

Repeat Administration of Adeno-Associated Virus 5 in Murine Airways

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Introduction

Recombinant AAV (rAAV) vectors have demonstrated long-term expression of transgenes in mouse, dog and non-human primate models amongst others, with little or no apparent toxicity.

rAAV5, in particular, is a relatively efficient vector for gene transfer to the lungs of rodents. We have shown that rAAV5 can also persist for at least 12 months in mouse lung and over 7 months in mouse nose (Sumner-Jones *et al.*, submitted for publication).

These facts combine to make rAAV5 an attractive candidate for sustained delivery of transgenes to the airways, for diseases such as Cystic Fibrosis. Indeed, a rAAV5 vector carrying the gene found to be defective in CF, *CFTR*, has recently been developed (Sirninger *et al.*, 2004).

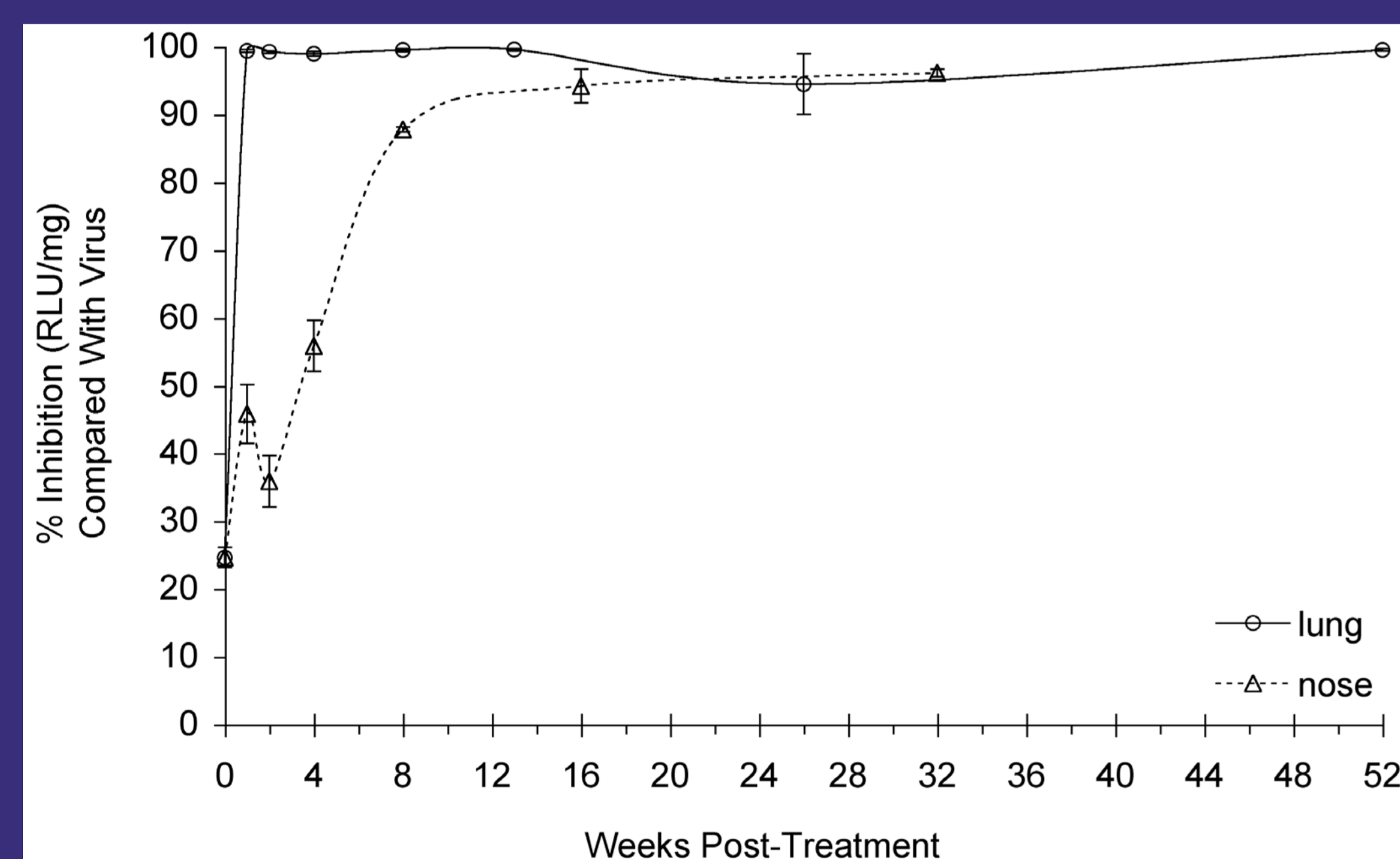
However despite its persistence, rAAV5 would most likely need to be administered more than once for the life-long treatment of chronic diseases such as CF, as there has been no evidence so far that stem cell niches have been targeted efficiently enough to maintain expression beyond the life-time of terminally differentiated cells of the airways.

We have used the mouse nose and lungs to investigate the feasibility of repeat administration of rAAV5.

Methods

- rAAV5/5 vectors containing Lux, EGFP, CAT or CTLA4lg under the control of the CMV Immediate-Early promoter/enhancer were produced by triple plasmid transfection of Human Embryonic Kidney 293T cells and purified by CsCl gradient
- Genomic titres (DNase-resistant GC) were established by TaqMan PCR.
- For *in vivo* studies 1×10^{11} GC of the appropriate virus were delivered to the lungs or nose of female C57BL6 mice (7-10 weeks old at week 0).
- Lungs, trachea and blood were harvested 4 weeks after delivery of the Lux vector. Luciferase activity was determined as previously described (Gill *et al.*, 2001).
- Pooled sera from the mice were analysed by ELISA for detection of antibodies against rAAV5/5 capsid proteins and against the proteins encoded by the different transgenes.
- Pooled sera were also analysed for the presence of neutralising antibody activity by *in vitro* transduction assays on African Green Monkey COS7 cells.

Results



Neutralising antibody activity persists in sera of mice exposed to a single dose of rAAV5/5Lux.

Mice received a single dose of rAAV5/5Lux by instillation to the lungs or perfusion to the nose and groups of 6-8 mice were sacrificed at the time-points indicated. Pooled sera were tested for anti-rAAV5/5Lux neutralising activity on COS7 cells. Data are presented as mean inhibition of transduction compared with cells transduced with virus alone (\pm s.e.m.).

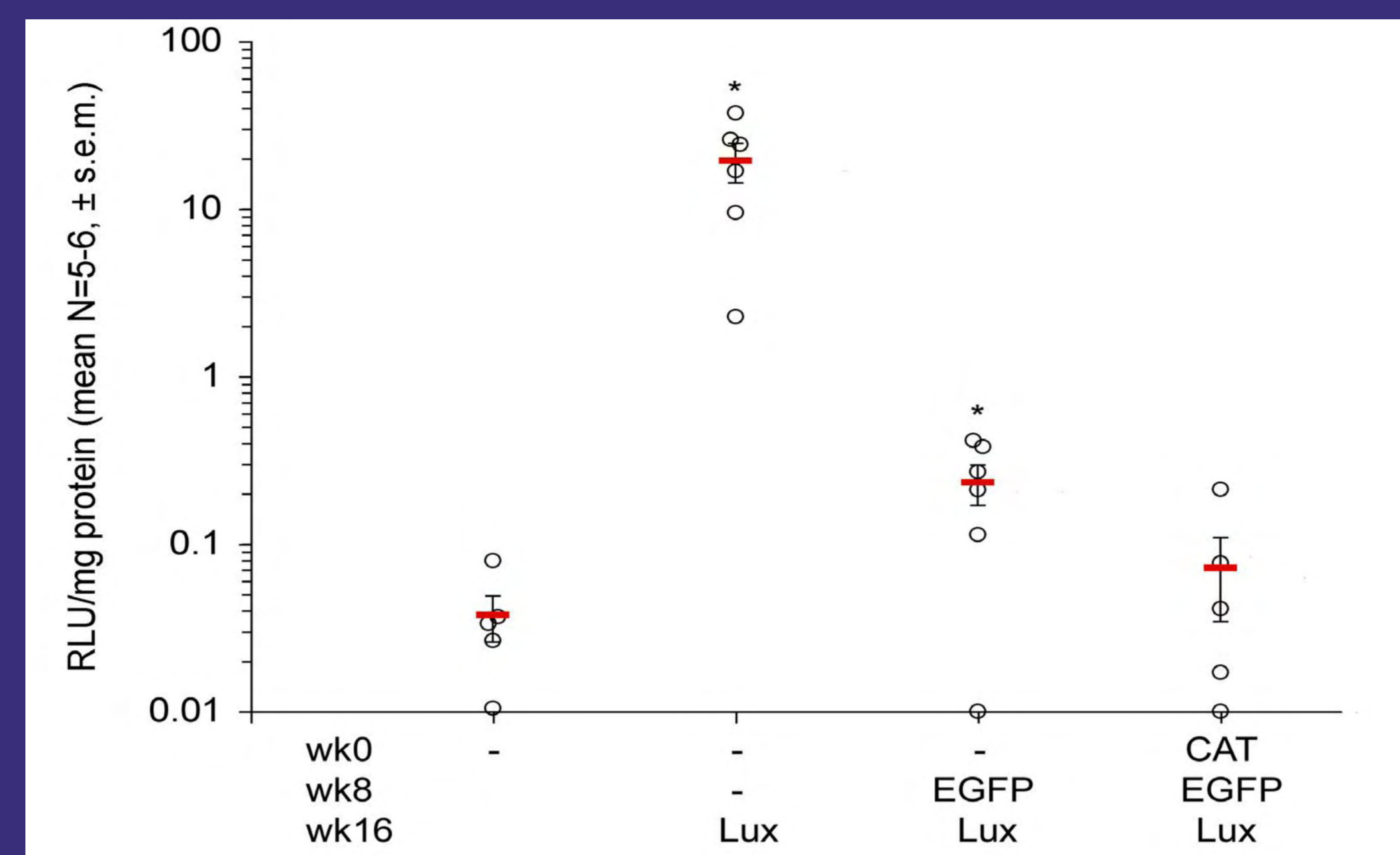
In order to investigate the consequences of this antibody response on subsequent transduction by the same rAAV5/5 serotype, we set up several repeat administration studies, in the mouse nose and lung, with short or long gaps between exposures to virus, and using viruses expressing either different transgenes to avoid compounding the issue with potential antigens in the reporter proteins, or expressing the immunosuppressor protein CTLA4lg.

References

Gill *et al.*, 2001. *Gene Therapy* 8, 539-1546
Sirninger *et al.*, 2004. *Human Gene Therapy* 15, 832-841

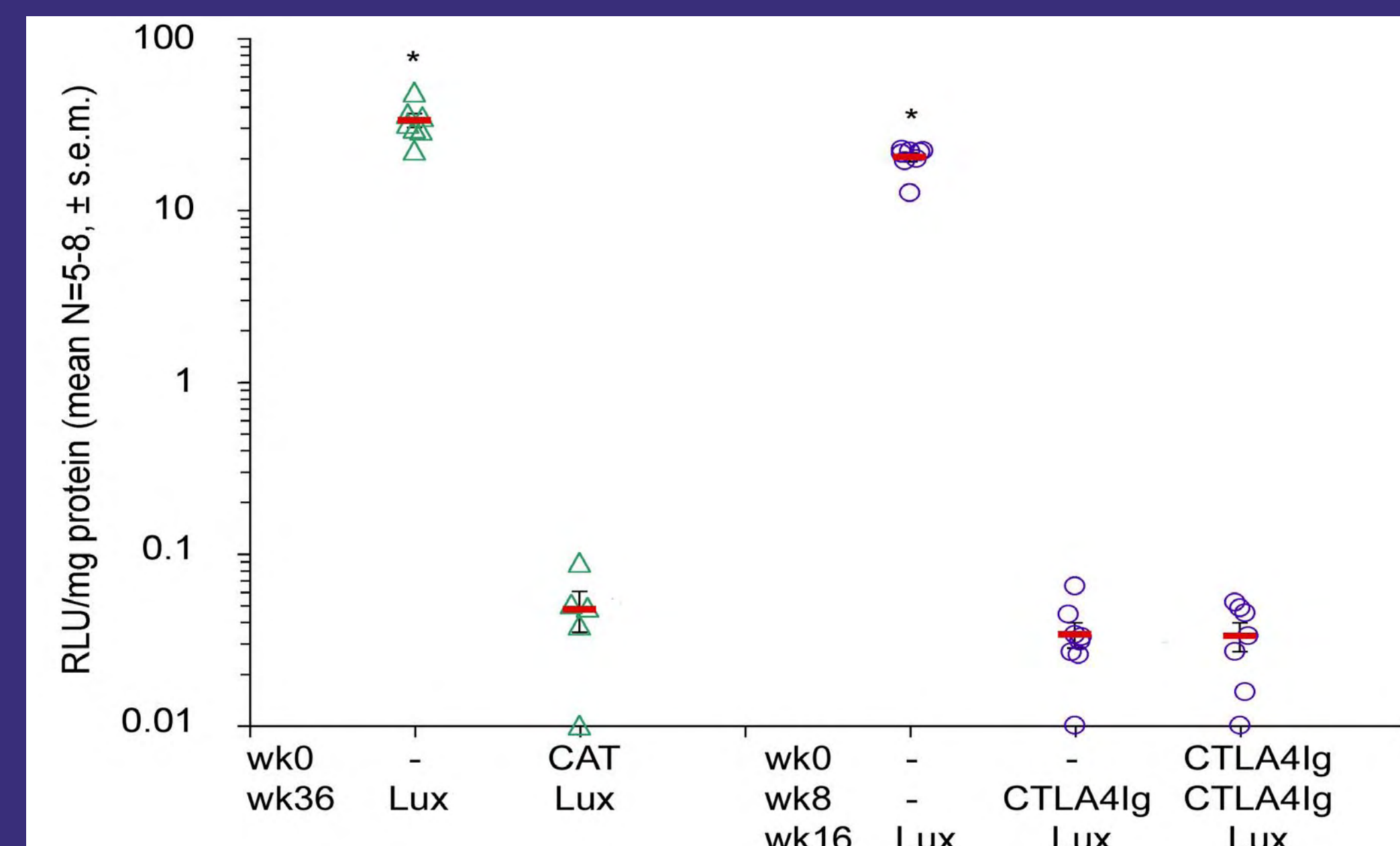
Abbreviations

GC: (DNase-resistant) genome copies, Lux: Luciferase, EGFP: Enhanced Green Fluorescent Protein, CAT: Chloramphenicol Acetyltransferase, CTLA4lg: chimerichumanCytotoxicTLymphocyte Associated antigen/human IgG Fc region, n/d: not done, nv: naïve.



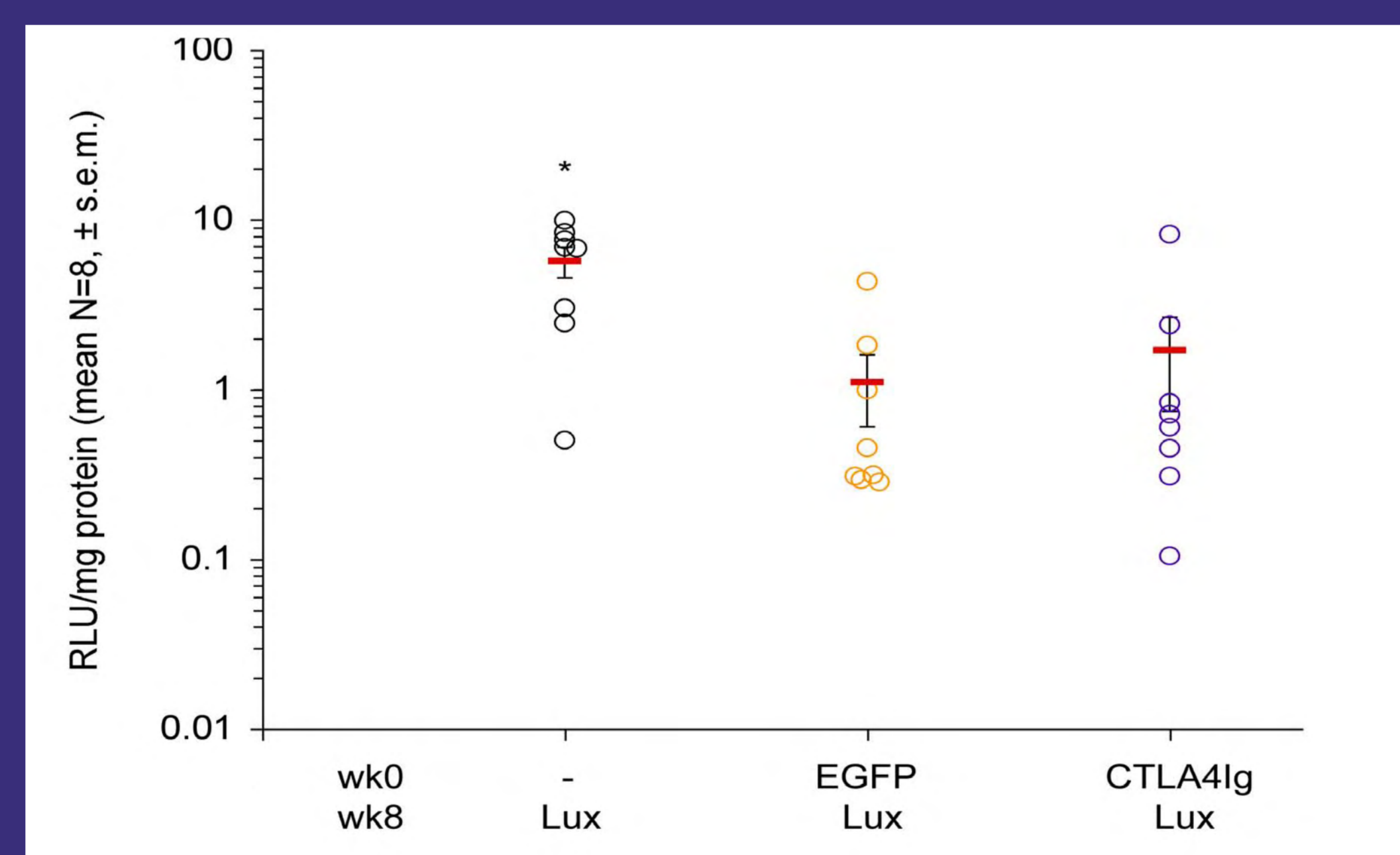
Triple vector administration of rAAV5/5 in the lung did not lead to significant transduction in the lungs.

Mice were exposed to one, two or three doses of virus expressing the transgene indicated at 8-week intervals. Open circles indicate each individual mouse, the bar indicates the group mean (\pm s.e.m.). Statistically significant differences between test samples and mocks are indicated by * ($p < 0.05$, ANOVA with Fisher's PLSD).



Increasing the gap between vector exposure to 36 weeks or delivering immunosuppressor molecule CTLA4lg by rAAV5/5 did not improve the efficiency of transduction.

Mice were exposed to the viruses indicated, at intervals of 36 weeks or 8 weeks. The symbols indicate each individual mouse, the bar indicates the group mean (\pm s.e.m.). Statistically significant differences between test samples and mocks are indicated by * ($p < 0.05$, ANOVA with Fisher's PLSD).



Repeated vector administration of rAAV5/5 did not lead to significant transduction in the nose.

Mice were exposed to one or two doses of virus expressing the transgenes indicated. Symbols indicate each individual mouse, the bar indicates the group mean (\pm s.e.m.). Statistically significant differences between test samples and mocks are indicated by * ($p < 0.05$, ANOVA with Fisher's PLSD).

Conclusions

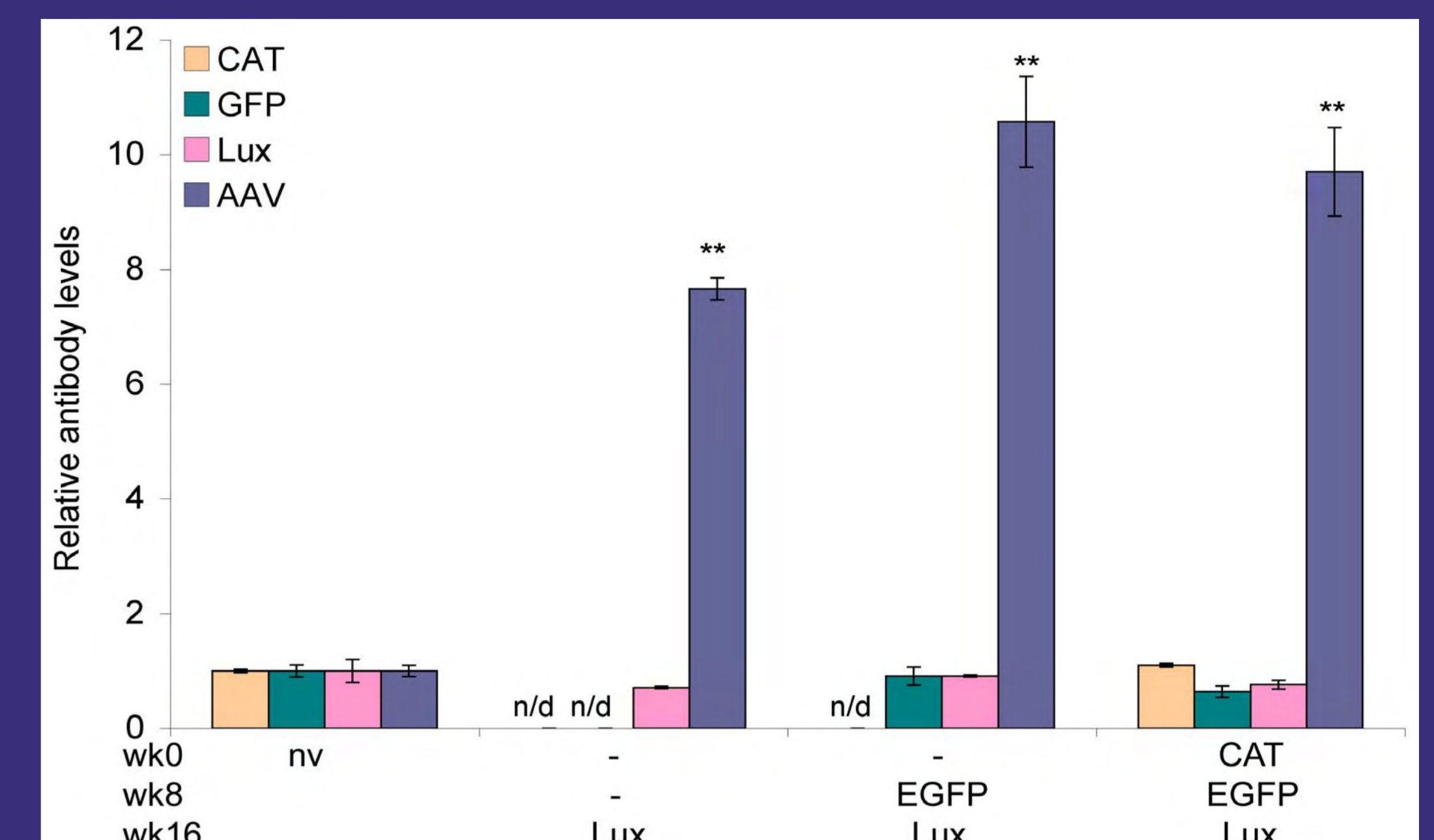
Administration of rAAV5/5 to the murine nose or lung induced a rapid and persistent antibody response against viral capsid proteins.

Anti-AAV5 antibodies prevented effective repeated administration of rAAV5/5.

rAAV5/5-mediated expression of the immunosuppressor molecule CTLA4lg was not sufficient to block the antibody response against the virus.

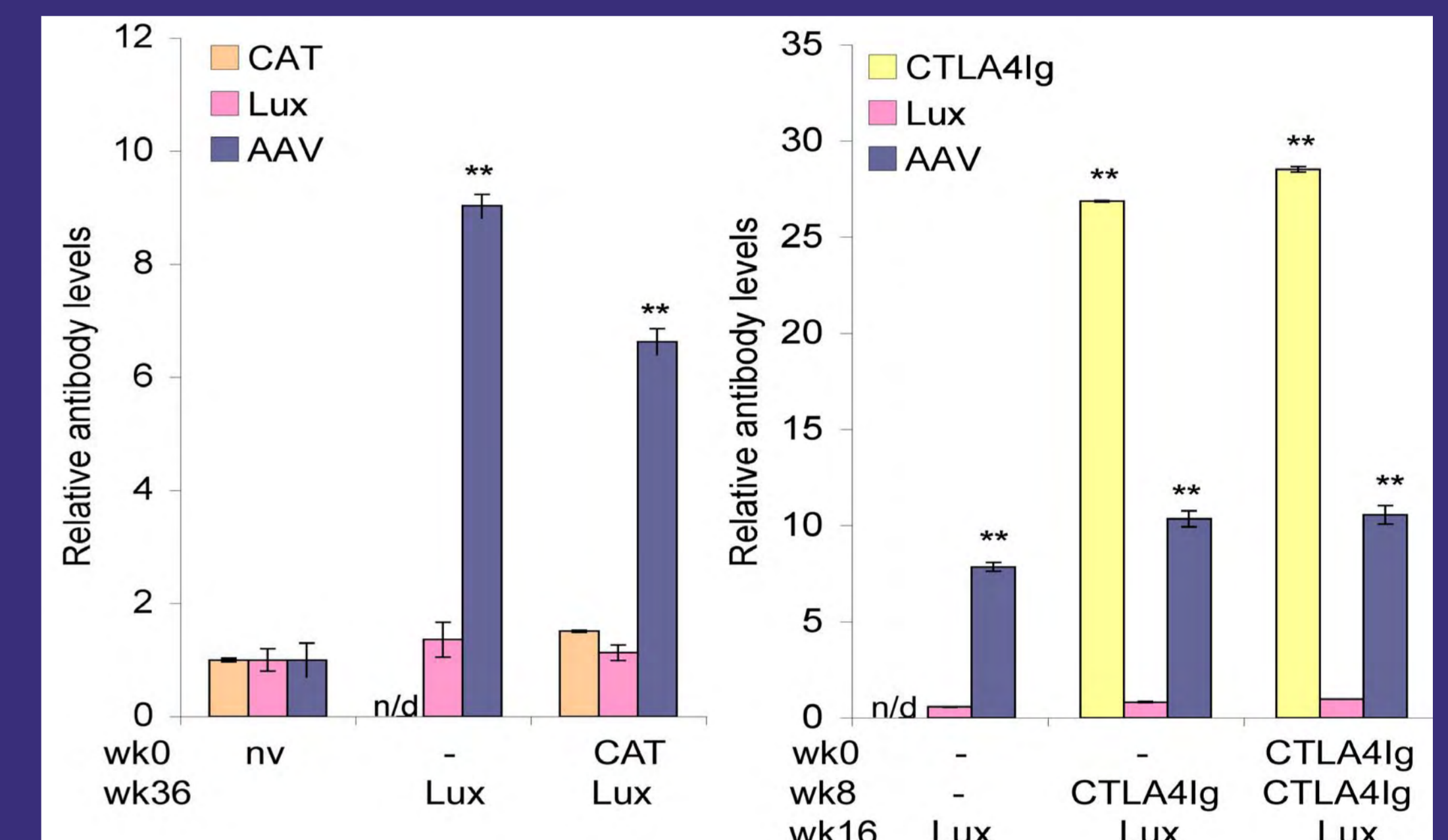
rAAV5/5-mediated expression of the immunosuppressor molecule CTLA4lg induced an anti-CTLA4lg immune response.

Administration of rAAV5/5 vectors expressing common reporter proteins (Lux, CAT, EGFP) did not induce an anti-reporter gene immune response.



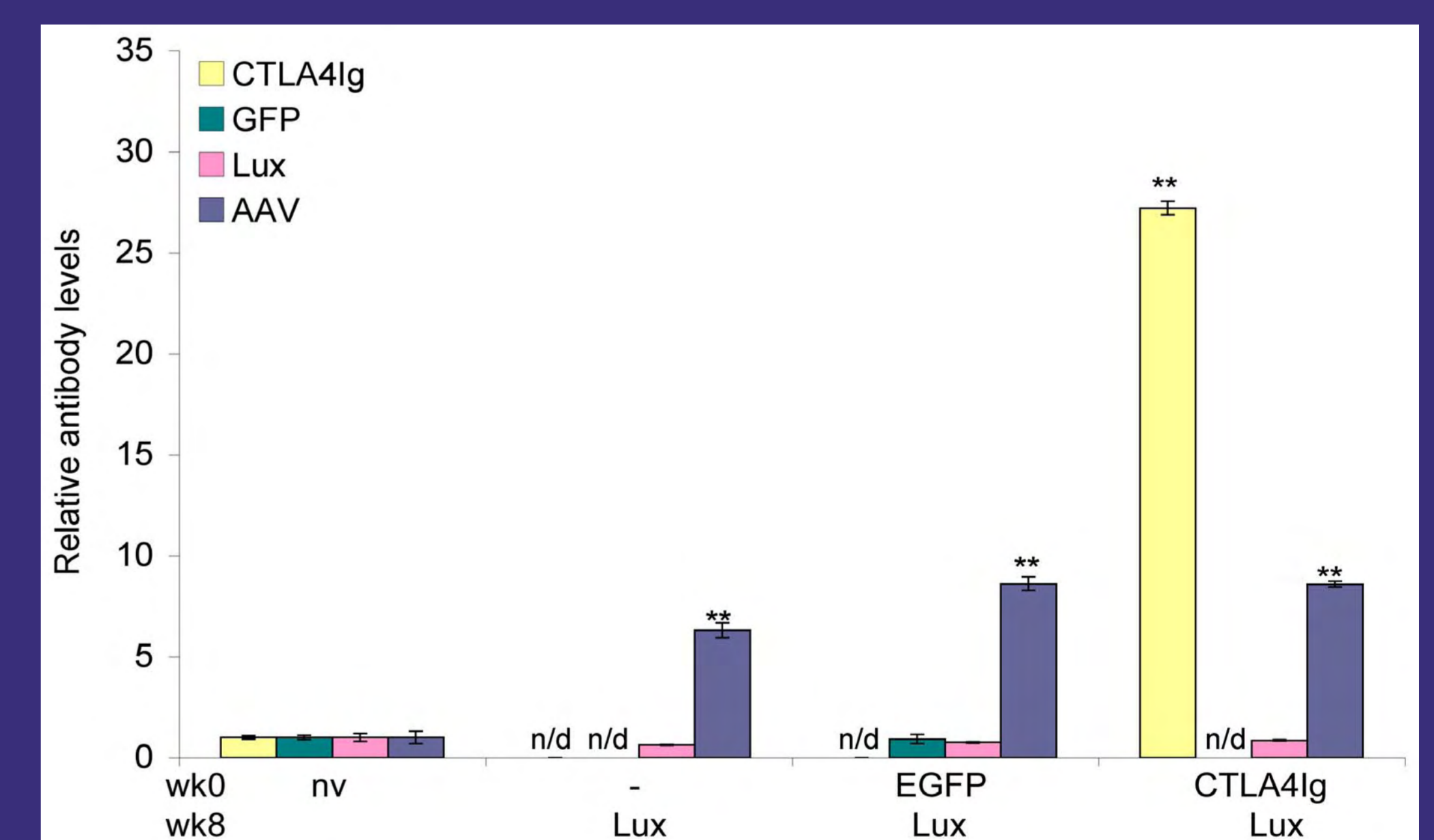
Lack of transduction correlated with levels of anti-rAAV5/5 antibodies, not anti-transgene antibodies.

Serum from the mice in the 8-week lung repeat administration study was tested for the presence of antibodies against rAAV5/5, or any of the reporter proteins by ELISA. Data are shown as mean relative ELISA absorbance (\pm s.e.m.) compared with sera from naïve mice (nv). Statistically significant differences between test samples and naïve samples are indicated by ** ($p < 0.05$, ANOVA with Fisher's PLSD).



Neither the extended gap nor the expression of CTLA4lg reduced the anti-rAAV5/5 response after delivery of the vectors to the lungs.

Serum from the mice in the 36-week repeat administration study and rAAV5/5CTLA4lg study was tested for the presence of antibodies against rAAV5/5, or any of the reporter proteins by ELISA. Data are shown as mean relative ELISA absorbance (\pm s.e.m.) compared with sera from naïve mice (nv). Statistically significant differences between test samples and naïve samples are indicated by ** ($p < 0.05$, ANOVA with Fisher's PLSD).



Lack of transduction in the nose also correlated with levels of anti-rAAV5/5 antibodies, not anti-transgene antibodies.

Serum from the mice in the nose repeat administration study was tested for the presence of antibodies against rAAV5/5, or any of the reporter proteins by ELISA. Data are shown as mean relative ELISA absorbance (\pm s.e.m.) compared with sera from naïve mice (nv). Statistically significant differences between test samples and naïve samples are indicated by ** ($p < 0.05$, ANOVA with Fisher's PLSD).