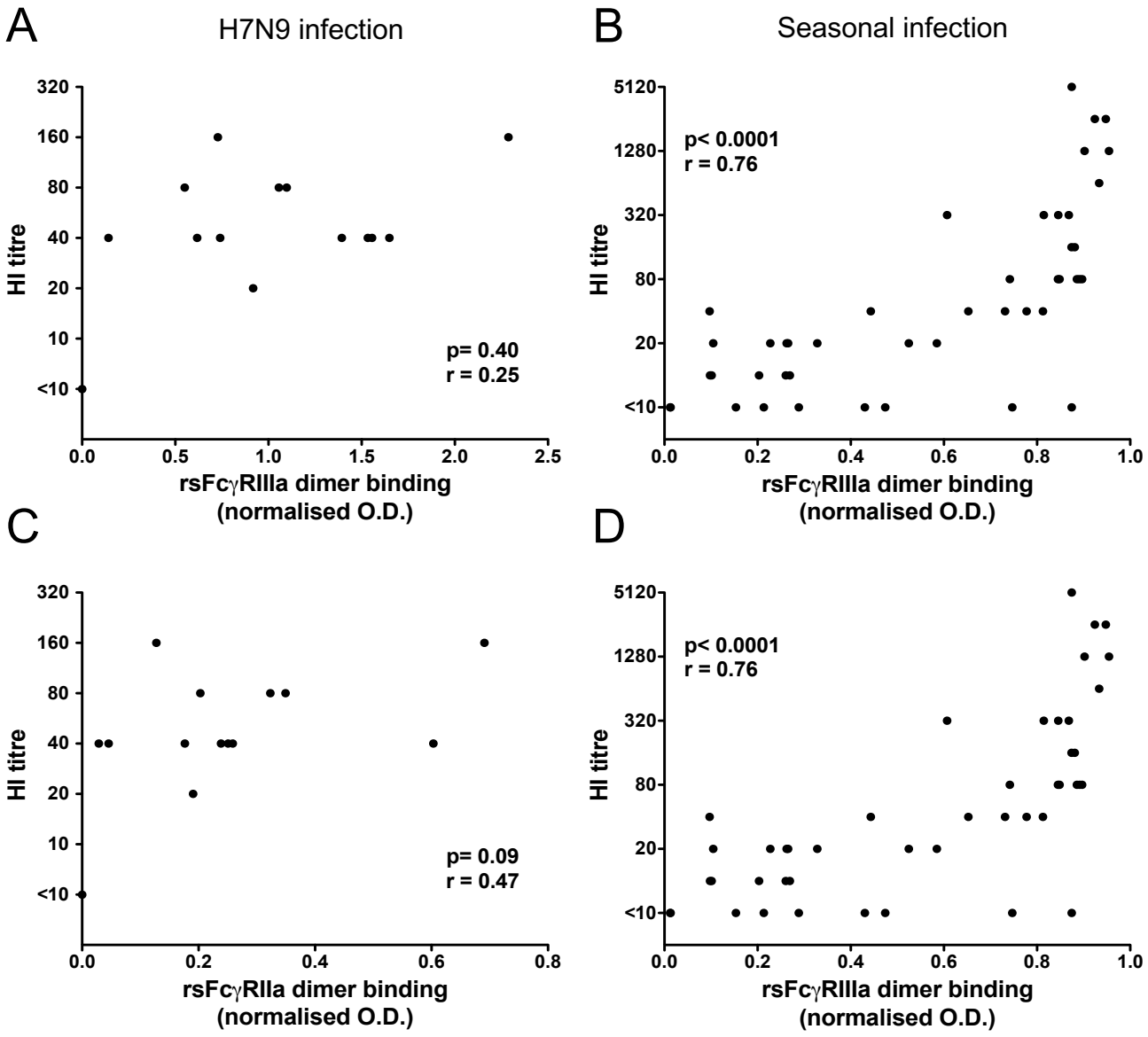


Figure S9. Correlation between HI and Fc functional Ab generation. HI Ab responses were plotted against rsFcγRIIIa binding (A, B) and rsFcγRIIa binding (C, D) Abs in severe H7N9 influenza using data from 18 subjects over multiple time points (A, C) and in severe seasonal influenza using data from 16 subjects over multiple time points (B, D). Viruses and HA proteins used for each cohort are described in the Methods and Table S1.