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 1x10^{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 IC$_{50}$ 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 IC$_{50}$ 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 $1 \times 10^{11}$ GC of vector expressing b12, F10, or CR6261 antibodies in BALB/c mice (plot shows mean and standard error, n=8).
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
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.
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 described\textsuperscript{14}. After producing the necessary vector stock, we administered 1x10\textsuperscript{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 µg/mL that was consistent with levels we had previously observed\textsuperscript{14}. 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 was 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 vivo\(^{14}\). 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.