

Figure S1 – VIP-expression of influenza neutralizing antibodies function both in vitro and in vivo.

a, Quantitation of F10 and CR6261 IgG by ELISA at four times following intramuscular injection of 1×10^{11} GC of the optimized expression vector into the same BALB/c mice challenged in Figure 2, demonstrating high variability of human IgG expression that is later resolved. **b**, Correlation of serum F10 antibody concentration as measured by ELISA five weeks post-AAV and neutralizing titer as determined by an in vitro GFP-reporter assay. **c**, Correlation of serum CR6261 antibody concentration as measured by ELISA five weeks post-AAV and neutralizing titer as determined by an in vitro GFP-reporter assay. **d**, Challenge of BALB/c mice expressing variable concentrations of CR6261 with 10,000 PFU of CA09 H1N1 influenza results in a range of pathology.

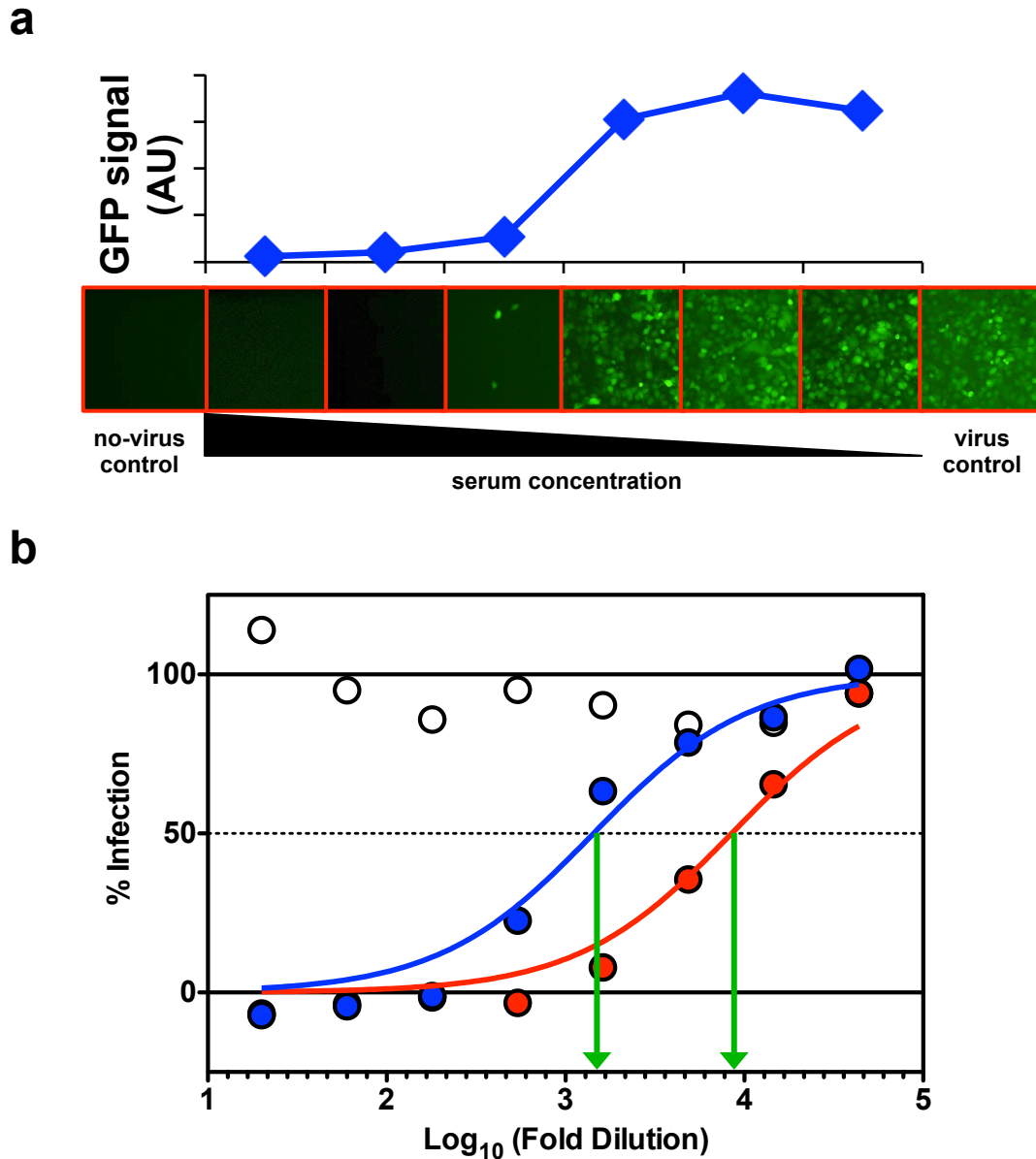


Figure S2 – In vitro neutralization assay and determination of serum IC₅₀ values.

a, Schematic representation of the in vitro neutralization assay in which MDCK-SIAT1 cells expressing influenza PB1 protein are infected with influenza virus carrying GFP in the PB1 segment. When performed in the presence of a titration of sera containing neutralizing activity, GFP expression is reduced in proportion to the decline in infected cells. **b**, Sample data from three animals exhibiting no neutralizing activity (white), moderate neutralizing activity (blue) and high neutralizing activity (red). Data from each mouse is fit using a nonlinear function and the fold-dilution calculated to result in 50% of GFP signal determines the IC₅₀ value (green arrows).

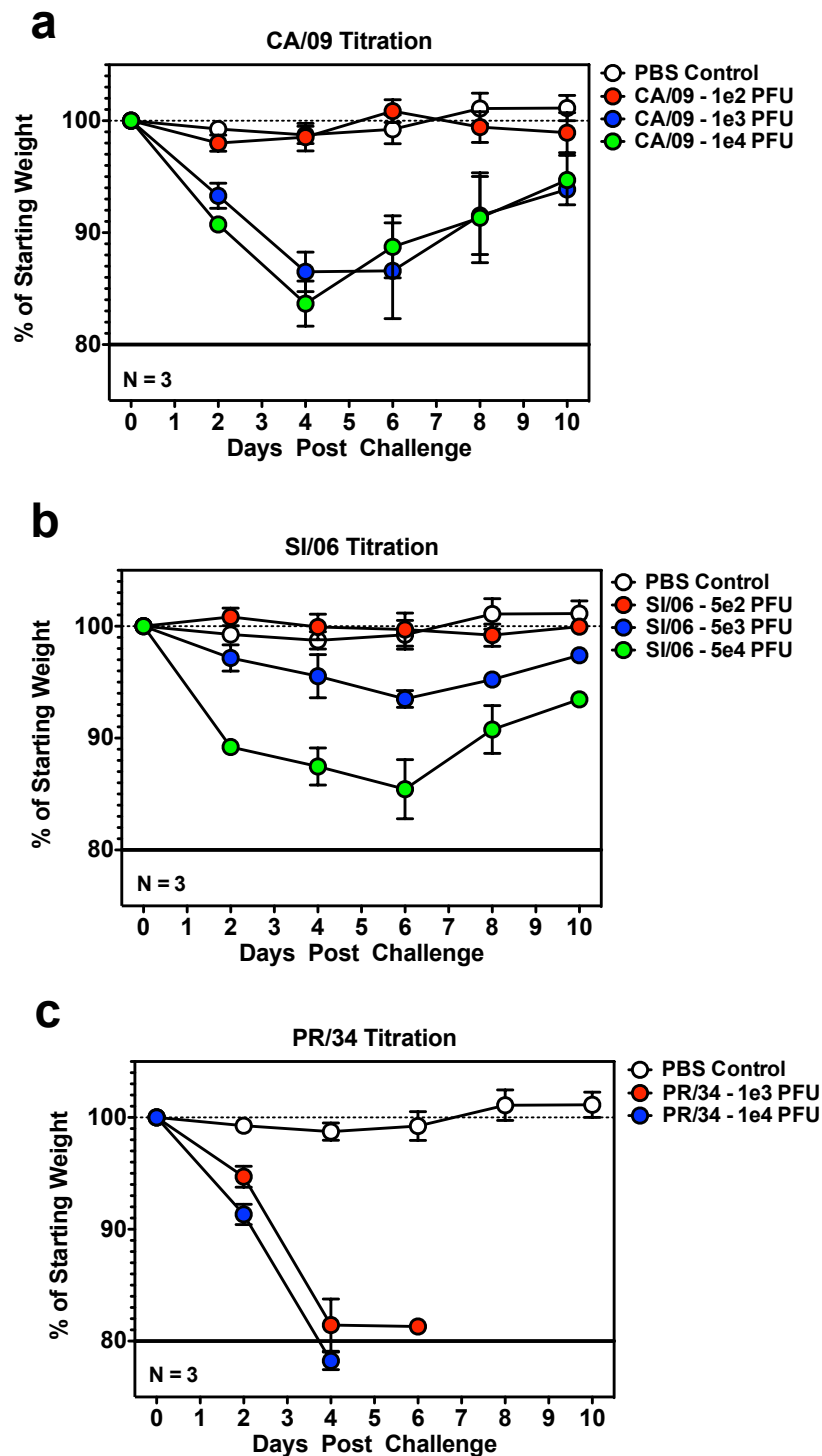


Figure S3 – Titration of Influenza Challenge Strains In Vivo

a, Weight loss resulting from administration of PBS, 100 PFU, 1000 PFU, or 10,000 PFU of CA/09 influenza to BALB/c mice (n=3). b, Weight loss resulting from administration of PBS, 500 PFU, 5000 PFU, or 50,000 PFU of SI/06 influenza to BALB/c mice (n=3). c, Weight loss resulting from administration of PBS, 1000 PFU, or 10,000 PFU of PR/34 influenza to BALB/c mice (n=3).

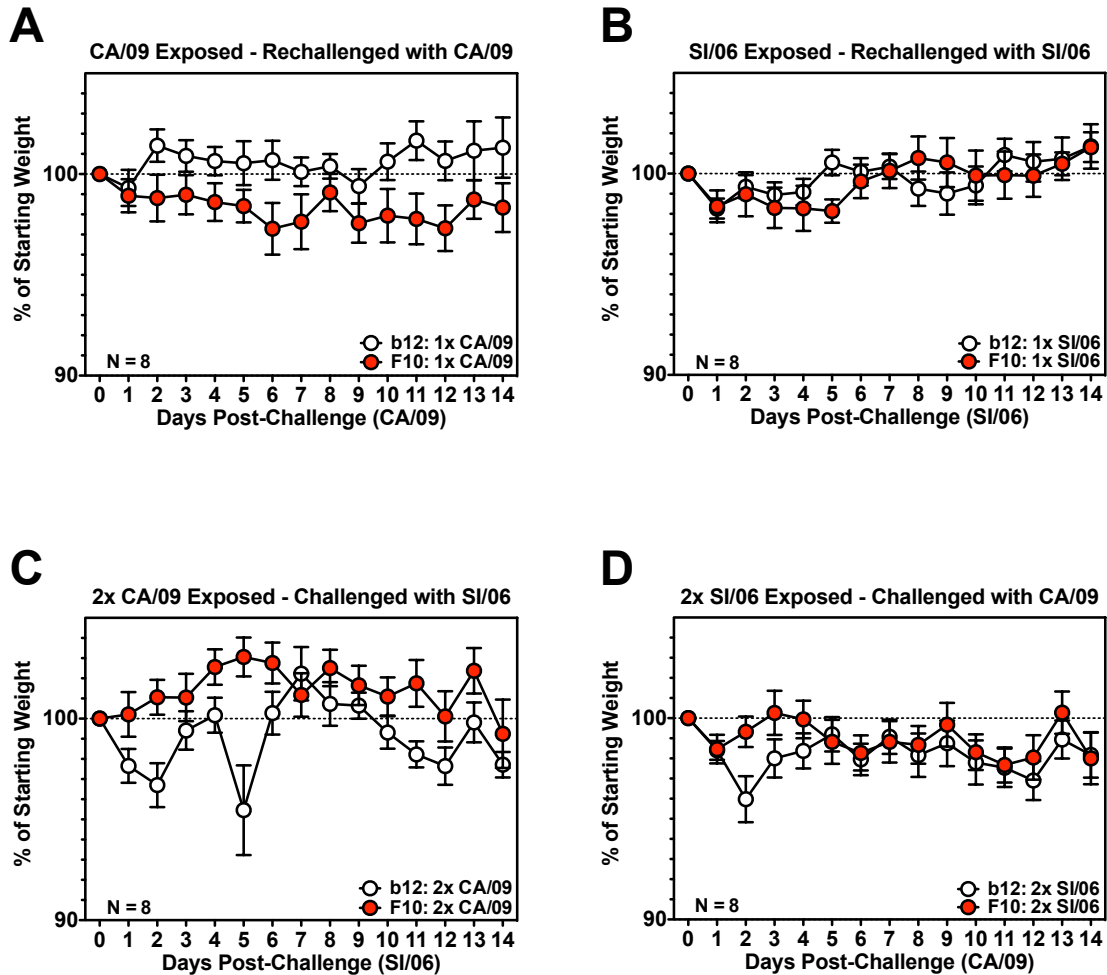


Figure S4 – Sensitivity of Previously Exposed Mice to Heterologous Influenza Challenge

a, Weight loss in BALB/c mice previously exposed to CA/09 resulting from administration of 10,000 PFU of CA/09 influenza (n=8). **b**, Weight loss in BALB/c mice previously exposed to SI/06 resulting from administration of 50,000 PFU of SI/06 influenza (n=8). **c**, Weight loss in BALB/c mice twice exposed to CA/09, resulting from administration of 50,000 PFU of SI/06 influenza (n=8). **d**, Weight loss in BALB/c mice twice exposed to SI/06, resulting from administration of 10,000 PFU of CA/09 influenza (n=8).

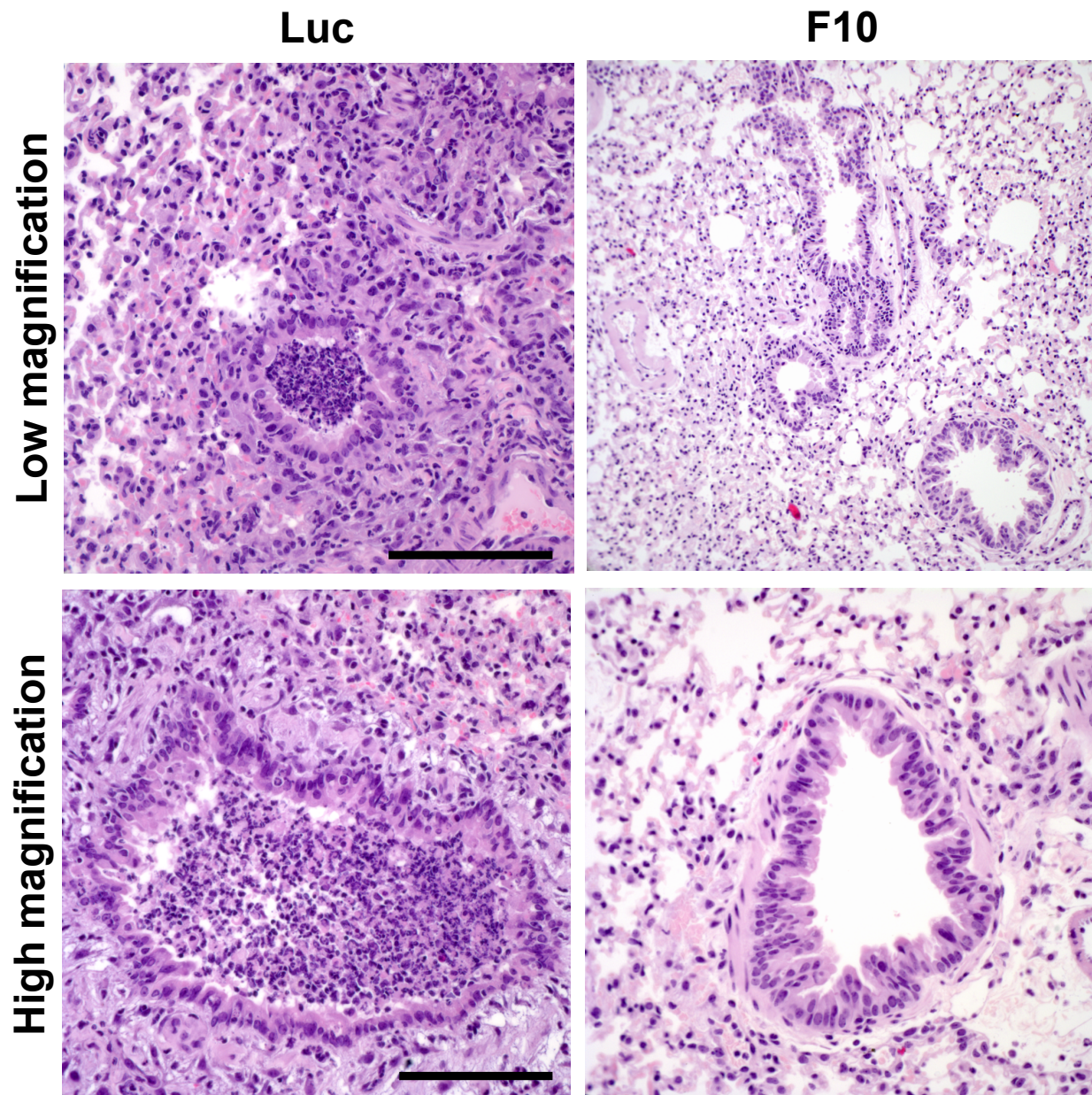


Figure S5 – VIP prevents inflammation following influenza challenge in young NSG mice.

Hematoxylin and eosin (H&E) staining of sections taken from paraffin-embedded lung tissues from young NSG mice challenged with 1000 PFU of PR8 H1N1 Influenza. Animals expressing either luciferase (left) or F10 (right) antibody. Extensive necrosis within the lumen of the bronchiole was observed in unprotected mice. Low magnification (top) scale bar = 200uM, High magnification (bottom) scale bar = 100uM

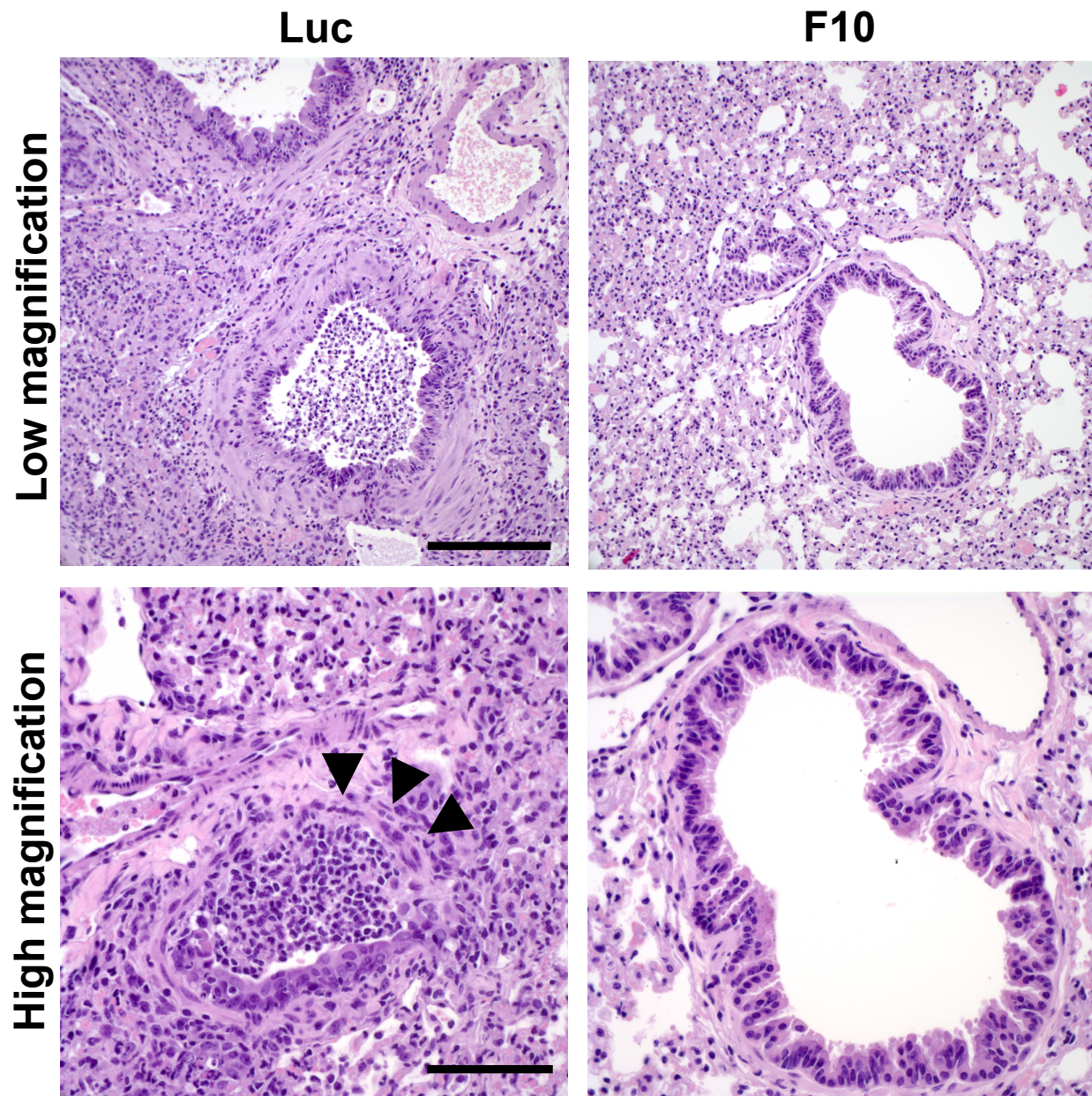


Figure S6 – VIP prevents inflammation following influenza challenge in old NSG mice. Hematoxylin and eosin (H&E) staining of sections taken from paraffin-embedded lung tissues from old NSG mice challenged with 1000 PFU of PR8 H1N1 Influenza. Animals expressing either luciferase (left) or F10 (right) antibody. Arrowheads show obliteration of the epithelial cells in addition to the luminal infiltrate. Low magnification (top) scale bar = 200uM, High magnification (bottom) scale bar = 100uM

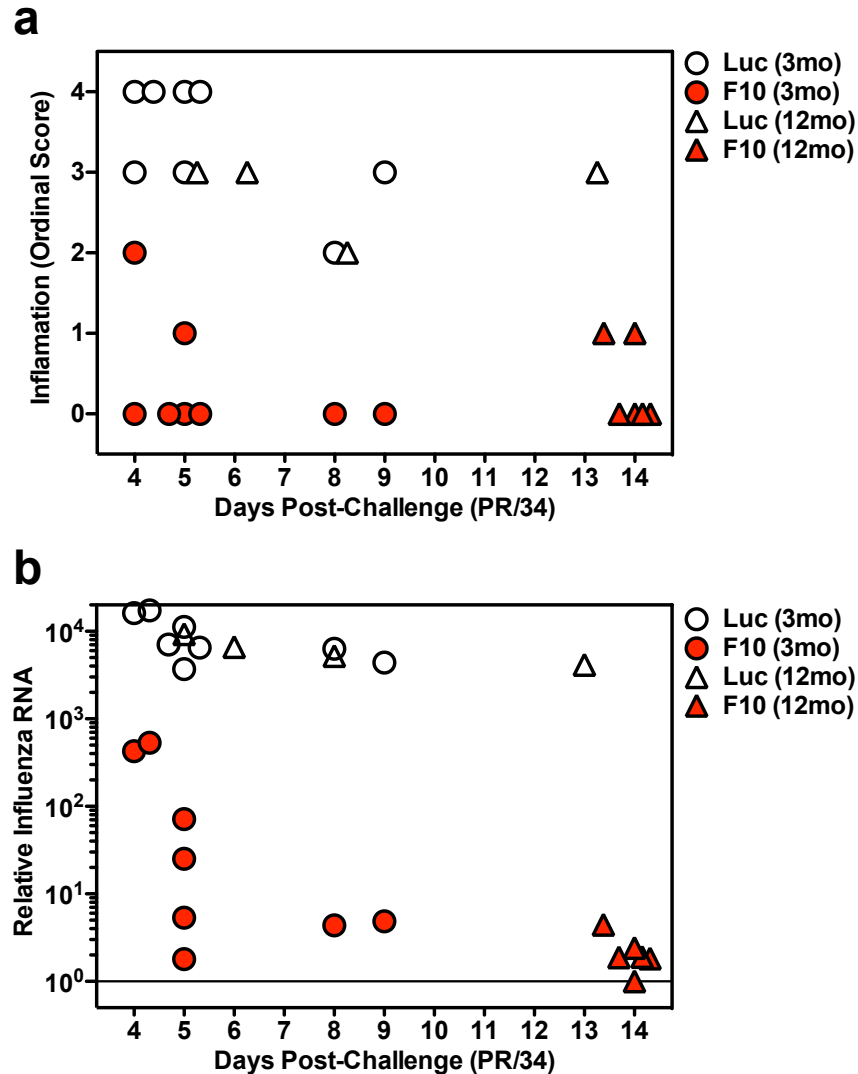


Figure S7 – Inflammation and Relative Influenza RNA in Lung of Old and Young NSG Challenged Mice
a, Ordinal score of inflammation in lungs of young (circles) or old (triangles) NSG mice given VIP expressing luciferase (white) or F10 antibody (red) as quantified by a pathologist. Scores were assigned from 0 (representing no inflammation) to a maximum of 4 (representing extensive inflammation and/or fibrosis). **b**, Quantitation of relative influenza RNA copies in lungs of young (circles) or old (triangles) NSG mice given VIP expressing luciferase (white) or F10 antibody (red). Samples were normalized to the endogenously expressed mouse ribosomal protein L32 gene.

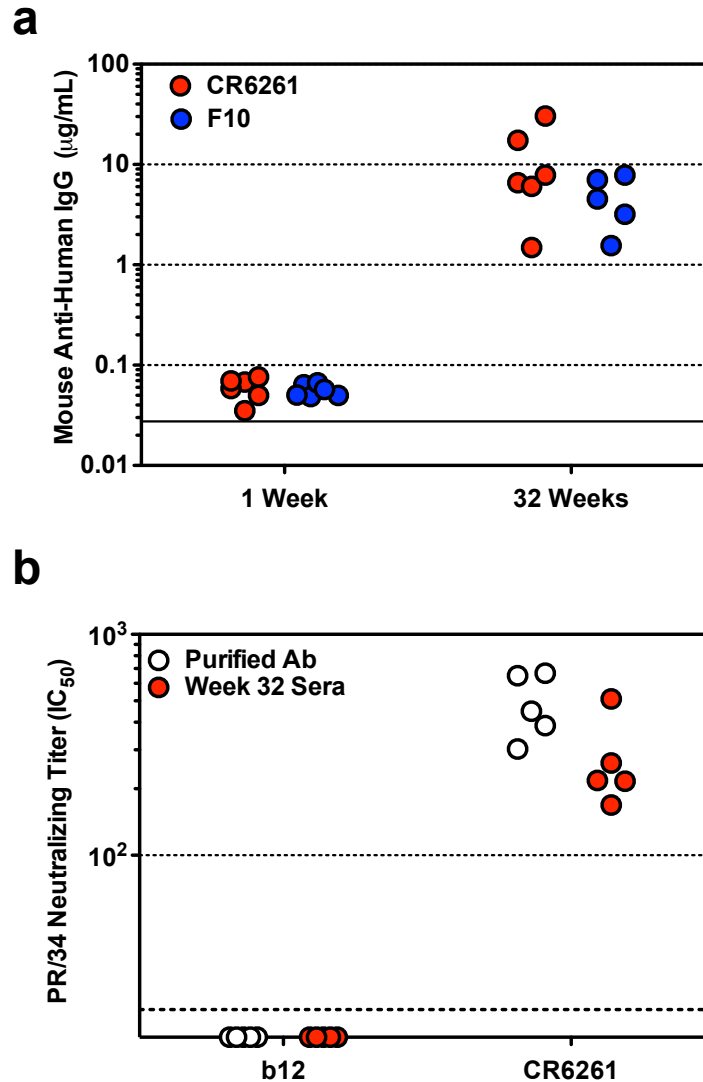


Figure S8 – Anti-Transgene Immune Response and Activity of In Vivo-Expressed Antibody

a, ELISA quantitation of mouse IgG recognizing human CR6261 or human F10 in sera taken from individual BALB/c mice either 1 week or 32 weeks following AAV administration. **b**, Activity of b12 or CR6261 antibody produced in individual BALB/c mice, 32 weeks after AAV administration as compared to purified proteins diluted in naïve mouse sera to an equivalent concentration. We observed neutralizing activity which was similar between these two samples, suggesting that endogenous antibodies which developed against CR6261 did not significantly interfere with neutralization activity.

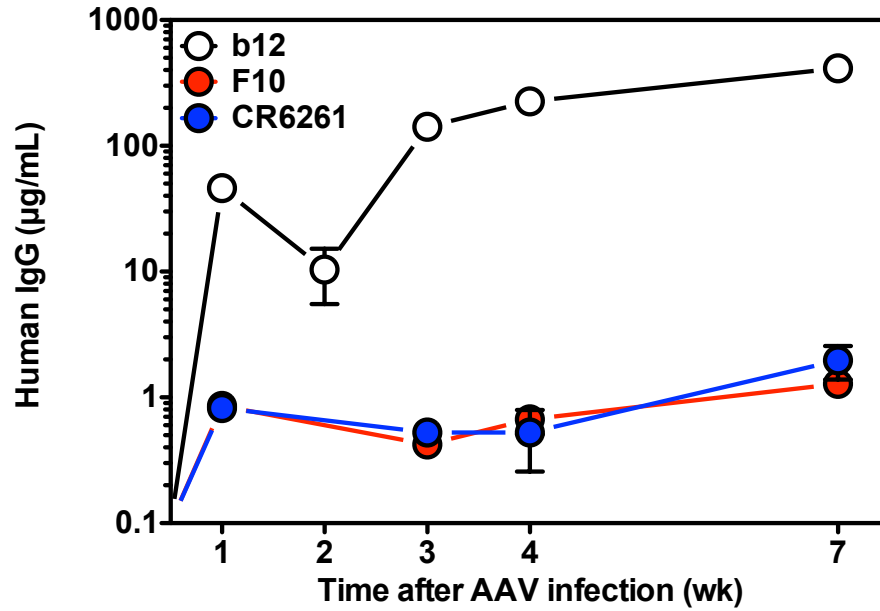
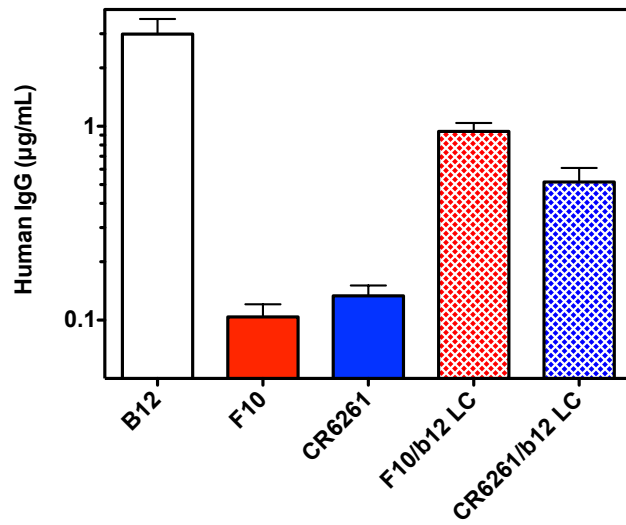


Figure S9 – Expression of antibodies in mice prior to optimization.

Quantitation of human IgG in serum by ELISA following intramuscular injection of 1×10^{11} GC of vector expressing b12, F10, or CR6261 antibodies in BALB/c mice (plot shows mean and standard error, n=8).

A

Abbreviation	Description of Substitution
LO1	5' b12 (6aa)
LO2	5' b12 (22aa)
LO3	3' b12 (6aa)
LO4	3' 4E10 (6aa)
LO13	5' b12 (6aa) + 3' b12 (6aa)
LO14	5' b12 (6aa) + 3' 4E10 (6aa)
LO23	5' b12 (22aa) + 3' b12 (6aa)
LO24	5' b12 (22aa) + 3' 4E10 (6aa)

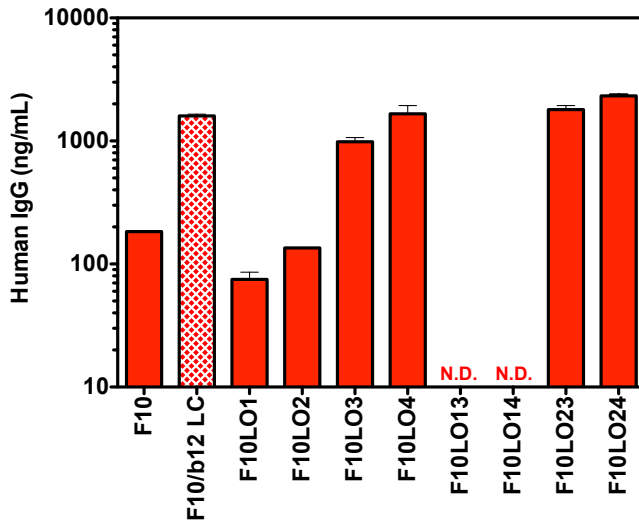
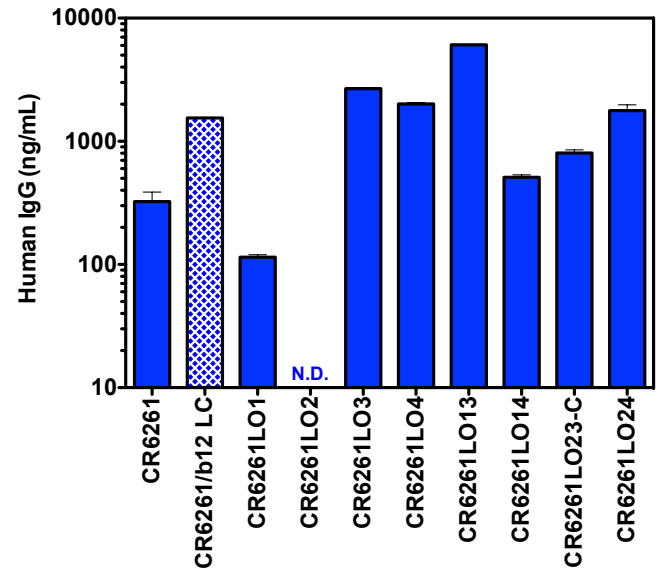
B**C**

Figure S10 – Optimization of antibody light chains.

Quantitation of human IgG in supernatant by ELISA following transfection of 293T cells with antibody expression constructs. **a**, Comparison of b12 antibody with F10 and CR6261 WT sequences as compared to chimeric constructs consisting of F10 or CR6261 heavy chain with b12 light chain. **b**, Comparison of F10 antibody light chain variants consisting of F10 VL sequences fused to b12 and/or 4E10 antibody light chain sequences. **c**, Comparison of CR6261 antibody light chain variants consisting of CR6261 VL sequences fused to b12 and/or 4E10 antibody light chain sequences. N.D. = Not Detected

A

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Thr Ser Ser
CAG GTT CAG CTT GTC CAA TCG GGT GCG GAG GTC AAG AAG CCG GGC TCA TCA GTC AAA GTA TCT TGC ACT TCT TCT

Glu Val Thr Phe Ser Ser Phe Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Leu Gly Gly
GAA GTT ACG TTC AGC TCT TTC GCT ATA TCG TGG GTG AGA CAA GCA CCG GGC CAG GGA TTA GAG TGG TTG GGA GGC

Ile Ser Pro Met Phe Gly Thr Pro Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Gln Ser
ATT TCA CCT ATG TTT GGC ACT CCT AAT TAC GCA CAG AAA TTT CAA GGC AGG GTT ACT ATC ACA GCC GAC CAA TCC

Thr Arg Thr Ala Tyr Met Asp Leu Arg Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Pro
ACA CGA ACT GCA TAT ATG GAC TTG CGA AGT TTG AGG TCT GAA GAC ACT GCG GTA TAT TAC TGC GCC CGA AGT CCT

Ser Tyr Ile Cys Ser Gly Gly Thr Cys Val Phe Asp His Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
TCA TAC ATT TGT TCG GGA GGT ACT TGC GTG TTT GAC CAT TGG GGT CAG GGA ACT TTA GTT ACT GTG AGC TCA GCC

B

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Phe Ser Cys Thr Gly
GAG ATC GTT CTC ACG CAG TCT CCA GGC ACC CTG TCT CTG TCT CCA GGG GAA AGA GCC ACC TTC TCC TGC ACA GGC

Asn Ser Asn Asn Val Gly Asn Gln Gly Ala Ala Trp Leu Gln Gln His Gln Gly His Pro Pro Lys Leu Leu Ser
AAT AGT AAT AAT GTG GGG AAT CAG GGA GCA GCA TGG TTA CAG CAA CAT CAA GGA CAT CCC CCG AAA CTG CTT TCC

Tyr Arg Asn Asn Asp Arg Pro Ser Gly Ile Ser Glu Arg Phe Ser Ala Ser Arg Ser Gly Asn Thr Ala Ser Leu
TAT CGT AAC AAC GAC CGC CCG TCG GGA ATC TCG GAA CGT TTT TCT TCT GCG TCA CGT TCA GGC AAC ACT GCC TCG CTG

Thr Ile Thr Gly Leu Gln Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Thr Trp Asp Ser Ser Leu Ser Ala Val
ACT ATA ACT GGC TTA CAG CCT GAA GAC GAA GCA GAC TAC TAT TGT TCA ACT TGG GAT TCT TCT CTG TCT GCG GTT

Val Phe Gly Gly Gly Thr Lys Val Glu Val Lys Arg Thr
GTG TTT GGC GGC GGC ACA AAA GTG GAG GTG AAG CGG ACC

Figure S11 – Sequence of final optimized variable regions of F10LO24.

a, Sequence of heavy chain variable region used in the optimized IgG expression vector for the F10 antibody. **b**, Sequence of the optimized hybrid light chain variable region used in the optimized IgG expression vector for the F10 antibody. Amino acids in red text represent sequence original to the F10 antibody. Amino acids in black text are derived from b12 antibody light chain sequence and those written in green text are derived from 4E10 antibody light chain sequence.

A

Glu Val Gln Leu Val Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser
 GAA GTC CAA CTC GTG GAA TCC GGA GCC GAG GTT AAA AAG CCC GGC TCC AGC GTG AAA GTC TCT TGC AAA GCA AGC

 Gly Gly Pro Phe Arg Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Pro Glu Trp Met Gly Gly
 GGT GGG CCG TTT CGA TCA TAC GCG ATA TCA TGG GTC CGG CAG GCA CCT GGA CAG GGG CCC GAA TGG ATG GGA GGC

 Ile Ile Pro Ile Phe Gly Thr Thr Lys Tyr Ala Pro Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Asp Phe
 ATA ATC CCA ATC TTT GGG ACC ACG AAG TAT GCC CCG AAA TTC CAG GGT AGG GTC ACT ATC ACC GCT GAC GAC TTC

 Ala Gly Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Lys His Met
 GCC GGA ACC GTC TAT ATG GAA CTT TCC AGC CTG CGC AGC GAG GAC ACC GCA ATG TAT TAC TGC GCA AAA CAC ATG

 Gly Tyr Gln Val Arg Glu Thr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser Ala
 GGA TAC CAA GTG AGA GAG ACC ATG GAT GTG TGG GGC AAG GGT ACT ACT GTG ACC GTG AGC TCA GCC

B

Glu Ile Val Leu Thr Gln Ser Pro Ser Val Ser Ala Ala Pro Gly Gln Lys Val Thr Ile Ser Cys Ser Gly Ser
 GAG ATC GTT CTC ACG CAG TCT CCA TCC GTA TCT GCA GCC CCG GGA CAG AAA GTG ACA ATT TCA TGC TCT GGG TCC

 Ser Ser Asn Ile Gly Asn Asp Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr
 TCA TCC AAC ATC GGA AAT GAC TAC GTT AGT TGG TAC CAA CAG CTC CCT GGG ACC GCG CCA AAA CTG CTG ATT TAT

 Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly
 GAC AAC AAC AAG AGG CCA AGT GGC ATC CCC GAC AGG TTT TCC GGC TCA AAG TCC GGC ACT TCA GCA ACA CTG GGC

 Ile Thr Gly Leu Gln Thr Gly Asp Glu Ala Asn Tyr Tyr Cys Ala Thr Trp Asp Arg Arg Pro Thr Ala Tyr Val
 ATC ACG GGT CTG CAA ACA GGC GAT GAG GCA AAC TAC TAT TGC GCA ACC TGG GAC AGG AGA CCG ACC GCT TAT GTT

 Val Phe Gly Gly Gly Thr Lys Leu Glu Arg Lys Arg Thr
 GTG TTC GGC GGC GGC ACT AAG CTG GAG AGG AAA CGT ACG

Figure S12 – Sequence of optimized variable regions of CR6261LO13.

a, Sequence of heavy chain variable region used in the optimized IgG expression vector for the CR6261 antibody. **b**, Sequence of the light chain variable region used in the optimized IgG expression vector for the CR6261 antibody. Amino acids in blue text represent sequence original to the CR6261 antibody. Amino acids in black text are derived from b12 antibody light chain sequence.

Supplementary Results:

To express b12, F10 and CR6261 antibodies in mice, we synthesized and cloned the variable regions of these antibodies into the VIP expression vector as previously described¹⁴. After producing the necessary vector stock, we administered 1×10^{11} GC of each vector to the gastrocnemius muscle of BALB/c animals. Serum samples were obtained weekly and human IgG was quantified by ELISA (fig. S9). We observed significant expression of the b12 antibody above 100 $\mu\text{g/mL}$ that was consistent with levels we had previously observed¹⁴. In contrast, F10 and CR6261 were present at much lower levels. We set out to improve F10 and CR626 expression by optimizing vector sequences.

Because the majority of the sequences were identical between the AAV encoding b12, F10 and CR6261, we hypothesized that some aspect of the heavy or light chain variable region sequences was responsible for poor expression of F10 and CR6261. While sequence alignment demonstrated that the b12, F10 and CR6261 heavy chain sequences as cloned were aligned in a similar manner, the light chain sequences differed substantially in the junctions between the signal sequences, the variable regions and the constant regions (not shown). To determine whether the F10 and CR6261 light chains were responsible for poor expression of these antibodies, we created chimeric antibody constructs in which the light chains of each antibody were replaced with the light chain of b12. When transfected into 293T cells, the chimeric antibodies were expressed in higher amounts than intact F10 and CR6261 antibodies, suggesting that the F10 and CR6261 light chains were contributing to poor expression (fig. S10a). To improve expression of the endogenous light chains of the F10 and CR6261 antibodies, we created a set of modified light chain variable regions containing 5' and 3' junctional sequences

derived from the light chains of either b12 or 4E10 antibodies, which we previously found to express well in vivo¹⁴. When transfected into 293T cells, F10 antibodies incorporating the F10LO24 light chain (which contains sequences from b12 as well as 4E10) was expressed at levels up to 12-fold higher than unmodified F10 antibodies (fig. S10b). Likewise CR6261 antibodies incorporating the CR6261LO13 light chain (which contains sequences from b12) was expressed at levels up to 20-fold higher than unmodified CR6261 antibodies (fig. S10c). We tested these modified antibodies using in vitro neutralization assays and confirmed that antibodies containing modified light chains maintained their ability to neutralize two strains of influenza (not shown). We carried out all subsequent experiments with the F10LO24 and CR6261LO13 modified sequences and refer to them as F10 and CR6261, respectively. The complete variable region sequences used, including the chimeric light chain variable regions, are provided in figures S11 and S12.