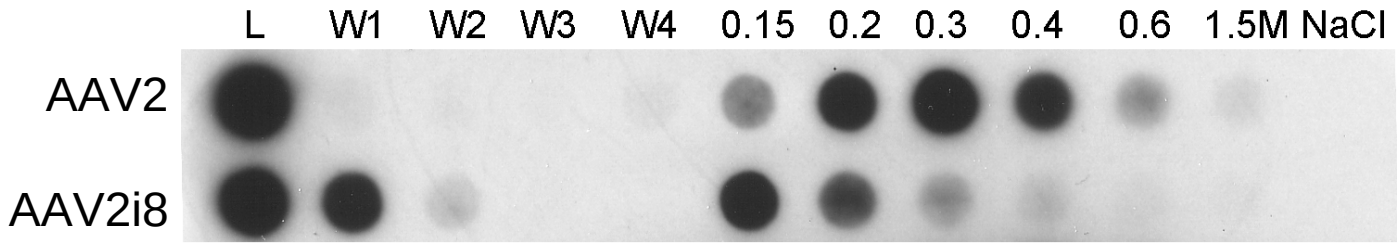
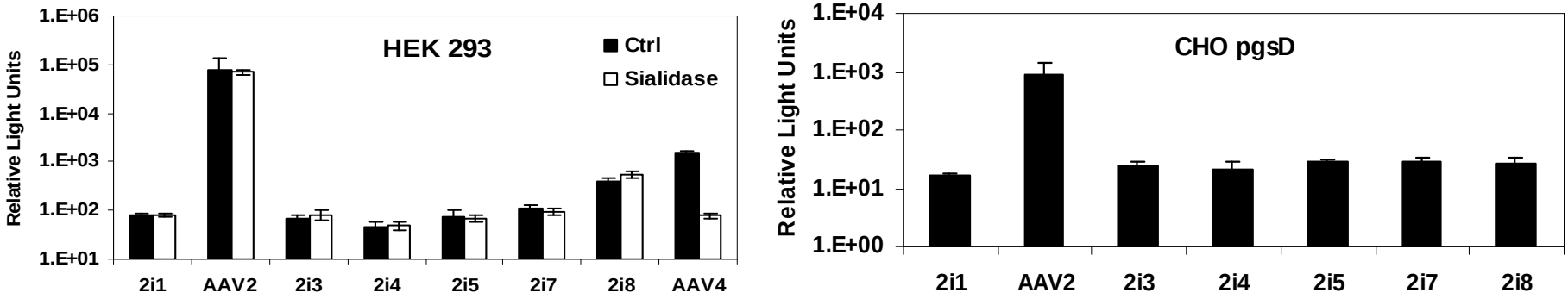



**Supplementary Figure 1: Heparin binding profile of parental AAV2 and AAV2i8 vectors**.

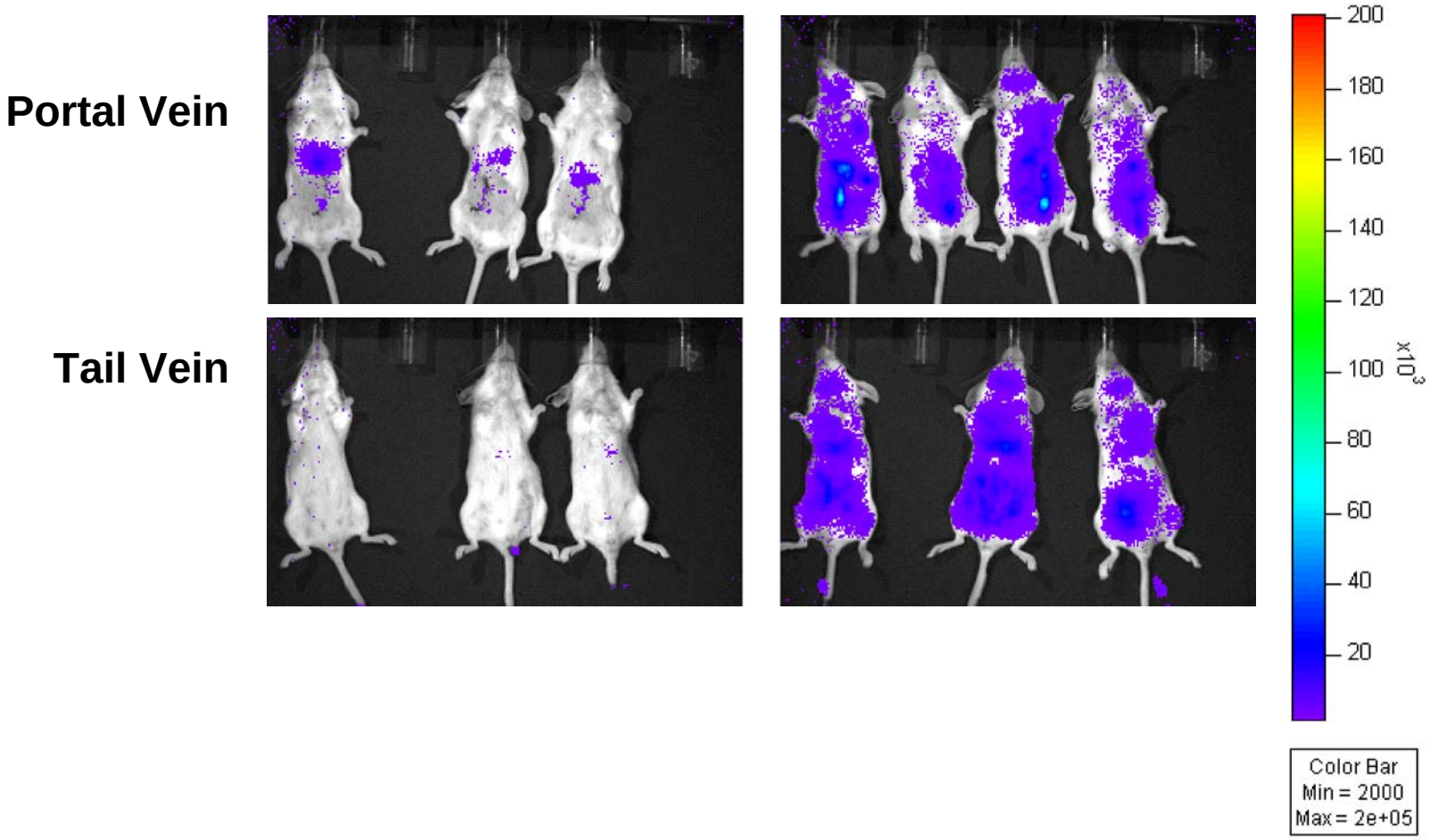
Samples were loaded (L) onto heparin-agarose affinity columns in 1xPBS, washed multiple times with 0.1xPBS (W1-4) and eluted at different salt concentrations (0.15M to 1.5M NaCl). Vector genomic DNA extracted from collected fractions were loaded onto a DNA-binding membrane using a dot blot manifold and detected using a 32P-labeled probe specific for the luciferase transgene. AAV2 peak fractions elute at ~300mM NaCl, while AAV2i8 capsids elute under physiological conditions (pH 7.4; 0.15M NaCl).




**Supplementary Figure 2: *In vitro* transduction profile of AAV2i vectors.**

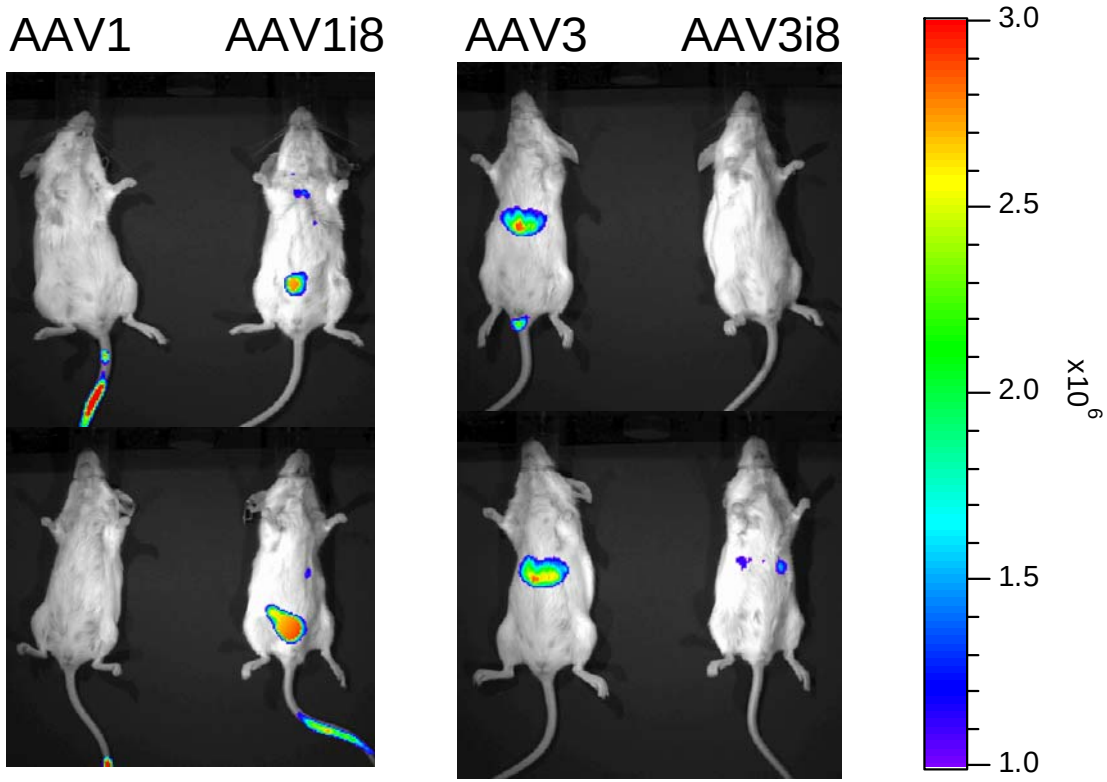
Transduction profile of AAV2i mutants in HEK 293 cells (left image) untreated (black bars, Ctrl) or treated with 50mU/mL sialidase (white bars). Luciferase transgene expression levels were determined in cell lysates harvested 24hrs post-infection with AAV2i mutants (MOI 1000). Transduction efficiencies of AAV2i mutants are decreased by 2-3 orders of magnitude in comparison with AAV2 vectors. AAV4 was included as positive control. All experiments were performed in triplicate and standard deviation is shown. Transduction profile of AAV2i mutants in heparin-deficient CHOpgsD cells (right image). Luciferase transgene expression levels determined 24hrs post-infection with AAV2i mutants (MOI 1000) are decreased by approximately two orders of magnitude in comparison with parental AAV2 vectors. All experiments were carried out in triplicate. Error bars indicate standard deviations.

**DOSE: 4E10 CMV-Luc vg/mouse; TIME: Week 1**



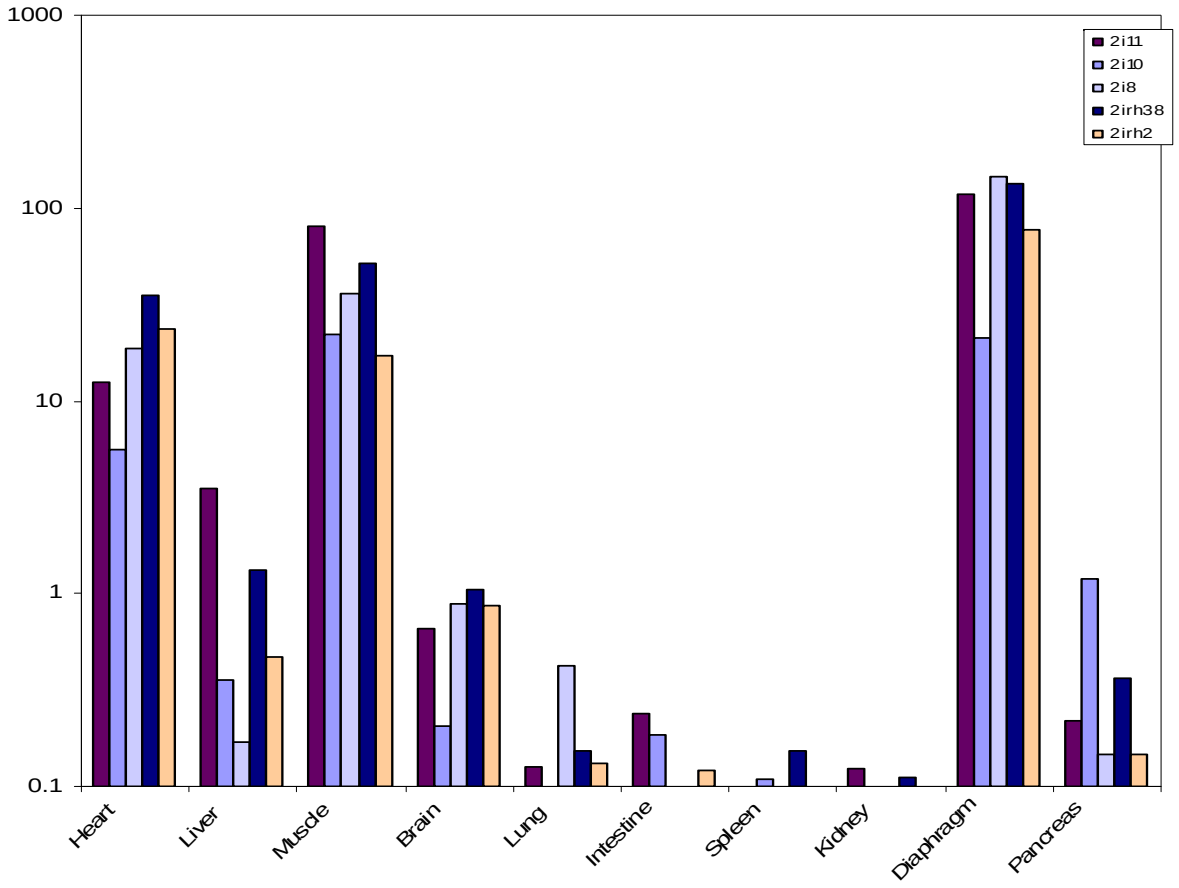

**Supplementary Figure 3: Effect of intravenous injection route on transduction profile of AAV2i8 vectors.**

Comparison of the effect of injection routes on transduction profile of AAV2i8 vectors packaging a firefly luciferase transgene cassette driven by the CMV promoter. Each animal received a dose of 4e10 viral genome-containing particles through the tail vein or portal vein. Animals were imaged at **1 wk** post-administration to determine transduction profiles using the Xenogen IVIS® imaging system. A systemic transduction profile is seen regardless of the route of administration.



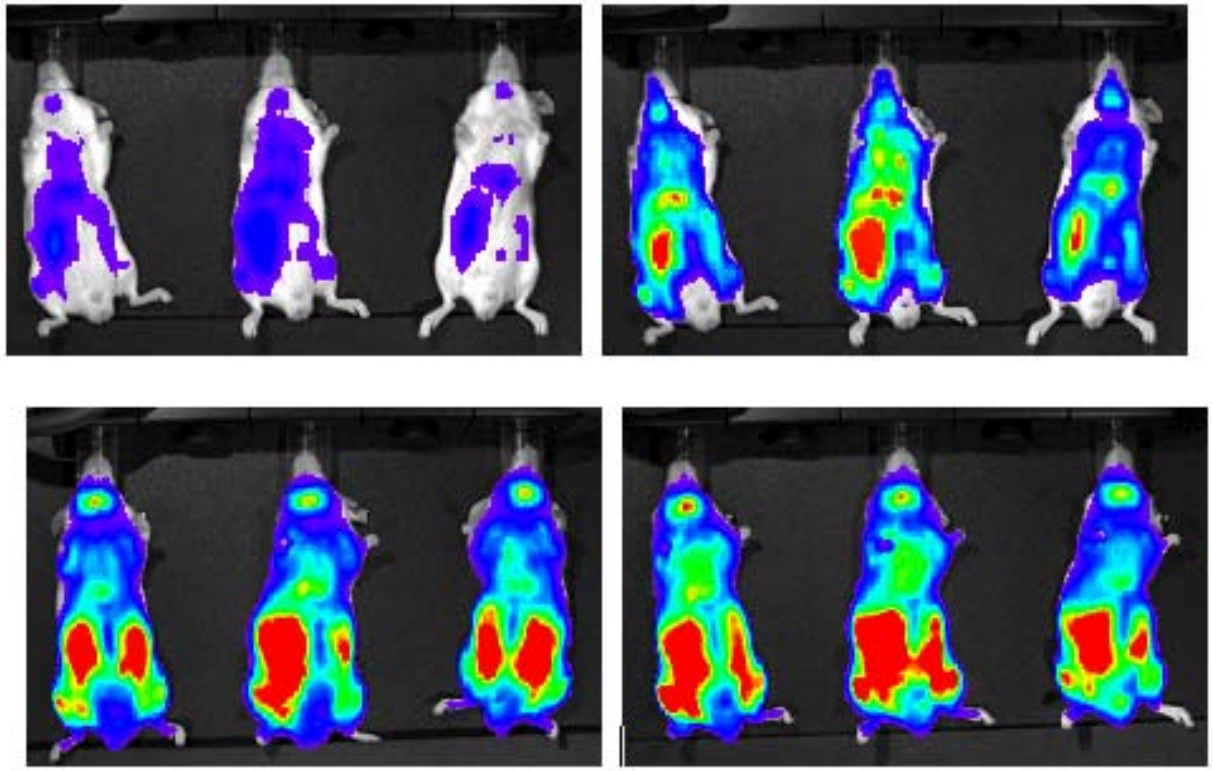

**Supplementary Figure 4: Transduction profile of AAV1i8 and AAV3i8 vectors.**

Effect of re-engineering the inner loop at the three-fold axis of symmetry on AAV1 and AAV3 capsids with the QQNTAP domain from AAV8 (AAV1i8 and AAV3i8, respectively). All animals were injected through the tail vein with a vector dose of 1e11 viral genome-containing particles packaging a firefly luciferase transgene cassette driven by the CBA promoter. Animals were imaged at 1 wk post-administration to determine transduction profiles using the Xenogen IVIS® imaging system. AAV1i8 and AAV3i8 mutants do not display any major changes in transduction profile in comparison with parental serotypes corroborating the notion that AAV2i8 vectors possess a unique receptor footprint.




**Supplementary Figure 5: Biodistribution of AAV2i8 and related chimeric vectors**

Comparison of the biodistribution of AAV2i8 and related vectors with a Q/NxxTxP motif packaging a firefly luciferase transgene cassette driven by the CBA promoter. Each animal received a dose of 1e11 viral genome-containing particles through the tail vein and genome copy numbers analyzed using Q-PCR as described in Materials & Methods.



**Supplementary Figure 6: Kinetics of luciferase transgene expression by AAV2i8 vectors.**

Bioluminescent images demonstrating kinetics of luciferase gene expression in mice following intravenous administration of AAV2i8 CBA-Luc vectors at1e11 vg/mouse. TOP LEFT: 3 days; TOP RIGHT: 1 week; BOTTOM LEFT: 4 weeks; BOTTOM RIGHT: 12 Weeks.

**Supplementary Table 1: Neutralizing antibody assay for AAV2i8**

| Vector | Sera AAV2 |
|---|---|
| AAV2 | 1:2500 |
| AAV8 | <1:10 |
| AAV2i8 | 1:40 |

**Supplementary Table 2: Neutralizing antibody to AAV2i8 from human sera**

| Subject ID | NA titer to AAV | | |
|---|---|---|---|
| | AAV2 | AAV8 | AAV2i8 |
| CHI 2-17 | 256 | <20 (8) | <20 (16) |
| DEN 4-8 | 256 | <20 (8) | <20 (8) |
| DEN 20-7 | 64 | <20 (<2) | <20 (<2) |
| IND 1-5 | 512 | <20 (16) | <20 (16) |

# Supplementary Table 3

| Nucleotide sequences of primers utilized in site-directed mutagenesis and Q-PCR analysis of vector genomes | |
|---|---|
| **Name** | **Primer Sequence** |
| 2i1/6 | 5’-ctaccaacctccagagtagcagcacagatccagctaccgcagatg-3’ |
| 2i3b | 5’-ctaccaacctccagagtagcaacacagcaccagctaccgcagatg-3’ |
| 2i4 | 5’-ctaccaacctccagagtaacagcaacctaccagctaccgcagatg-3’ |
| 2i5 | 5’-ctaccaacctccagagtagcaccacagcaccagctaccgcagatg-3’ |
| 2i7 | 5’-ctaccaacctccaggcagccaacacagcagcagctaccgcagatg-3’ |
| 2i8,rh43, rh49-53, rh57, rh58, rh64 | 5’ctaccaacctccagcaacagaacacagcaccagctaccgcagatg-3’ |
| 2i9 | 5’-ctaccaacctccagagtgcccaggcacaagcagctaccgcagatg-3’ |
| 2i10, rh40 | 5’-tctaccaacctccagcaggcaaacacgggtcctgctaccgcagat-3’ |
| 2i11, 12, rh32-34 | 5’-tctaccaacctccagaacgccaccactgcccccgctaccgcagat-3’ |
| 2irh2 | 5’-tctaccaacctccagcagacaaacggggctcctgctaccgcagat-3’ |
| 2irh38 | 5’-tctaccaacctccagcagacaaacacgggtcctgctaccgcagat-3’ |
| Fluc-fwd | 5’-aaaagcactctgattgacaaatac-3’ |
| FLuc-rev | 5’-ccttcgcttcaaaaaatggaac-3’ |