Supplementary information for

Broadly protective murine monoclonal antibodies against influenza B virus target highly conserved neuraminidase epitopes

Authors: Teddy John Wohlbold[1,2], Kira A. Podolsky[3], Veronika Chromikova[1], Ericka Kirkpatrick[1,2], Veronica Falconieri[3], Philip Meade[1,2], Fatima Amanat[1], Jessica Tan[1,2], Benjamin R. tenOever[1], Gene S. Tan[1], Sriram Subramaniam[3], Peter Palese[1,4], and Florian Krammer[1]*

Affiliations:
[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, NY 10029, USA
[2]Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, NY 10029, USA
[3]Center for Cancer Research, National Cancer Institute Building 50, Room 4306, National Institutes of Health, Bethesda, MD, USA
[4]Department of Medicine, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, NY 10029, USA
*Correspondence to: florian.krammer@mssm.edu

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Supplementary notes

IgG sequences and comparison to IgG germlines

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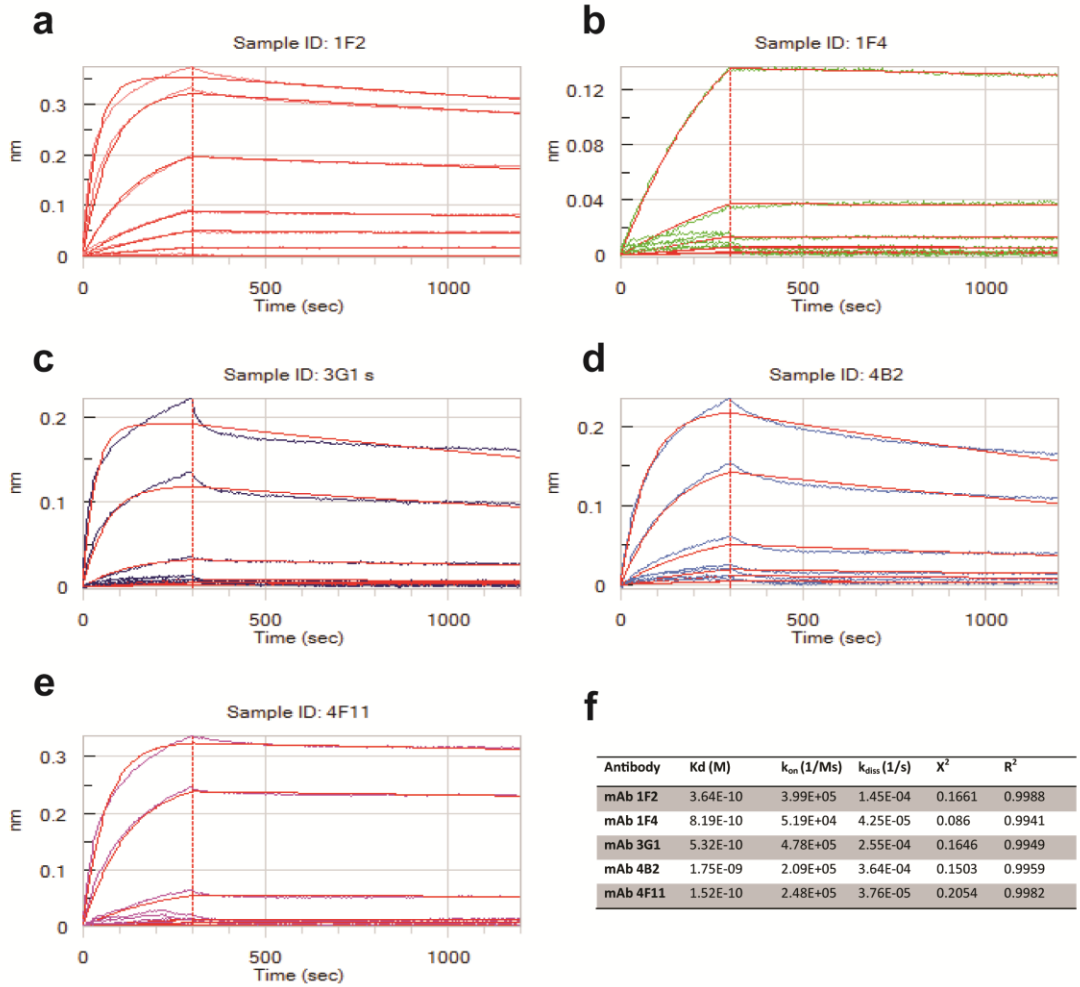
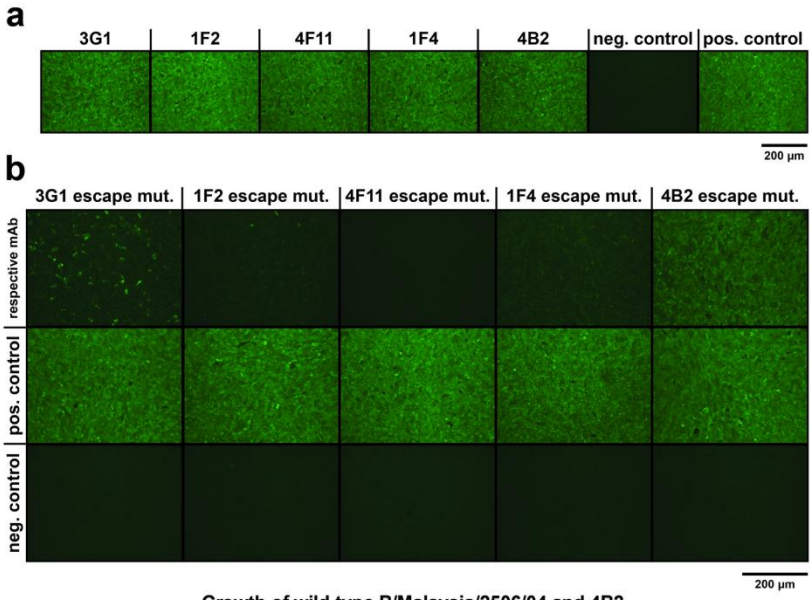
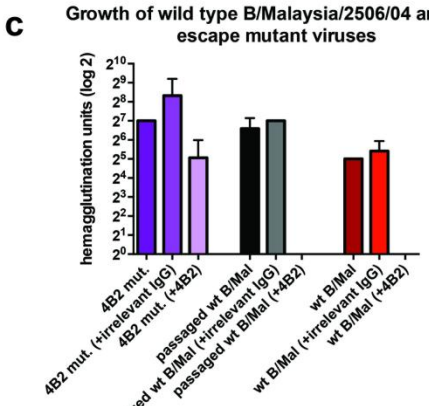


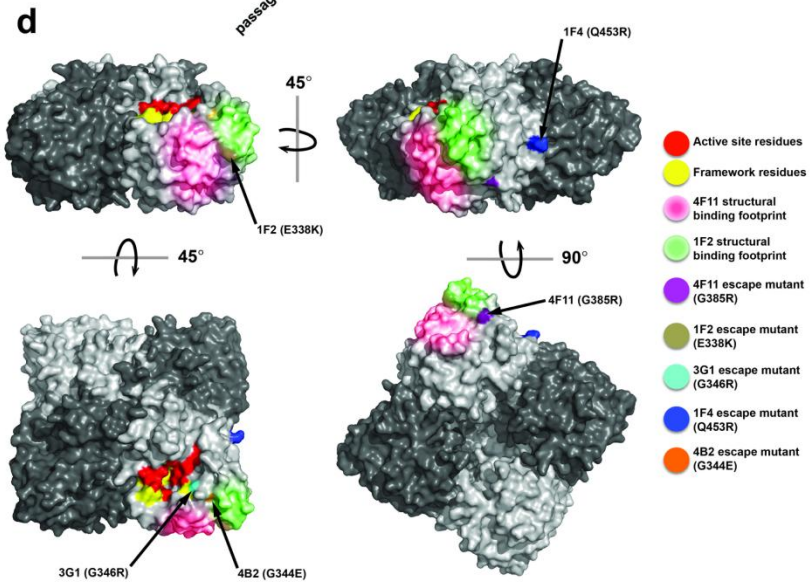
f

Antibody	Kd (M)	k_{on} (1/Ms)	k_{diss} (1/s)	X^2	R^2
mAb 1F2	3.64E-10	3.99E+05	1.45E-04	0.1661	0.9988
mAb 1F4	8.19E-10	5.19E+04	4.25E-05	0.086	0.9941
mAb 3G1	5.32E-10	4.78E+05	2.55E-04	0.1646	0.9949
mAb 4B2	1.75E-09	2.09E+05	3.64E-04	0.1503	0.9959
mAb 4F11	1.52E-10	2.48E+05	3.76E-05	0.2054	0.9982

Supplementary Figure 1. **Antibody affinity as measured by bio-layer interferometry.** Antibody binding kinetics to recombinant NA from B/Malaysia/2406/04 of mAbs 1F2 **(a)**, 1F4 **(b)**, 3G1 **(c)**, 4B2 **(d)** and 4F11 **(e)** as measured by bio-layer interferometry. **(F)** shows a summary of the measured Kd, k_{on}, k_{diss} as well as curve fitting parameters.



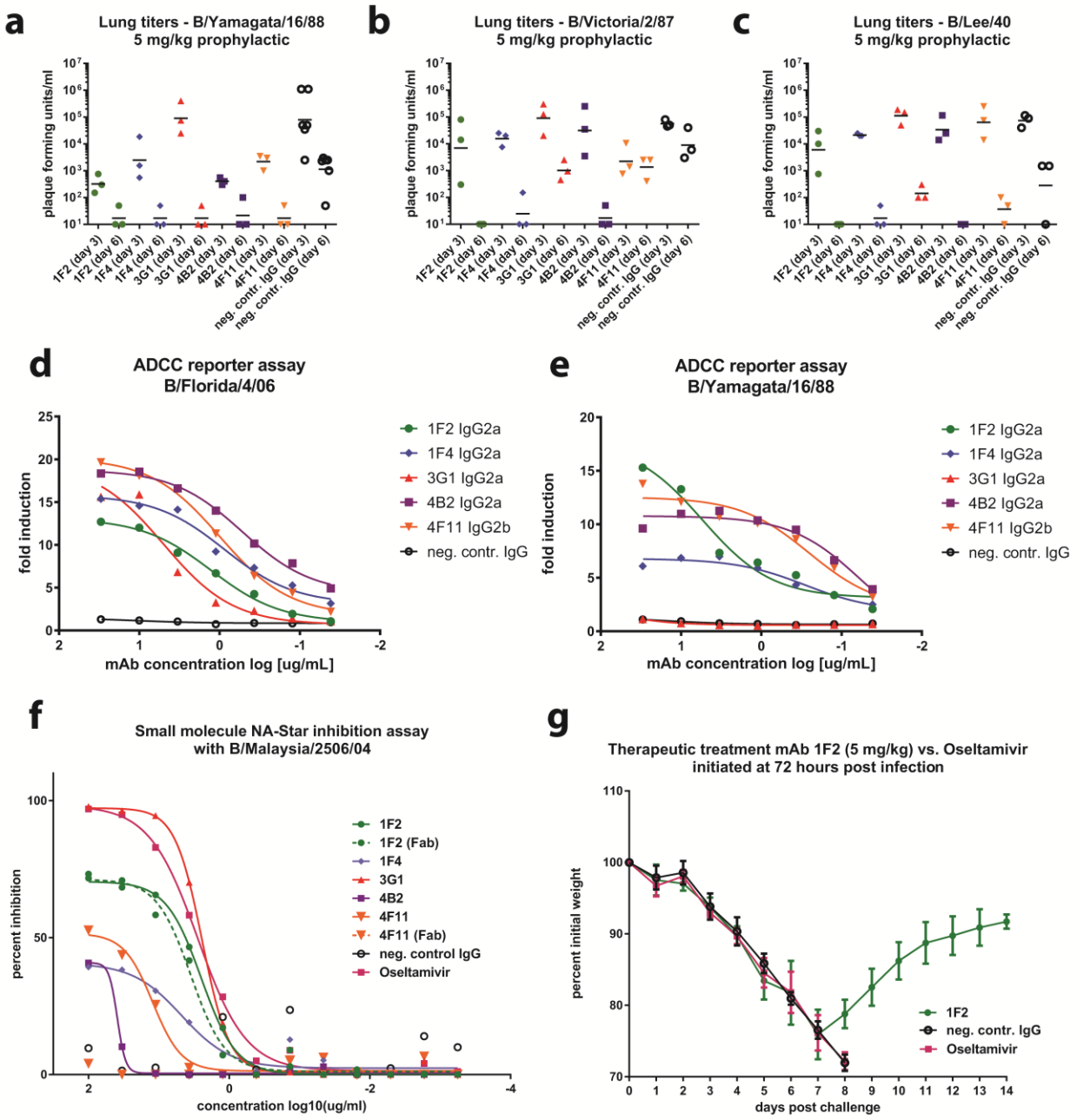




e

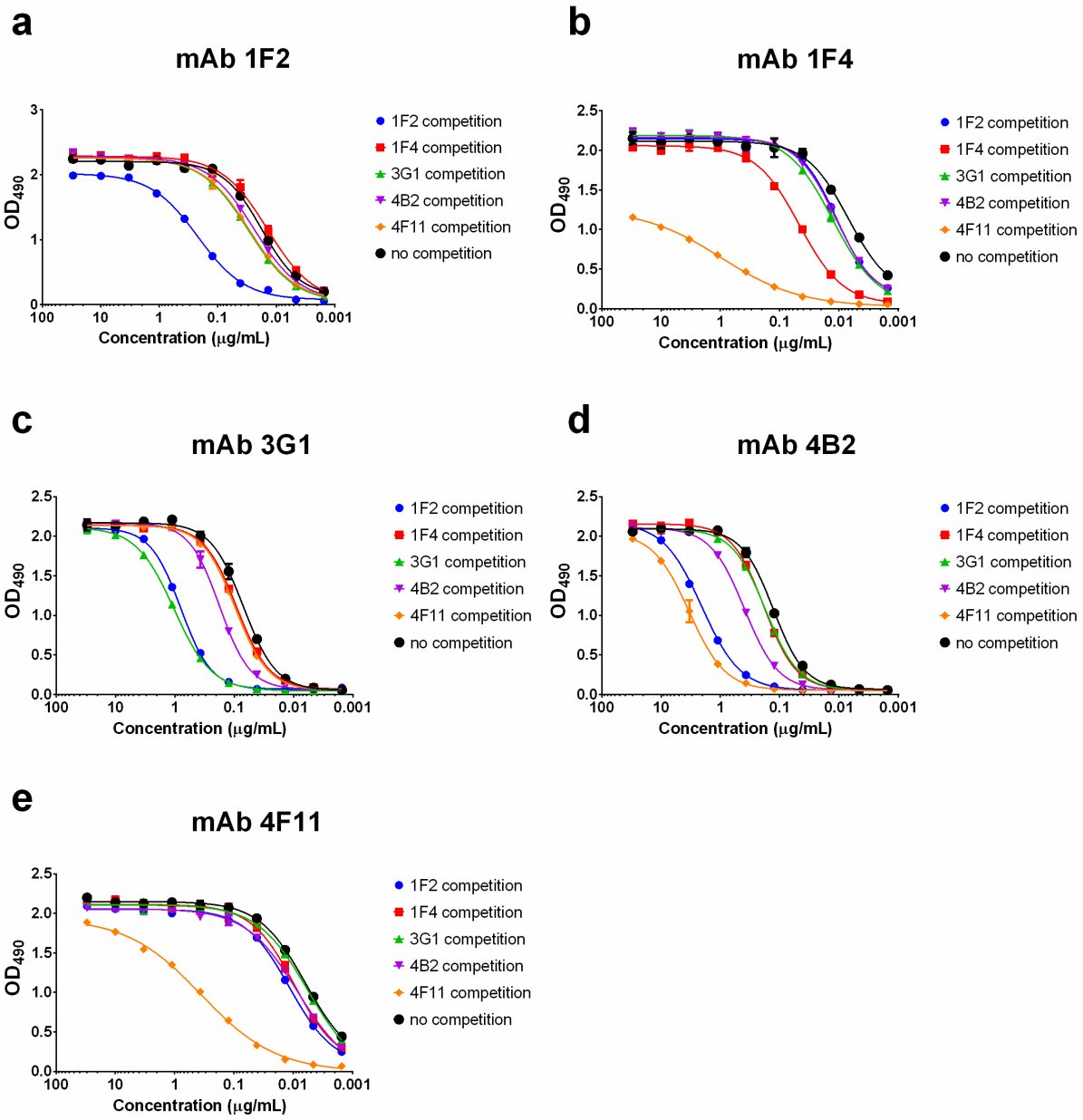
	Position	Amino Acid Residue	% Conservation
4F11 escape mutant	385	G	100
1F2 escape mutant	338	E	99.36
3G1 escape mutant	346	G	99.89
1F4 escape mutant	453	Q	99.89
4B2 escape mutant	344	G	99.89

Supplementary Figure 2. **IBV escape mutants reveal amino acid residues critical for mAb binding. (a)** To demonstrate mAb reactivity via immunofluorescence, MDCK cells were infected with wt B/Malaysia/2506/04 virus (MOI = 10), fixed, and stained using anti-NA mAbs (30 ug/ml). All mAbs displayed clear surface staining, allowing this assay to be used as a screen for potential binding mutants. A polyclonal cocktail of purified mouse mAb IgGs against the IBV HA was used as a positive infection control (pos. control). An irrelevant mouse mAb, 8H9 was used as a negative control (neg. control). **(b)** MDCK cells were infected with the generated B/Malaysia/2506/04 escape mutant viruses and stained with the respective mAb to which the escape mutant was generated, in a similar fashion to panel A. All mutant viruses – except that generated to mAb 4B2 – displayed clear loss of binding to the corresponding mAb. Scale bars represent 200 um. **(c)** HA titers of wt B/Malaysia/2506/04 virus (wt B/Mal), 4B2 escape mutant virus (4B2 mut.), and passaged wt B/Malaysia/2506/04 virus (passaged wt B/Mal) in the presence of mAb 4B2 at 10 ug/ml, irrelevant mouse mAb 3C12 (anti-N8) at 10 ug/ml, or no mAb at 72 hpi. Only the generated 4B2 escape mutant virus grew to detectable titers in the presence of 4B2. Passaged wt B/Malaysia/2506/04 virus, as explained in detail in the materials and methods section, is a control virus produced by serially passaging wt B/Malaysia/2506/04 virus in MDCK cells in the presence of irrelevant mouse mAb 3C12 alongside wt B/Malaysia/2506/04 virus in the presence of increasing concentrations of 4B2. The mean and standard error of the mean, obtained from biological triplicates, are displayed graphically. **(d)** Critical binding residues identified in IBV escape mutants – along with the structurally defined binding footprints from Fig. 2 and the NA enzymatic active site/framework residues – were mapped on one of the four monomers of the 3D structure of the NA from B/Brisbane/60/2008 virus (PDB ID: 4CPL). The remaining three monomers of the tetramer are shown in either light or dark grey. Degrees of rotation are approximate. **(e)** List of amino acid residues (position, identity, and percent conservation) identified as critical binding residues by escape mutant generation. Percent conservation was determined using 944 subsampled IBVs. B/Malaysia/2506/04 numbering is used throughout.

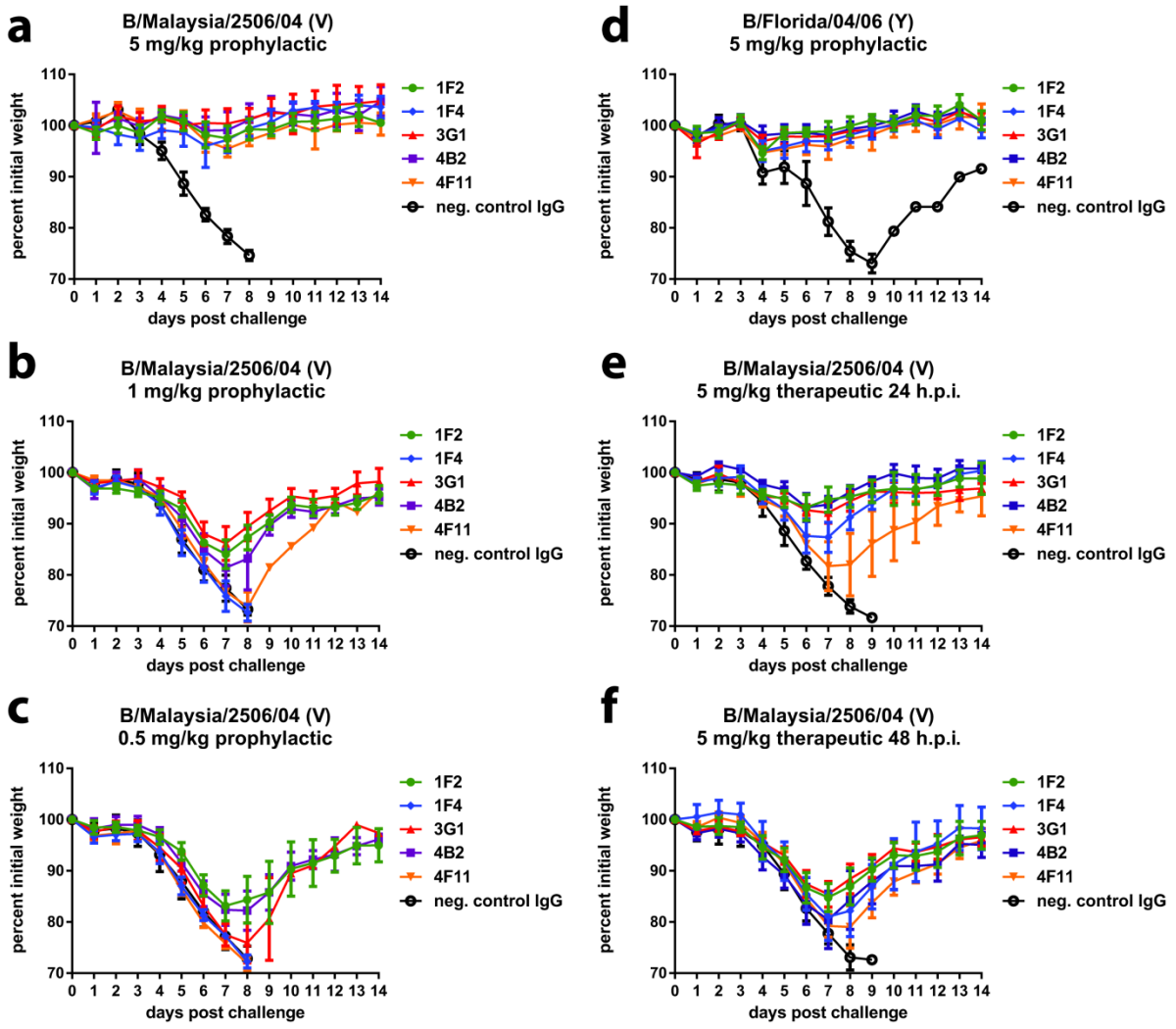


Supplementary Figure 3. **IBV anti-NA mAbs reduce viral lung titers in mice, activate *ADCC*, inhibit NA activity of drug-resistant IBV, and demonstrate superior effectiveness to oseltamivir. (a-c)** Female BALB/c mice (3 per group) were administered 5 mg/kg antibody prophylactically, challenged with B/Yamagata/16/88, B/Victoria/2/87, or B/Lee/40 viruses respectively and in identical fashion to Figure 4A. Mice were sacrificed on day 3 or 6 post-infection for lung titer analysis. Lung titers in groups treated with anti-NA mAbs are most reduced on day 6 post-infection compared to negative control mAb 8H9. **(d, e)** Anti-NA mAb incubated with MDCK cells infected with B/Florida/04/06 or B/Yamagata/16/88 viruses, respectively (MOI = 3), are able to engage Fc receptors and activate ADCC *in vitro*. Fold induction is defined as RLU

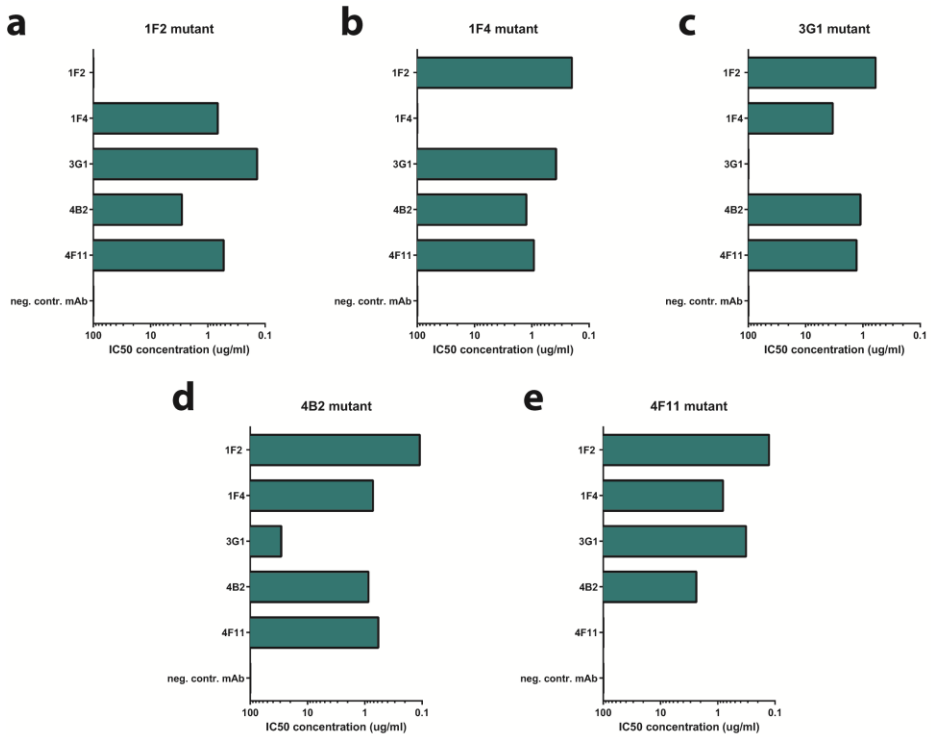
(induced by antibody)/ RLU (no antibody control background). Murine mAb 2G12 (anti-Ebolavirus Gp) is used as a negative control. Technical triplicates were performed. **(f)** NI activity against B/Malaysia/2506/04 using the NA-*Star* assay. Data points are presented as percent inhibition. Technical duplicates were performed. **(g)** Female BALB/c mice (5 per group) were administered either 5 mg/kg of mAb 1F2 intraperitoneally, 5 mg/kg negative control mAb 8H9 intraperitoneally, or placed on a twice daily, 20 mg/kg, 6 day-long regimen of oseltamivir delivered via oral gavage and initiated at 72 hpi with B/Malaysia/2506/04. Percent weight is shown and is calculated based on the initial body weight on day 0.



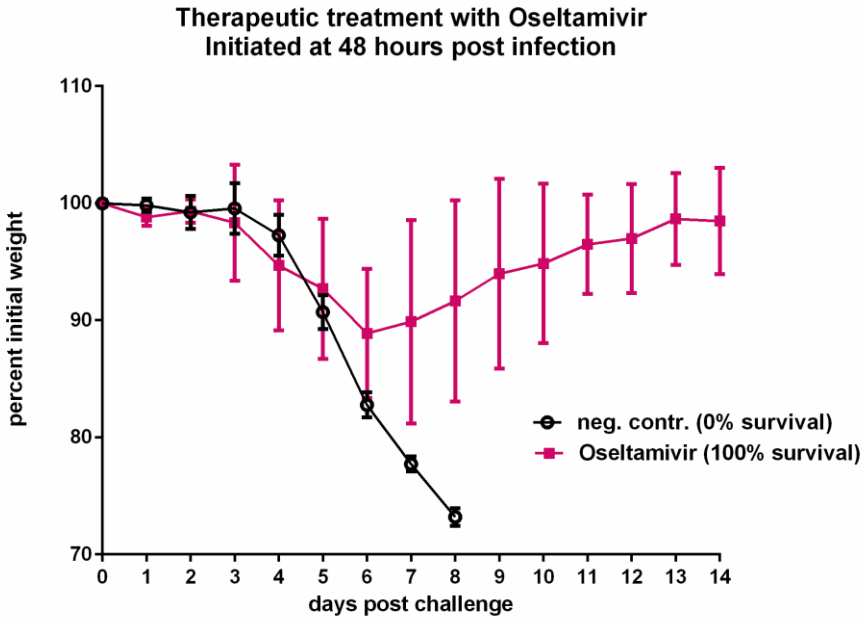
Supplementary Figure 4. **Competition between different mAbs for binding to B NA.** Antibodies 1F2 **(a)**, 1F4 **(b)**, 3G1 **(c)**, 4B2 **(d)** and 4F11 **(e)** were competed against themselves and each other using an ELISA-based assay. Technical duplicates were performed.



Supplementary Figure 5. **IBV anti-NA mAbs protect mice from morbidity when administered prophylactically or therapeutically.** Displayed are the weight loss curves corresponding to the survival curves in **Fig. 3.** Mice (5 per group) were administered either 5, 1, or 0.5 mg/kg of mAb intraperitoneally 2 h prior to a 5 mLD_{50} challenge with B/Malaysia/2506/04 virus **(a-c)** or administered 5 mg/kg of mAb intraperitoneally 2 h prior to a 5 mLD_{50} challenge with B/Florida/04/06 virus **(d)**. In therapeutic studies, mice were administered 5 mg/kg of each antibody either 24 **(e)** or 48 **(f)** h after challenge with 5 mLD_{50} of B/Malaysia/2506/04 virus. Percent weight is calculated based on the initial body weight on day 0. Mice that lost more than 25% of their initial body weights were humanely euthanized, and the remaining curves were generated from the weights of the surviving mice only. Murine mAb 8H9 (anti-H6) was used as a negative control in all experiments.



Supplementary Figure 6. **Neuraminidase inhibition assay against the escape mutants raised with the five mAbs.** Escape mutants generated with 1F2 **(a)**, 1F4 **(b)**, 3G1 **(c)**, 4B2 **(d)** and 4F11 **(e)** were each tested for sensitivity to the panel of five mAbs. The means obtained from technical duplicates are displayed graphically.



Supplementary Figure 7. **Oseltamivir treatment of mice initiated 48 hours post infection with B/Malaysia/2506/04 virus does not protect from weight loss but**

leads to survival of the infection. Female BALB/c mice (5 per group) were administered 5 mg/kg negative control mAb 8H9 intraperitoneally or were placed on a twice daily, 20 mg/kg, 6 day-long regimen of oseltamivir delivered via oral gavage and initiated at 48 hpi. Percent weight is shown and is calculated based on the initial body weight on day 0. Percentages next to the legend indicate survival.

Supplementary notes

IgG sequences and comparison to IgG germlines

mAb 1F2

IGHV-1 77*01

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Query  1    QVHLQQSGPEVARPGASVKLSCKASGYTFTDYYLNWVKQRPRQGLEWIGQIHPGSTNTYY  60
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IGHJ4*01

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IGHD3*01

```
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IGKJ4*01

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mAb 1F4

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IGHJ3*01

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IGKV6-25*01

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Query  61   RFTGIISGTDYTLTISSVQAEDRALYYCQQHYSAP-----WTFGGGTKLEIK  107
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Germ.  61   RFTGSGSGTDYTLTISSVQAEDLALYYCQQHYSTPIGKJ*WTFGGGTKLEIK  112
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IGKJ1*01

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Germ.  1    WTFGGGTKLEIK  12
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mAb 3G1

IGHV1-9*01

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Query  1   QVQLQQSGAELMKPGASVKISCKATGYKFTSYWIGWVKQRPGHGLEWCGEIFPGSGSINY  60
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Query  61  NEKFKGKATFTADTSSNTAYLQLTSLTSEDSAVYYCAR  98
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IGHJ4*01

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IGKJ1*01

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IGHJ3*01

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IGHJ3*02

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IGKJ2*01

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mAb 4F11

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IGHJ3*01

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IGKJ2*01

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