

# Glycoengineering of AAV Delivered Monoclonal Antibodies to Increase ADCC Activity

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## Abstract

The constant domain of human IgG contains a single N-linked oligosaccharide at Asn-297. The absence of fucose on the carbohydrate at this site can have a dramatic impact on antibody-dependent cellular cytotoxicity (ADCC) activity. Glycoengineered versions of several therapeutic antibodies for cancer are already in clinical trials. Our lab is studying the use of adeno-associated virus (AAV) as a vector for delivery of HIV and SIV-specific antibodies for therapeutic purposes. Since the antibody is produced by vector-transduced muscle cells *in vivo*, current techniques of glycoengineering do not apply. AAV-delivered IgG with markedly increased ADCC activity could possess significantly increased therapeutic potential. In this study we designed and tested shRNA clones targeting fucosyltransferase-8 (FUT8), the glycosyltransferase responsible for fucosylation at Asn-297 on IgG. Multiple shRNAs were found that effectively knocked down both human and macaque FUT8. shRNA clones that exhibited the highest levels of knock-down by real-time PCR were used to clone constructs that expressed either 2 or 3 shRNAs under the control of individual pol III promoters. When shRNA constructs were cotransfected with 4L6 antibody expression vectors,  $\alpha$ 1-6 fucose was almost undetectable on 4L6 by lectin western blot. Due to the efficacy of the constructs during cotransfection, constructs #1 and #2 were cloned into AAV vectors used to deliver SIV-specific broadly neutralizing antibodies. When these glycoengineered-AAV(GE-AAV) vector plasmids were transfected into HEK-293T cells, we observed a 70-80% knockdown in FUT8 by real-time PCR. We are now ready to assess the ADCC activity of antibody produced by our GE-AAV vectors. Due to the correlation of decreased fucose content and ADCC, we believe these antibodies will have greatly enhanced effector functions. By increasing the ADCC potential of HIV-specific antibodies, we increase the likelihood of targeting and destroying viral reservoirs, a critical hurdle in the search for a functional cure.

## Background

The Desrosiers lab has been exploring AAV to deliver broadly neutralizing antibodies. By expressing these antibodies by viral vector, protection is independent of the host's immune response. Our lab has been able to consistently obtain high levels of IgG by AAV vaccination (Fig1). These levels can vary from 10 to over 200  $\mu$ g/ml in serum and have been demonstrated to be very long lived.<sup>1</sup>

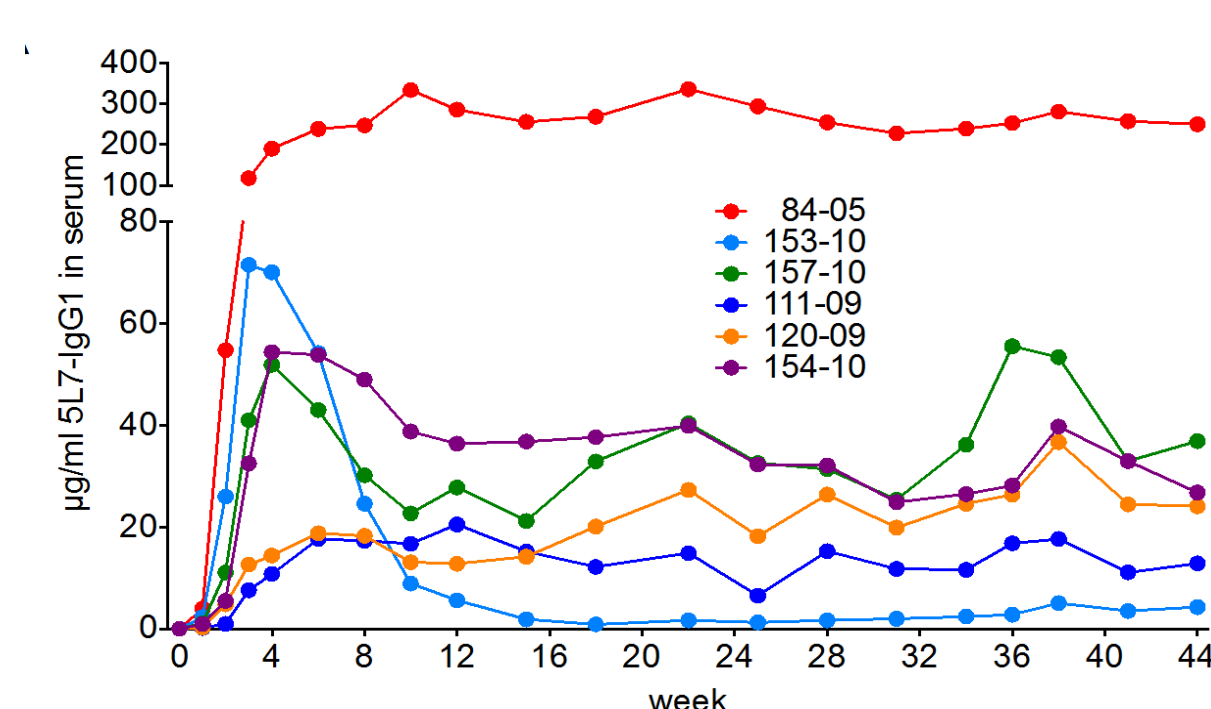


Figure 1: 5L7 levels after AAV-delivery in rhesus monkeys. Antibody levels were determined by ELISA.

Despite the promising success with AAV delivery, there is room for optimization. Therapeutic antibodies are commonly used for the treatment in cancer. To maximize the efficiency of cancer treatment, many groups have explored modification to the structure and glycan content of IgG. These modifications can increase antibody FC binding and effector functions. One of the most effective modifications is glycoengineering. IgG contains a single N-linked oligosaccharide at asn-297. By far, the most drastic increase in effector function comes from the removal of fucose at asn-297.<sup>2-4</sup> ADCC has been identified clinically as a key mechanism of anti-cancer therapeutic antibodies.<sup>3</sup> When anti-CD20 IgG1 (rituximab) was made in a non-fucosylated form, a 100-fold increase in B-cell depleting activity was observed (Fig. 2).<sup>5</sup> Much lower concentrations of antibody were necessary to achieve identical clinical efficacy.

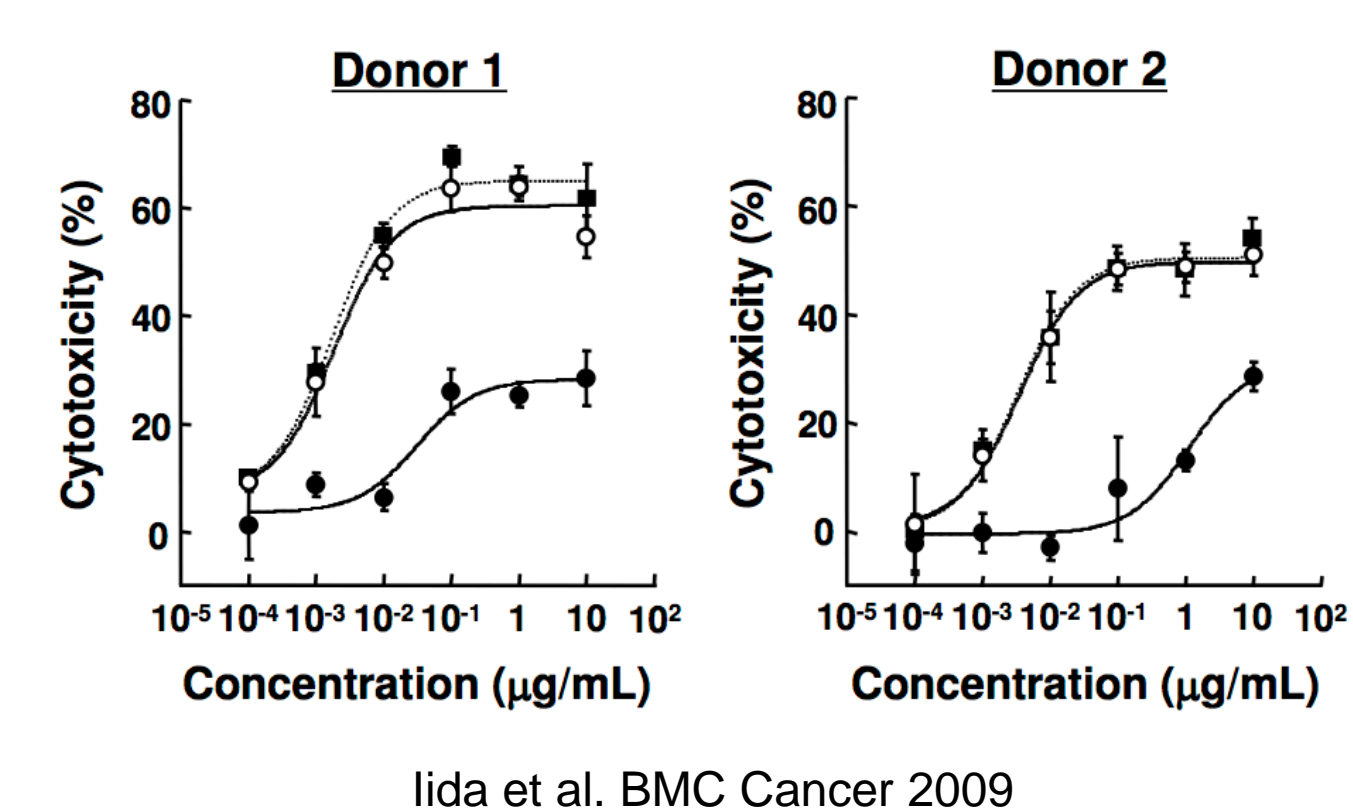


Figure 2: *Ex vivo* B-cell depletion activity of anti-CD20 IgG1 rituximab variants in a whole blood matrix. (a) Heparinized peripheral blood from healthy donors 1 and 2 were incubated with serial dilutions of anti-CD20 IgG1 rituximab variants (fucosylated (closed circle), non-fucosylated (open circle), triple amino acid-substituted mutant S239D/S298A/I332E (closed square)).

Glycoengineering has enormous potential for treatment of HIV. High levels of ADCC have been associated with slowed progression, better viral control, and lower viral set points.<sup>6-8</sup> Glycoengineering has also been successful in enhancing anti-HIV antibodies. When b12 was produced devoid of fucosylation, 10-fold higher viral inhibition was observed when compared to wild-type-b12.<sup>9</sup> These findings suggest that glycoengineering may be an important avenue to pursue in the search for a functional cure for HIV. ADCC has been suggested to be effective in the clearance of reactivated latent HIV-1 reservoirs.<sup>10</sup> By increasing the ADCC potential of HIV-specific antibodies 10-100 fold, we seek to increase the antibodies' ability to target and destroy latent viral reservoirs.

## Methods

shRNAs targeted to human and rhesus FUT8 were constructed under the control of individual pol III promoters. These constructs were cloned downstream of the IgG PolyA tail. Following transduction, shRNA expression will knockdown FUT8 in antibody producing cells, ensuring reduced fucose content of the expressed IgG

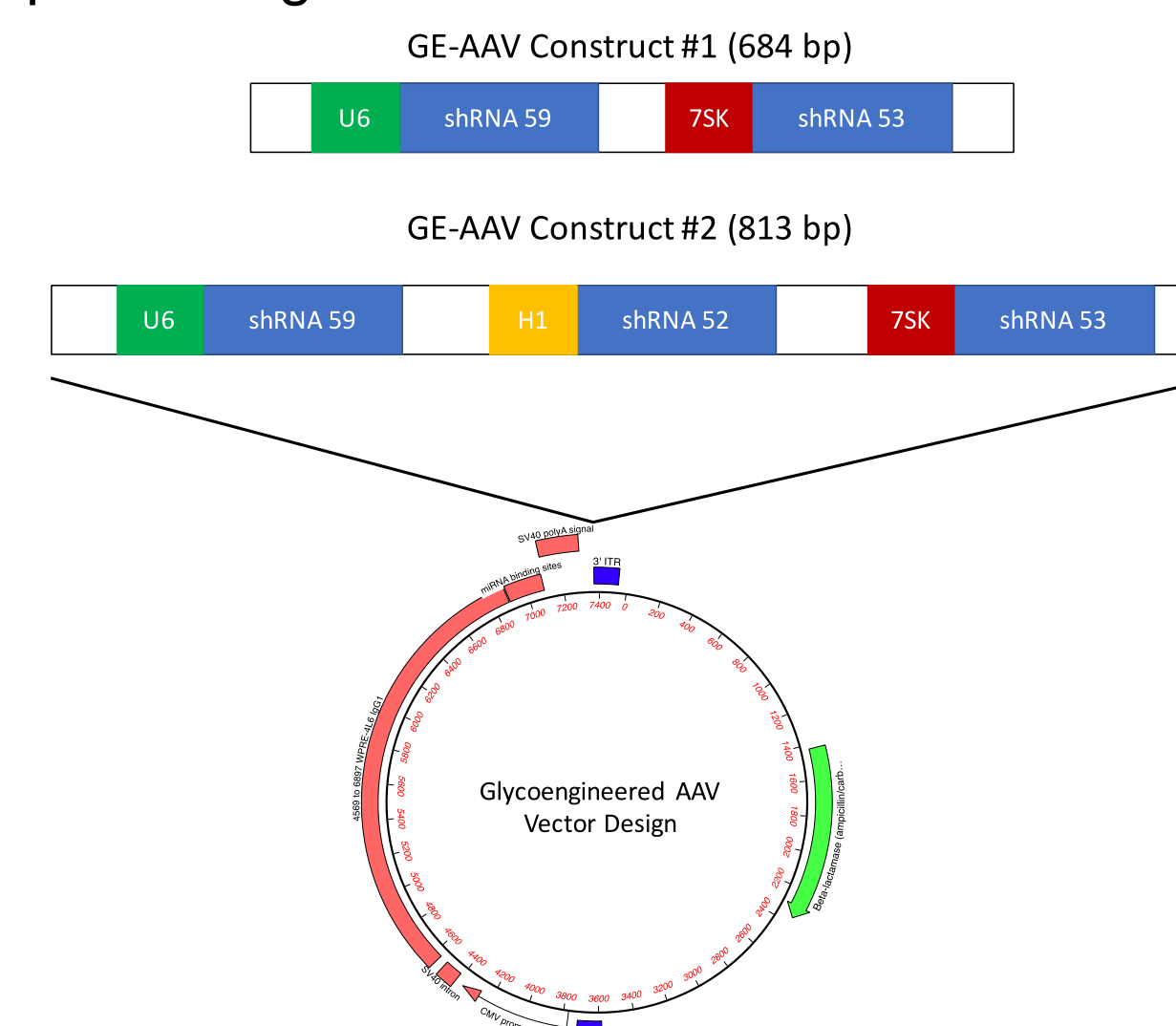


Figure 3: Cloning Strategy for GE-AAV Constructs.

## Results

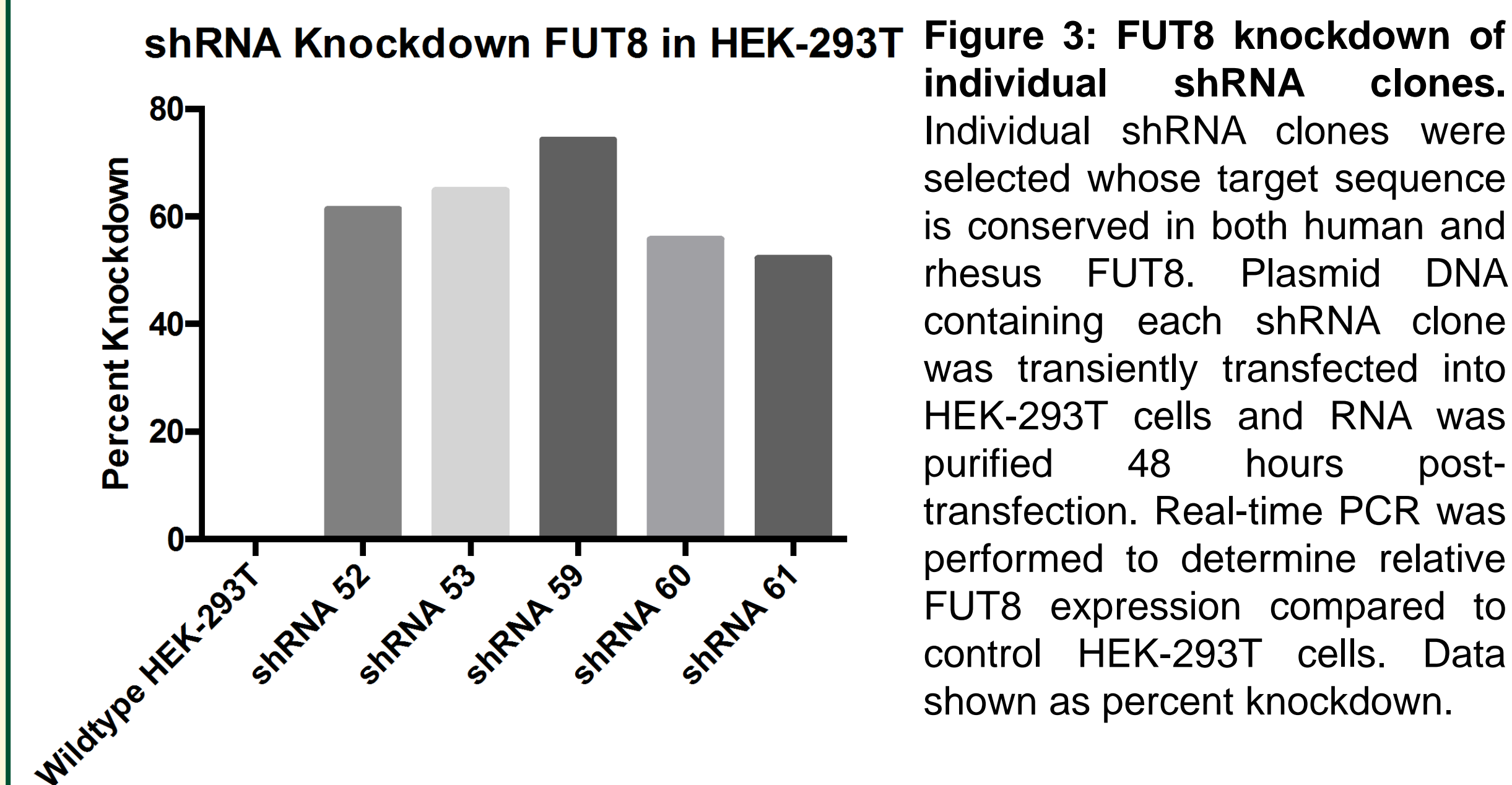


Figure 3: FUT8 knockdown of individual shRNA clones. Individual shRNA clones were selected whose target sequence is conserved in both human and rhesus FUT8. Plasmid DNA containing each shRNA clone was transiently transfected into HEK-293T cells and RNA was purified 48 hours post-transfection. Real-time PCR was performed to determine relative FUT8 expression compared to control HEK-293T cells. Data shown as percent knockdown.

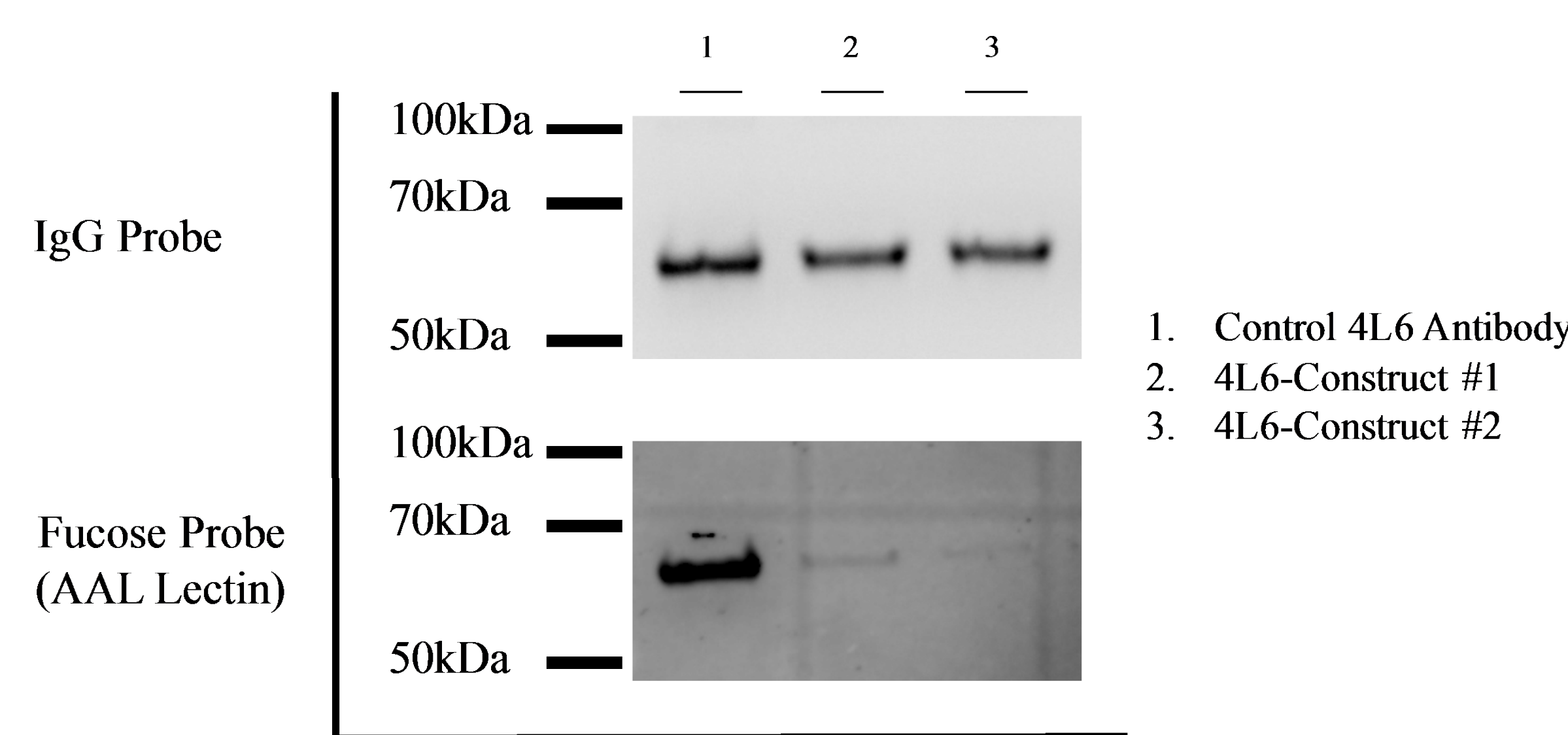


Figure 4: AAL lectin western blot to detect fucose content of 4L6 antibody. Constructs 1 and 2 were cotransfected along with an expression plasmid for 4L6 IgG into HEK-293T cells. Antibodies were purified, quantified, and 2ug were loaded in parallel into two SDS-PAGE gels. One blot was probed with an anti-mouse IgG antibody while the second was probed with an AAL lectin specific for  $\alpha$ 1-6 fucose. Fucose knockdown in lane 2 and 3 was compared to 4L6 produced in absence of shRNA (Lane 1).

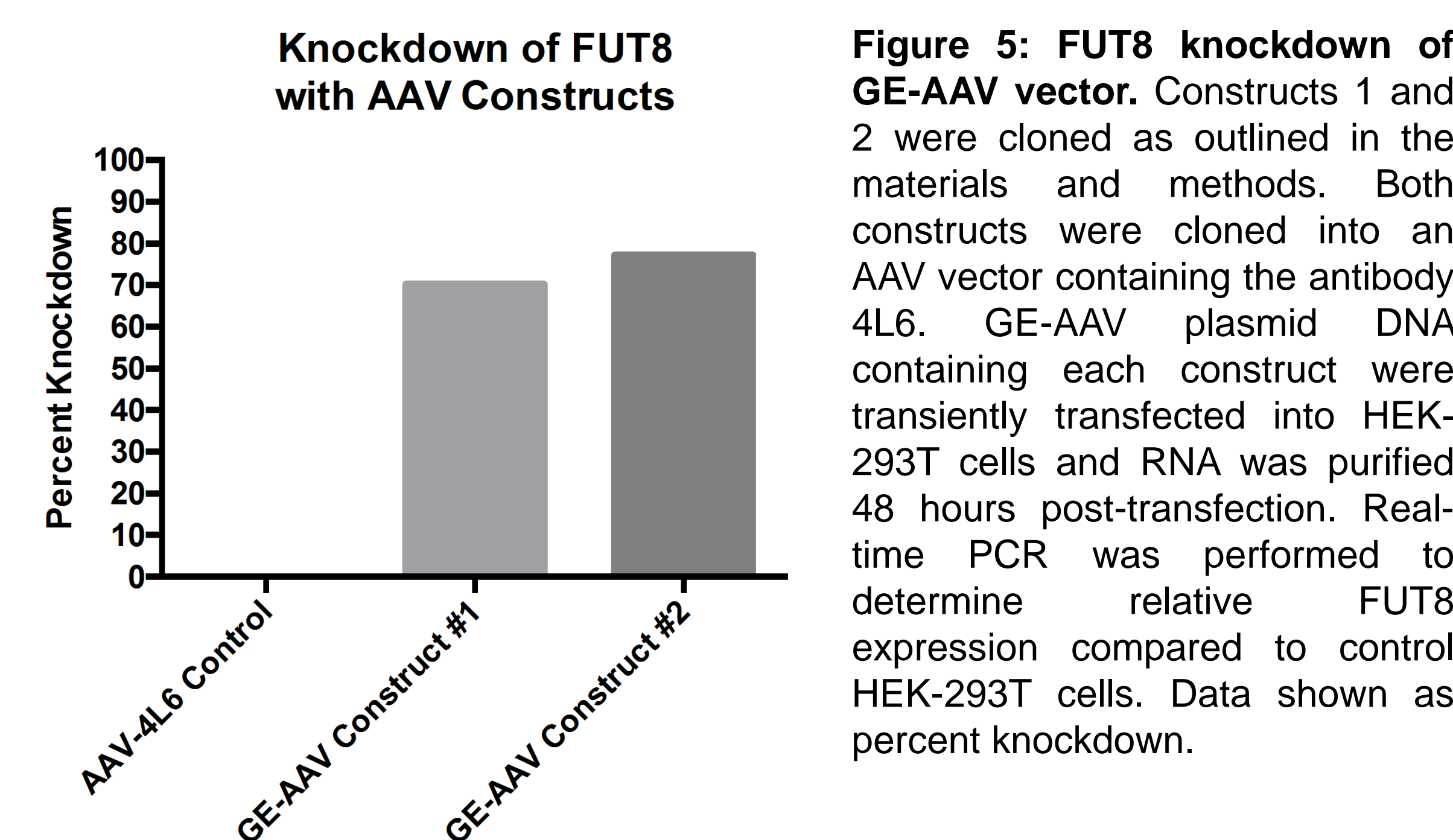


Figure 5: FUT8 knockdown of GE-AAV vector. Constructs 1 and 2 were cloned as outlined in the materials and methods. Both constructs were cloned into an AAV vector containing the antibody 4L6. GE-AAV plasmid DNA containing each construct were transiently transfected into HEK-293T cells and RNA was purified 48 hours post-transfection. Real-time PCR was performed to determine relative FUT8 expression compared to control HEK-293T cells. Data shown as percent knockdown.

## Conclusions

- The concept of glycoengineering antibody through manipulation of the AAV vector appears to have promising clinical applications.
- Fucose content of delivered antibodies can be drastically reduced by FUT8 knockdown *in vitro*.
- Individual shRNA can successfully target both human and rhesus FUT8 making our chosen shRNA sequences clinically relevant.
- When cloned into an AAV vector downstream of the poly-A tail, an 80% knockdown of FUT8 was observed in construct #2.

## Future Directions

- Large scale prep of 4L6 antibody will be made from the GE-AAV-4L6 vector.
- Mass spectrometry will be performed on the N-glycan content of GE-AAV produced antibodies.
- In vitro* ADCC assays will be performed to assess the increased antibody effector function due to fucose removal.
- AAV viral stock will be prepared for planned monkey experiments.
- Macaque prevention study to determine if glycoengineering of broadly neutralizing antibodies enhances protection when challenged with SIV.

## References

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