



Comparative Efficiency of HA and VSV-G Pseudotyped Lentiviral Vectors for Cystic Fibrosis Airway Gene Therapy

Chantelle Carpentieri¹⁻³, Nigel Farrow¹⁻³, Patricia Cmielewski¹⁻³, Chantelle McIntyre¹⁻³,
Alexandra McCarron¹⁻³, Nathan Rout-Pitt¹⁻³, David Parsons¹⁻³ & Martin Donnelley¹⁻³

1. Robinson Research Institute, University of Adelaide, South Australia

2. Adelaide Medical School, University of Adelaide, South Australia

3. Department of Respiratory & Sleep Medicine, Women's & Children's Hospital, South Australia

Introduction:

- Lentiviral (LV) vectors are a promising option for treating cystic fibrosis (CF) airway disease by delivering a functional copy of the CFTR gene into airway epithelial cells.
- The choice of vector pseudotype is important to ensure that the correct cells and locations are effectively targeted. The VSV-G and HA envelope proteins target airway receptors on the basolateral and apical surfaces, respectively.
- Conditioning the airway surface with the compound lysophosphatidylcholine (LPC) prior to LV vector delivery may increase transduction efficiency by facilitating access to the basolateral surface.

Aims:

- Use LV vectors carrying either the LacZ or Luciferase (Luc) reporter genes, and pseudotyped with VSV-G or HA, to determine:
 - Which pseudotype is more effective.
 - The effect of LPC airway conditioning on the transduction levels.

Methods:

- Normal C57Bl/6 female mice were anaesthetised and intubated.
- The airways of mice were conditioned with 10 µl of PBS (control, n=7-12) or LPC (n=7-12), followed one hour later by two 15 µl aliquots of VSV-G (n=7-12) or HA (n=7-12) pseudotyped LV vector containing either the Luc or LacZ reporter genes.
- One week post-instillation, LacZ treated mice were humanely killed via CO₂ asphyxiation and their lungs inflation fixed. LacZ transduction was assessed by *en face* after histochemical (X-gal) analysis, while cell types were determined by histological methods.
- Bioluminescence imaging (BLI; Xenogen, IVIS) was performed at 1 week, and then monthly for up to nine months after LV vector instillation to assess Luc gene expression levels in the lung airways over time.

Results:

- En face* LacZ staining assessment of mouse lungs indicated that airway conditioning with LPC resulted in stronger initial transduction levels than PBS, independent of which pseudotype was used (Fig 1).
- In the VSV-G treated mice, LacZ transduction was typically more pronounced in the trachea and upper bronchioles, regardless of whether the conditioning was PBS or LPC (Fig 1a & b).
- In the HA treated mice, there was a difference in the transduction efficiency observed between PBS and LPC, with higher levels of transduction in the trachea and bronchioles with LPC conditioning (Fig 1d) compared to PBS (Fig 1c).
- Preliminary histological analysis of the upper and middle conducting airways indicated that ciliated cells were the most predominant LacZ expressing cell type regardless of pseudotype, however LPC showed to benefit HA.

- For both pseudotypes, rare numbers of Type I and II alveolar cells were transduced regardless of the conditioning treatment.

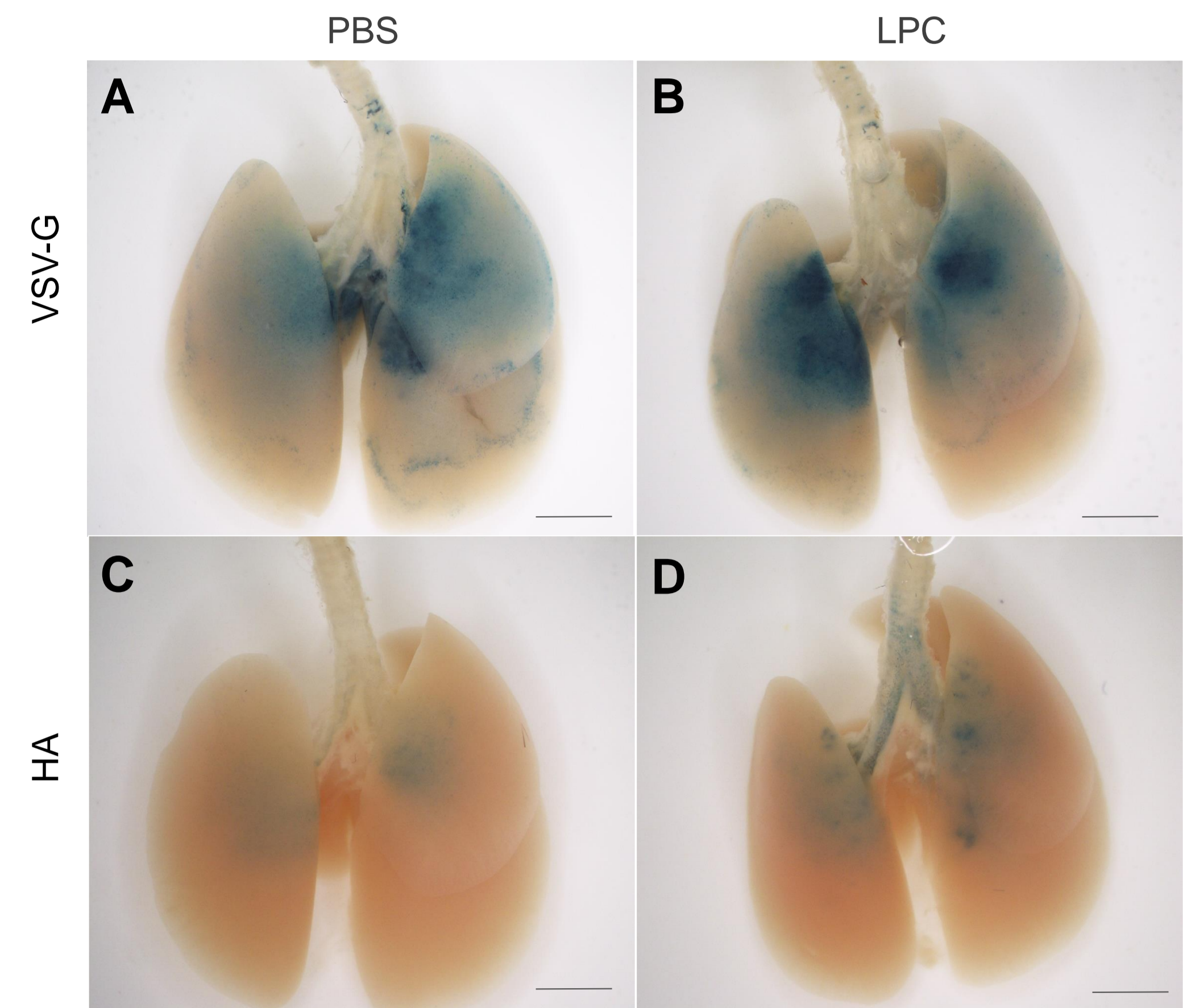


Figure 1: Examples of LV-LacZ transduction of mice lungs at 1 week following LV vector instillation with (A) PBS VSV-G, (B) LPC VSV-G, (C) PBS HA and (D) LPC HA. (x7.5 magnification). Scale bar 2mm.

- Lung luminescence was detected by BLI at all imaging time points in the PBS and LPC conditioned mice that received either pseudotyped LV-Luc vector (Fig 2).
- At one week, the VSV-G pseudotype group had significantly higher expression levels than the HA group, regardless of whether LPC conditioning was used.
- Over the longer term (1-9 months) there was no quantifiable difference in luminescence between groups pre-treated for either PBS or LPC, the VSV-G or HA pseudotypes.

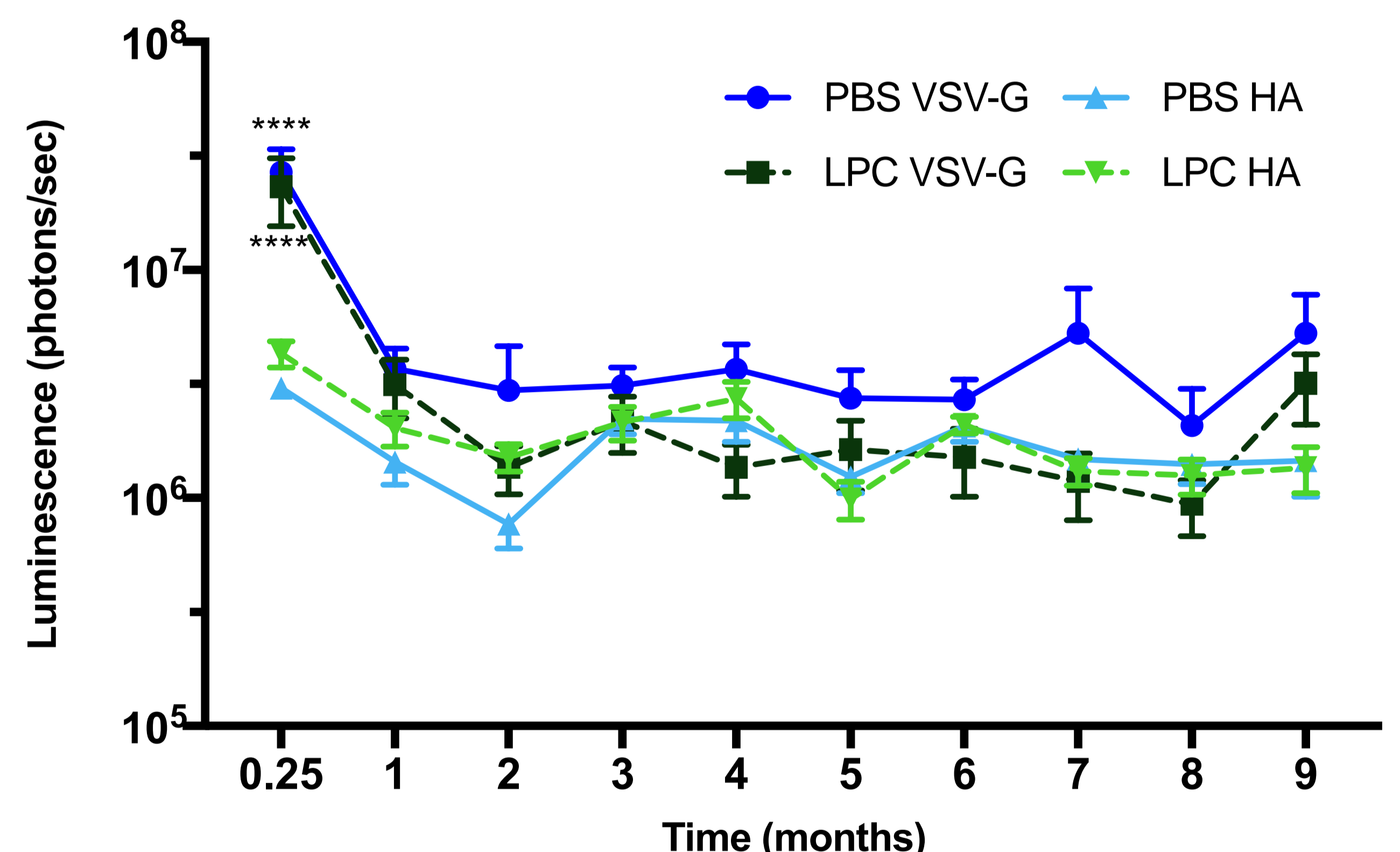


Figure 2: Luc gene expression (flux bioluminescence) in the lung airways of mice conditioned with either PBS or LPC followed by VSV-G or HA LV vector instillation. (**** p < 0.0001, two-way ANOVA, Tukey's at 1 week, n=7-8 per group).

Conclusion:

- At one week the Luc and LacZ data both suggest that the VSV-G pseudotype is more effective at transducing airway cells than the HA pseudotype.
- Both pseudotypes transduced the correct cell types in the upper conducting airways for the treatment of CF.
- The long term Luc results suggest that conditioning the airways with LPC prior to LV vector delivery does not increase the total lung transduction level with either pseudotype.
- Monthly Luc imaging will be continued (~18 months) to observe total lung gene expression levels over time.

Acknowledgements

Channel 7 Children's Research Foundation and Cure4 Cystic Fibrosis Foundation.