Research Square

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# Lentiviral and AAV-mediated Expression of Palivizumab Offer protection against Respiratory Syncytial Virus infection

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# Abstract

Respiratory syncytial virus (RSV) infection is a common cause of hospitalisation in infants and the elderly. Palivizumab prophylaxis is the only approved treatment modality but is costly and only offered to select vulnerable populations. Here, we investigated gene delivery approaches via recombinant adeno-associated virus (rAAV2/8) and simian immunodeficiency virus (rSIV.F/HN) vectors to achieve sustained *in vivo* production of palivizumab in a murine model. Delivery of palivizumab-expressing vectors 28 days prior to RSV challenge resulted in complete protection from RSV-induced weight loss. This approach offers prophylaxis against RSV infection, allowing for wider use and reduction in treatment costs in vulnerable populations.


# Introduction

Respiratory syncytial virus (RSV) is the most common cause of acute lower respiratory tract infections in infants and young children. In 2015, global RSV infection in children younger than 5 years was estimated at 33.1 million, of which about 3.2 million required hospitalisation with 59,600 in-hospital deaths (1). Infection with RSV in children is also associated with long-term complications, such as recurrent wheeze and potentially asthma (2), rendering the virus a severe burden in the paediatric population. Other high-risk populations include the elderly, those with chronic lung or heart disease and the immunosuppressed (3).

To date, no licensed RSV vaccine is available, and the only approved intervention is passive immunisation with the monoclonal antibody (mAb) palivizumab (Synagis®). To provide protection throughout the RSV season, multiple doses of palivizumab at monthly intervals are required to achieve sufficient mAb bioavailability. Due to the relatively high cost, estimated between £2500-£4200 per person per season (4), its use has been limited to select, vulnerable infant populations. Adopting gene therapy for passive immunisation via mAb gene transfer, also known as vectored-immunoprophylaxis (5, 6), could provide sustained *in vivo* mAb production, offering protection throughout the entire RSV season, making the treatment more widely accessible.

Here, we investigated two viral vector platforms to direct *in vivo* expression of palivizumab via either the intranasal or intramuscular route. The first is a recombinant lentiviral vector based on simian immunodeficiency virus (SIV) pseudotyped with Sendai virus envelope proteins F and HN (known as rSIV.F/HN) (7). A single intranasal rSIV.F/HN dose directs sustained expression of α1-antitrypsin in both the lung lumen and systemic circulation of mice for at least 19 months (8), highlighting this platform as a candidate for long-term expression of palivizumab. Recombinant adeno-associated virus serotype 8 (rAAV2/8) vector delivered intramuscularly also directs long-term expression of antibodies and other transgenes in both mice and man (9). We hypothesised that these vectors would direct robust, long-term expression of palivizumab to protect animals against an RSV challenge.

# Methods

## Lentiviral vector production and titration

rSIV.F/HN lentiviral vector particles were produced essentially as described previously (8), via a five-plasmid transient transfection method using HEK293T cells grown in suspension. Briefly, the single ORF cDNA of palivizumab was configured using publicly available sequence (10) as described previously (11), and CpG-depleted, human codon-optimized, and synthesized using the GeneArt Gene Synthesis service (Thermo Fisher Scientific). The palivizumab, or a CpG-free Gaussia Luciferase (soGLux), cDNA was inserted via unique NheI and PsPOMI sites into a recombinant simian immunodeficiency virus (SIV) genome plasmid backbone under the transcriptional control of the hCEF promoter (18). Vectors were purified using anion exchange chromatography and tangential flow filtration and formulated into either FreeStyle293 media or TSSM buffer (tromethamine 20 mM, NaCl 100 mM, sucrose 10 mg/mL, and mannitol 10 mg/mL). The functional titre in Transducing Units per mL (TU/mL) of lentiviral vectors was determined based on the genomic integration of WPRE DNA sequence after transduction of HEK293F cells *in vitro*.

## rAAV vector production and titration

Recombinant rAAV was produced as described (19). Briefly, the CpG-free palivizumab and Gaussia Luciferase (soGLux) cDNAs were inserted, via unique NheI and PsPOMI site, into a recombinant adeno-associated virus (AAV) genome plasmid backbone with AAV2 ITRs under the transcriptional control of the CASI promoter (6) . HEK293T cells were transfected with the plasmids pAdDeltaF6, pAAVRep2/Cap8, and prAAV2ITR with transgene using polyethylenimine (PEI; Polysciences Inc.). After 72 hours, cells were resuspended in lysis buffer (1M Tris(hydroxymethyl)aminomethane, 150 mM NaCl) and EDTA-free protease inhibitor cocktail and underwent four freeze-thaw cycles. Cell lysates were incubated (37ºC for 30 min) with Benzonase (50 U/mL final concentration) and clarified via centrifugation, and purified using iodixanol gradient fractionation and diafiltration into D-PBS using Amicon Ultra-15 100K MWCO filters. The number of Genome Copies (GC/mL) was determined by qPCR.

## Animal studies

All procedures involving laboratory mice were carried out in accordance with UK Home Office approved project and personal licenses under the terms of the Animals (Scientific Procedures) Act 1986 (ASPA 1986), were approved by the University of Oxford or Imperial College Animal Welfare Ethical Review Body as appropriate, and are reported in compliance with the ARRIVE guidelines (https://arriveguidelines.org). Female BALB/c mice, 6-8 weeks old at the initiation of studies, were used. Animals were arbitrarily assigned to study groups using an open-label randomised block approach. Overall, 259 animals were used (Figure 2: n=12/group, 3 groups, n=11/group, 3 groups, n=22/group 1 group, n=16/group 1 group; Figure 3 & 4: n=16/group 7 groups; Figure 5; n=5/group, 8 groups). Group sizes reduced during the study as effect sizes became more predictable. Animals were euthanised at the end of each study by cervical dislocation or intraperitoneal injection of 100 µL pentobarbital.

## Administration of vector and virus to mice

Anaesthesia was induced using inhalation of 4-4.5% isoflurane (Abbott, Maidenhead, UK) and maintained with 2.5-3.5% isofluorane. For delivery of lentiviral vector and for RSV infection, a total volume of 100 µL was administered by nasal sniffing as previously described (20) Adeno-associated viral vector was administered by injection into the gastrocnemius or quadriceps muscle using a 500 µL insulin syringe with 29G needle in a total volume of 40 µL.

### Collection of samples from mice

Blood was collected from the tail vein of mice, stored overnight at 4$^{o}$C and centrifuged to isolate serum. For collection of broncho-alveolar lavage fluid (BALF), mice were euthanised and dissected to expose the trachea. A small tear was made in the trachea and a 0.75 mm cannula used to infuse the lung with 1 mL BALF solution (PBS, 50 µM EDTA; 1% BSA was included except for leukocyte quantification) and then fluid collected by gentle aspiration. Flushing the lung was performed three times using the same (for studies with monoclonal antibodies), or fresh (for studies with GLux) BALF solution. For quantification of protein expression, the collected BALF was centrifuged to sediment cells, and the supernatant used for ELISA or luciferase assay as described below.

### Quantification of protein expression

Palivizumab levels in the cell culture media, serum and BALF were measured using Human IgG ELISA kit (Bethyl, Cambridge, UK) according to the manufacturer's instructions. GLux activity in the serum was measured using BioLux *Gaussia* Luciferase Assay Kit (NEB, Ipswich, USA) according to the manufacturer's instructions. Expression levels in ELF were corrected for the dilution using lavage fluid collection urea assay as described previously (8).

### Total and differential leukocyte quantification

The BALF was centrifuged at 3,500 rcf and the cell pellet resuspended in Ammonium-Chloride-Potassium buffer (150 mM NH4Cl, 10 mM KHCO3, 0.1 mM EDTA) to lyse red blood cells. After 2 minutes, DMEM (Sigma-Aldrich) was added, the samples were centrifuged at 3,500 rcf and the pellet was resuspended in 500 µl of DMEM. For total leukocyte quantification, an aliquot of the cell suspension was mixed with 0.1% trypan blue and cells manually counted using a haemocytometer. For differential leukocyte quantification, 100 µL of the cell suspension was transferred onto a glass slide (Tharmac, Waldsoms, Germany) using Cytospin III Cytocentrifuge (Thermo Fisher Scientific) and allowed to air-dry. The slides were fixed and stained using Reastain Quick-Diff Kit (Reagena, Toivala, Finland) and visualised using a light microscope. At least 300 cells per slide were counted, differentiating between macrophages, lymphocytes and neutrophils based on their morphology.

### Statistics

Group sizes were initially projected from previous data (13), using IgG expression and RSV-induced weight loss as the primary endpoints and were reduced throughout the study as estimates of effect size

became more robust. G*Power software v3.1.9.6 (21) was used to project group sizes and establish achieved power. Statistical analysis was performed using Prism software v8.4.2 for Mac (GraphPad Software). The non-parametric Kruskal-Wallis test with Dunn's multiple comparisons post-hoc test was used to compare experimental groups with the indicated negative control group throughout. For weight loss studies, area under the curve values for the 7 days post RSV challenge were first calculated for each animal prior to Kruskal-Wallis evaluation. Numerical values in text are presented as mean±standard deviation. Symbols and error bars in figures represent the mean and standard deviation unless stated otherwise. A calculated p-value of $p<0.05$ was deemed a significant difference and indicated on figures where appropriate. In figures, the symbols ns, *, **, *** and **** represent $p>0.05$, $<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively.

# Results

In vivo **palivizumab production using intramuscular rAAV2/8 and intranasal rSIV.F/HN delivery.**

A single-open reading frame (ORF) of palivizumab (10), was constructed in which the light and heavy chain sequences were fused via a protease cleavage site that combined a 2A self-processing peptide and a furin target sequence (11). To improve palivizumab expression, the transgene was codon-optimised for *Homo sapiens* and depleted of CpG dinucleotides. Vector genome plasmids for two palivizumab-expressing viral vectors, rAAV2 with muscle-active CASI promoter and rSIV with lung-active hCEF promoter, were generated. Similar vector genome plasmids where the palivizumab ORF was replaced with the ORF for *Gaussia* luciferase (GLux) were also created. Transfection of HEK293T cells with the palivizumab vector genome plasmids resulted in robust palivizumab protein expression (Fig. 1A). The rAAV2 vector genomes were used to produce rAAV2/8 serotype vector particles, and the rSIV vector genomes were used to produce rSIV.F/HN pseudotyped vector particles. Transduction of HEK293T cells with the palivizumab vector particles also resulted in robust palivizumab protein expression (Fig. 1B).

As a simple surrogate for palivizumab expression, we first assessed the activity of the GLux vector particles to direct expression of GLux protein after *in vivo* delivery. Mice were administered three ascending, single doses of rAAV2/8 GLux vector via intramuscular (IM) delivery, or rSIV.F/HN GLux vector via intranasal (IN) delivery. Mid and high doses ($10^{10}$ or $10^{11}$ Genome Copies (GC)) of rAAV2/8 GLux resulted in abundant serum GLux activity, detectable from as early as day 7, which was sustained for at least 12 months at 63,521 ± 33,399 and 1,931,382 ± 570,309 RLU/µL, respectively ($p = 0.0009$ and $p < 0.0001$) (Fig. 2A). The low rAAV2/8 GLux dose ($10^9$ GC) also tended to direct detectable GLux activity (18,911 ± 24,857 RLU/µL), but this level was not significantly different from animals naïve to treatment (419 ± 142 RLU/µL; $p = 0.0745$). Serum levels achieved at 12 months with the mid and high doses (1e6 or 1e7 Transducing Units (TU)) of the rSIV.F/HN GLux vector (1,829 ± 839 or 3,043 ± 2,908 RLU/µL) were significantly higher than naïve animals ($p = 0.0004$ and $p = 0.0031$ respectively) (Fig. 2B), but markedly lower than those achieved with rAAV GLux vectors. As with rAAV expressing GLux, serum levels with the low dose of rSIV.F/HN GLux (1e6 TU) achieved 514 ± 75 RLU/µ, but were not significantly different from naïve animals ($p > 0.9999$).

We were also interested in the expression levels achieved in bonchoalveolar lavage fluid (BALF), a sample representing the fluid space in which RSV infections occur. While levels of serum GLux achieved after rAAV2/8 IM vector delivery (Fig. 2A) eclipsed the levels achieved after rSIV.F/HN IN vector delivery (Fig. 2B), the profile of GLux activity in BALF was reversed, such that expression levels achieved with rSIV.F/HN vectors ranged up to 71e6 ± 38e6 RLU/µL (naïve 3e3 ± 0.8e3 RLU/µL) (Fig. 2D) that were markedly higher than the peak levels achieved with rAAV2/8 GLux (4e3 ± 1e3 RLU/µL).

These results established that robust, long-lasting, *in vivo* expression could be achieved for a simple reporter protein using our rAAV2/8 and rSIV.F/HN vector systems and we next evaluated *in vivo* palivizumab expression. Mice were administered three ascending, single doses of rAAV2/8 palivizumab vector via IM delivery, or rSIV.F/HN vector via IN delivery. Mid and high doses ($10^{10}$ or $10^{11}$ Genome Copies) of rAAV2/8 resulted in marked serum palivizumab, detectable as early as day 14, which were sustained for at least 6 months at 15.0 ± 3.7 and 89.3 ± 5.0 µg/mL, respectively ($p = 0.0356$ and $p < 0.0001$) (Fig. 3A). The low rAAV2/8 dose ($10^9$ GC) was undetectable after day 28 (Fig. 3B). As anticipated, only marginal levels of palivizumab were detected in the serum at any dose of rSIV.F/HN at 6 months (Fig. 3C) since IN delivery of this vector typically results in higher transgene levels in the lungs compared with systemic circulation (8).

Significant palivizumab expression was observed in the bronchoalveolar lavage fluid (BALF) of mice administered mid and high ($10^7$ and $10^8$) doses of rSIV.F/HN, with palivizumab levels reaching 9.1 ± 1.3 and 22.6 ± 7.2 ng/mL) at 1 month (Fig. 4B; $p = 0.0034$ and $p = 0.0006$). This expression was sustained for the duration of the study with comparable palivizumab levels of 14.5 ± 3.8 and 17.6 ± 8.9 ng/mL at 6 months (Fig. 4D; $p = 0.0006$ and $p = 0.0228$). In mice administered $10^{10}$ and $10^{11}$ GC of rAAV2/8, palivizumab levels in the BALF reached 20.8 ± 4.8 and 230 ± 28.9 ng/mL at 1 month (Fig. 4A; $p = 0.0296$ and $p < 0.0001$), sustained for the duration of the experiment, averaging 55 ± 11.4 and 313.6 ± 40.1 ng/mL respectively, in the BALF at 6 months (Fig. 4C; $p = 0.0423$ and $p < 0.0001$). Using an approximate conversion technique (8), the epithelial lining fluid (ELF) levels of the palivizumab at 6 months post-delivery was estimated as 9 µg/mL for rAAV2/8 and 0.5 µg/mL for rSIV.F/HN at the highest doses. The results indicate these vector platforms can provide robust palivizumab expression *in vivo*.

### Protection from weight loss in a mouse model of RSV infection

In our mouse model of RSV infection, animals challenged with $7x10^5$ focus-forming units (FFU) of RSV delivered IN experienced pronounced weight loss over a ~ 7 day period. In the majority of animals, weight loss was reversible and was recovered over the next ~ 7 days (Fig. 5A). Animals losing > 20% of their initial body weight for more than 2 days are killed at a humane endpoint, to limit the severity of their experience.

We hypothesised that *in vivo* palivizumab expression by rAAV8 and rSIV.F/HN vectors would protect mice against RSV-induced weight loss. Mice were therefore administered a single dose of vector expressing palivizumab, either rAAV2/8 via the IM route ($10^{10}$ or $10^{11}$ GC) or rSIV.F/HN ($10^8$ or the maximal feasible

dose $2x10^8$ TTU) via the IN route. Two groups received matched high doses of vectors expressing control GLux reporter gene. Twenty-eight days post-dosing, mice were challenged with RSV as described above. Mice dosed with either rAAV2/8 or rSIV.F/HN expressing GLux were not protected from weight loss ($p = 0.5986$ and $p > 0.9999$; Fig. 5A and 5B respectively). In contrast, complete protection from RSV-induced weight loss was achieved in mice administered the highest dose of rAAV2/8 ($p = 0.0032$; Fig. 5A), as well as both doses of rSIV.F/HN, expressing palivizumab ($p = 0.0159$ and $p = 0.0159$ respectively; Fig. 5B). Interestingly, mice dosed with $10^{10}$ GC of rAAV2/8 expressing palivizumab were not protected from weight loss ($p > 0.9999$; Fig. 5A).

We also evaluated immune cell recruitment into the airways following RSV infection in these animals. The relative proportion of macrophages, lymphocytes and neutrophils as well as the absolute numbers of lymphocytes in the BALF were determined at the end of the study (Fig. 5C & 5D). As expected, lymphocytes were increased by exposure to RSV ($p = 0.0183$) (12). There was a dose-dependent trend for palivizumab expression to reduce RSV-associated lymphocytes, but this result did not reach significance due to lack of statistical power. Overall, these results indicate that vector-mediated expression of palivizumab in mice can help protect against weight loss due to RSV infection.

# Discussion

In this study we sought to evaluate the ability of two different gene transfer vectors systems to direct long-term, prophylactic expression of a mAb to inhibit lung infection by RSV. We chose to evaluate the rAAV2/8 and rSIV.F/HN vector systems as they have alternate preferred delivery routes, and they have also been used by us and others to establish *in vivo* expression of other therapeutic proteins (6, 7, 13).

Here, we first confirmed that both rAAV2/8, via IM injection, and rSIV.F/HN, via IN delivery, could direct long-term expression of GLux, a common reporter gene. While dose-dependent serum GLux expression was achieved with both vector systems, the peak levels achieved with rAAV2/8 were approximately 100-fold higher than achieved with rSIV.F/HN. After rAAV2/8 vector delivery, serum Glux activity rose to a plateau after approximately 30 days and was sustained essentially unchanged for at least 1 year. Sustained GLux expression was also seen after rSIV.F/HN vector delivery although the kinetics were somewhat different; with GLux levels falling from a peak after approximately 30 days post-delivery to a plateau after approximately 60 days which was then sustained essentially unchanged for at least 1 year. GLux activity was also measured in the BALF, a sample representing the fluid lining the lung epithelium that is the site of RSV infection. At 1 year after vector delivery GLux activity was approximately 10,000-fold higher with the rSIV.F/HN vector system, suggesting that local expression within the lung allowed GLux protein accumulation.

Subsequently, we established that both vector systems could direct expression of palivizumab, a prototype anti-RSV mAb, after both *in vitro* and *in vivo* transduction. As with the GLux reporter protein, palivizumab serum levels were highest after rAAV2/8 delivery. In contrast to the results with the GLux reporter protein, BALF palivizumab levels were approximately 10-fold higher with the rAAV2/8 than the

rSIV.F/HN vector system. Together, these data suggest that GLux is not necessarily a representative reporter protein when developing therapeutic proteins with IgG properties. Importantly, active transport processes exist to aid selective redistribution of immune complexes (14) an advantage that the GLux reporter may lack. Nevertheless, both vectors demonstrated significant palivizumab expression in BALF for at least 6 months post-delivery - the duration of the study.

Finally, both vector systems directed sufficient palivizumab expression such that at the higher end of the dose ranges used, both vectors offered complete protection from the weight loss observed in our RSV challenge model. This suggests that clinical benefit could be offered by translating this novel treatment modality to at-risk human populations. Interestingly, in these challenge studies, treatment with rAAV2/8 GLux vector appeared to increase the weight loss experienced after RSV infection, while rSIV.F/HN GLux vector treatment appeared to have no effect. This might be indicative of some perturbation in physiology induced by either the rAAV2/8 vector system itself, or to high serum levels of the GLux reporter protein. Further studies are perhaps warranted to more fully understand this phenomenon prior to development of rAAV2/8 -based therapeutics.

The kinetics of palivizumab expression with both vector systems following a single vector administration are consistent with protection for a complete 31-week RSV season, an improvement over passive vaccination with palivizumab protein, which is typically repeated monthly. Intriguingly, transgene expression from both vectors has been observed for the life-time of experimental animals, suggesting that protection in humans could reasonably be expected to extend into subsequent RSV seasons, a distinct advantage in the context of vulnerable populations suffering repeated RSV infections (3). In the studies presented here, protection against RSV challenge was only determined at a single time-point, 4 weeks after palivizumab vector delivery; further studies are warranted to establish that the duration of RSV protection matches the longevity of palivizumab expression observed.

While the degree of protection offered by both vectors was similar, rAAV2/8 directed higher levels of palivizumab both in the serum and BALF, and offered an easily translatable IM delivery route. In contrast, the rSIV.F/HN platform requires inhalation, which might be challenging in the context of a child with a history of respiratory disorders. However, the rSIV.F/HN vector has a crucial advantage, the ability to offer repeated efficacy after at least three doses (15, 16), which has proven difficult to achieve with a single serotype of rAAV in the lung (17).

In conclusion, we demonstrate here that gene transfer, mediated by rAAV2/8 and rSIV.F/HN, can direct therapeutically relevant levels of palivizumab in the murine circulation and lung lumen, which is protective against RSV infection.

# Declarations

### Author Contributions

SH, DG and CJ designed and supervised the studies. AA, OH, FK and CJ performed the studies. AA, OH, CJ, DG and SH prepared the manuscript. All authors reviewed the manuscript.


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AA was supported by an MRC DTA studentship awarded to the University of Oxford. OH was supported by a Merdeka Scholarship, Khazanah-OxCIS. FK was supported by a PhD Fellowship from the Wellcome Trust (109058/Z/15/Z). DG and SH were supported by a Wellcome Trust Portfolio grant (110579/Z/15/Z).


### Competing Interests

SH and DG hold patents on rSIV.F/HN and related technology. All other authors declare no competing interests.

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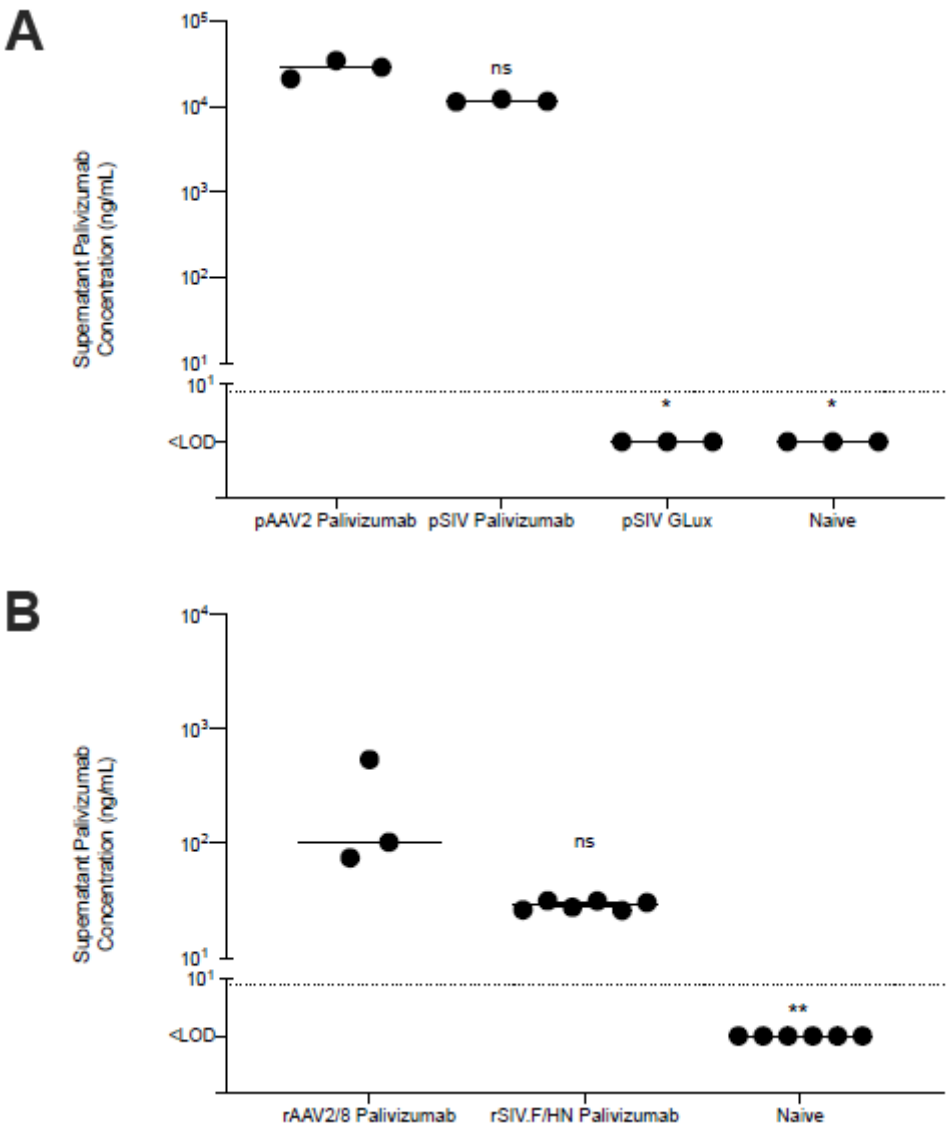
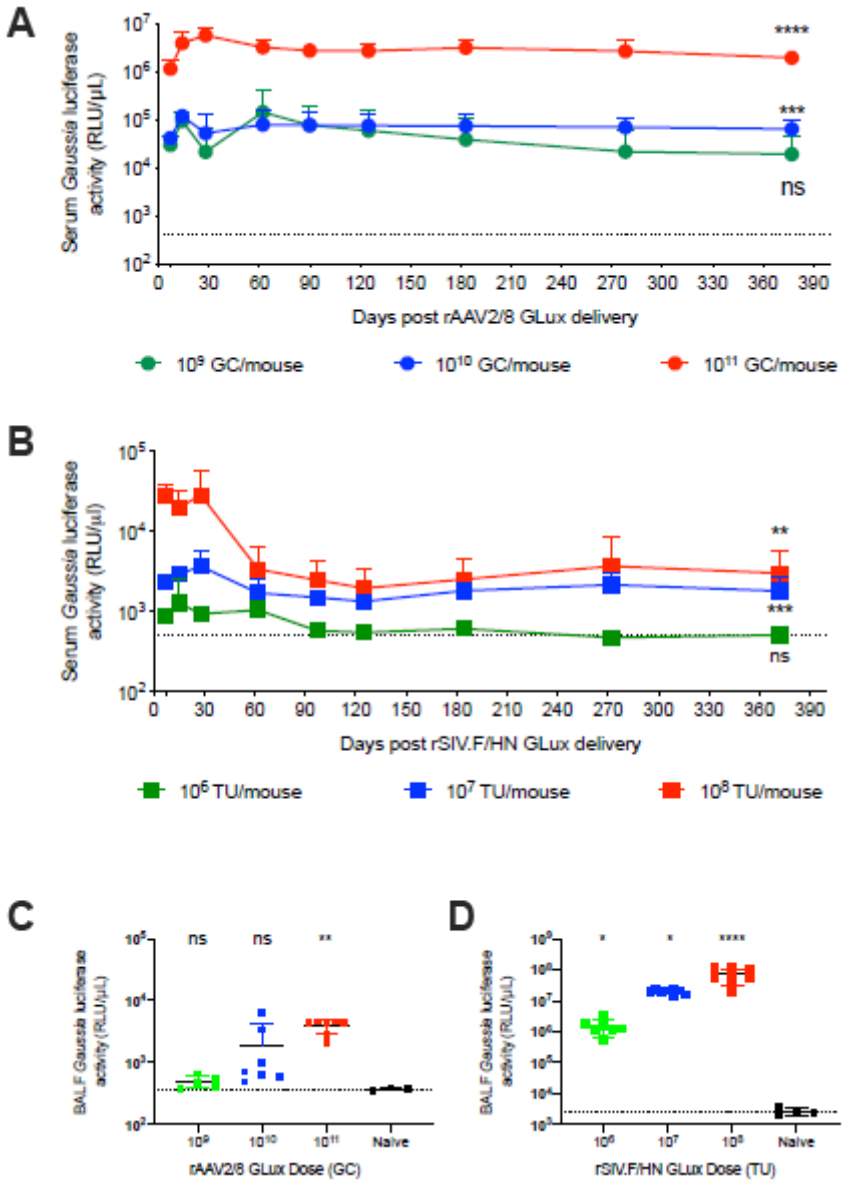
# Figures



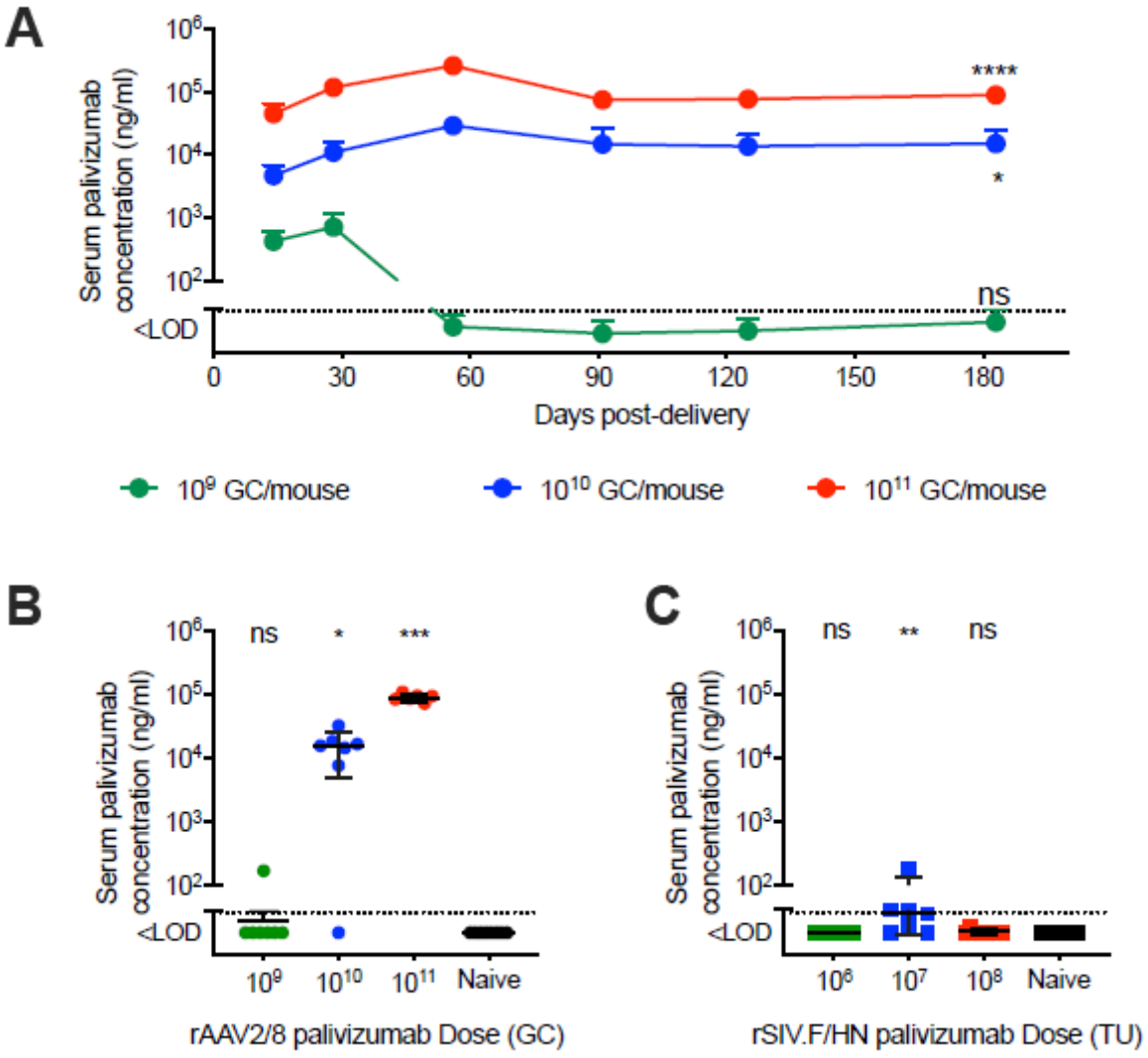

## Figure 1

In vitro production of palivizumab from rAAV2/8 and rSIV.F/HN HEK293T cells were transfected with rAAV and rSIV vector genomes expressing palivizumab or GLux (A), were transduced with rAAV and rSIV vector particles expressing palivizumab (B) or remained naïve to treatment; 48 hours post-transfection/transduction, palivizumab levels in tissue culture supernatant was measured using a Human IgG ELISA. Bars represent group median values. The dotted line represents the limit of detection (LOD). Differences between treatment and naïve control groups were evaluated using the Kruskal-Wallis test with Dunn's post hoc multiple comparison test.



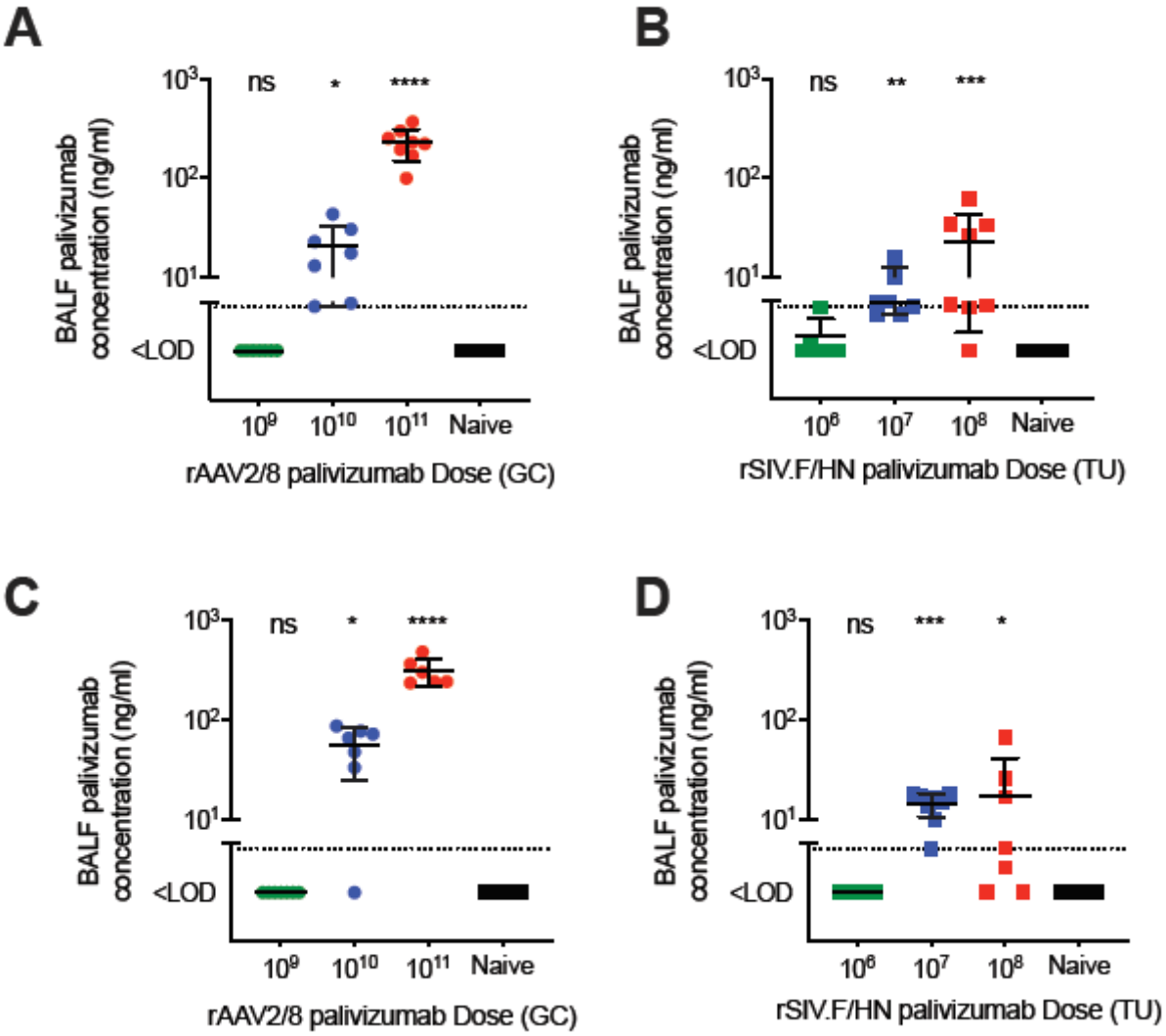

## Figure 2

In vivo production of Gaussia Luciferase from rAAV2/8 and rSIV.F/HN Female BALB/c mice (n=11-16/group) were administered 109 (green circles), 1010 (blue circles) or 1011 (red circles) Genome Copies (GC) of rAAV2/8 CASI Glux via intramuscular (IM) injection (A), 106 (green squares), 107 (blue squares) or 108 (red squares) Transducing Units (TU) of rSIV.F/HN hCEF palivizumab via intranasal (IN) instillation (B) or were naïve to treatment. GLux activity was determined in serum was obtained via tail vein bleeding at the indicated time-points. GLux activity in BALF samples from rAAV2/8 (C) and rSIV.F/HN (D) treatment groups was determined at the end of the study (approximately 12 months post vector delivery). Symbols represent group mean, bars represent SEM. The dotted line represents the mean naïve value. Differences between treatment groups and naïve animals were evaluated using the Kruskal-Wallis test with Dunn's post hoc multiple comparison test.



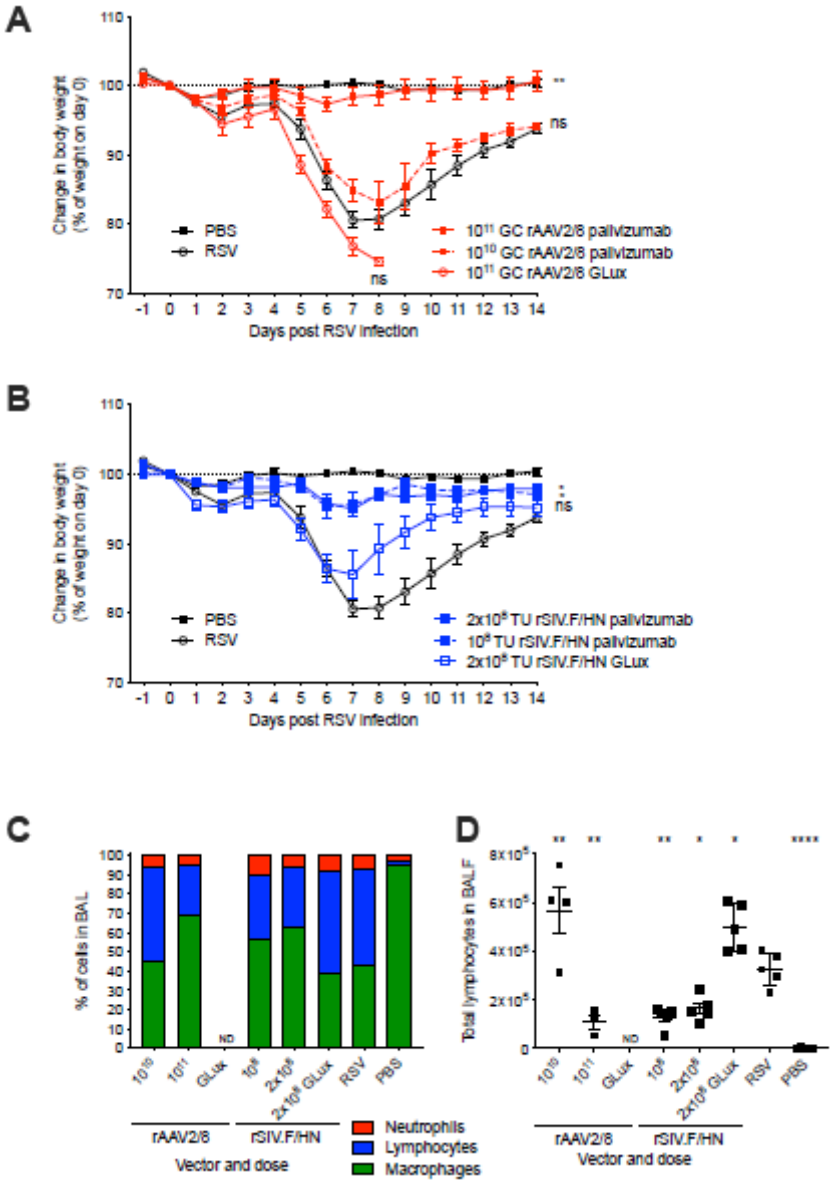

## Figure 3

In vivo production of palivizumab from rAAV2/8 and rSIV.F/HN: serum Female BALB/c mice (n=16/group) were administered 109 (green circles), 1010 (blue circles) or 1011 (red circles) Genome Copies (GC) of rAAV2/8 CASI Glux via intramuscular (IM) injection, 106 (green squares), 107 (blue squares) or 108 (red squares) Transducing Units (TU) of rSIV.F/HN hCEF palivizumab via intranasal (IN) instillation or were naïve to treatment. Serum was obtained via tail vein bleeding at the indicated time-points and palivizumab levels determined using a human IgG ELISA. A time-course of Palivizumab concentration in the serum throughout the duration of the experiment is shown for rAAV2/8 only (A), as serum antibody levels for mice treated with rSIV.F/HN were not significantly different from naive at most time-points (not shown). The individual values for serum palivizumab levels at 6 months post-delivery are shown for both rAAV2/8 (B) and rSIV.F/HN (C) treatment groups. The dotted line represents the mean naïve value. Differences between treatment groups and naïve animals were evaluated using the Kruskal-Wallis test with Dunn's post hoc multiple comparison test.




## Figure 4

In vivo production of palivizumab from rAAV2/8 and rSIV.F/HN: BALF Female BALB/c mice (n=16/group) were administered 109 (green circles), 1010 (blue circles) or 1011 (red circles) Genome Copies (GC) of rAAV2/8 CASI Glux via intramuscular (IM) injection (A and C), 106 (green squares), 107 (blue squares) or 108 (red squares) Transducing Units (TU) of rSIV.F/HN hCEF palivizumab via intranasal (IN) instillation (B and D) or were naïve to treatment. Serum was obtained via tail vein bleeding at the indicated time-points. Eight mice from each group were culled at 1 month (A and B) and 6 months (C and D) post-delivery to obtain BALF. Palivizumab levels in the BALF was measured using a Human IgG ELISA. The dotted line represents the LOD. Differences between treatment groups and naïve animals were evaluated using the Kruskal-Wallis test with Dunn's post hoc multiple comparison test.




## Figure 5

Protection against RSV infection with vector-mediated palivizumab gene transfer Female BALB/c mice (n=5/group) were administered rAAV2/8 vector expressing palivizumab (1010 or 1011 GC), or GLux (1011 GC) via IM injection (A), or were administered rSIV.F/HN expressing palivizumab (108 or 2x108 TTU) or GLux (2x108 TTU) via IN instillation (B). Twenty-eight days later, the mice received 7 x 105 FFU of RSV or PBS via IN instillation and their weight was recorded for 14 days. The percentage of animal weight prior to RSV or PBS challenge was determined daily for up to 14 days post-challenge. For clarity, symbols and error bars represent mean±standard error of the mean. Missing weight data after day 7 for the rAAV2/8 GLux dosed mice is due to the application of a pre-determined humane endpoint in this treatment group. The area under the weight loss curve up to day 7 was calculated for each animal and the differences between treatment groups and RSV challenged animals were evaluated using the Kruskal-Wallis test with Dunn's post hoc multiple comparison test. Differential leukocyte quantification was carried out on BALF cells on H&E stained cytospin slides recovered 14 days post RSV infection, with the relative proportions of

each cell type (C) and absolute numbers of lymphocytes (D) shown. Missing leucocyte (ND: C & D) data for rAAV2/8 GLux dosed mice is due to the application of a pre-determined humane endpoint in this treatment group.