SUPPLEMENTARY INFORMATION

Adeno-associated virus-binding antibodies detected in cats living in the Northeastern United States lack neutralizing activity

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Supplementary Figure S1. ODc values of AAV-binding antibody (Ab)-positive samples selected for anti-AAV NAb assays. (a) AAV2-binding Ab ELISA ODc values obtained from a total of 99 cats are sorted in a descending order from the left to the right. The AAV2-binding antibody-positive samples used for the anti-AAV2 neutralizing antibody (NAb) assay are indicated as solid black bars with the following animal IDs from the left to the right: G1-1, G1-18, G1-17, G1-8, G1-14, G2-16, G2-9, G2-2, G2-18, G2-12, G3-16, G3-26, G3-8, and G3-1. (b) The same graphic representation for AAV6-binding antibody ELISA ODc values. The AAV6-binding antibody-positive samples used for the anti-AAV6 NAb assay are indicated as solid black bars with the following animal IDs from the left to the right: G1-1, G1-8, G3-22, G3-18, G1-9, G3-14, G3-1, G1-3, G2-2, G4v-1, G4v2, G2-9, G4v-12, G4v-4, G2-13, and G2-16. (c) The same graphic representation for AAV9-binding antibody ELISA ODc values. The AAV9-binding antibody-positive samples used for the anti-AAV9 NAb assay are indicated as solid black bars with the following animal IDs from the left to the right: G1-31, G2-5, G1-25, G1-32, G1-33, G1-35, G1-12, G2-4, G3-8, G2-2, G2-1, G3-17, G3-12, and G3-15.

Supplementary Figure S2. Anti-AAV2 and AAV6-NAb assays at a lower MOI. (a) In vitro anti-AAV2 NAb assay was performed as described in Methods using an MOI of 2 x 10^3 to investigate the presence or absence of anti-AAV2 NAbs in cat sera. The Y-axis shows transduction efficiency of reporter cells with AAV2 vector pre-incubated with sample sera diluted at 1:2.5. Results of individual animals are shown with animal identification numbers (IDs). Values are averages of technical duplicates. ID Gx-y indicates x=Group number and y=animal number specific to each animal in each group. G4 and G4v indicates Group 4 Pre-vac and Post-vac animals, respectively. (b) The same assay was performed for anti-AAV6 NAbs.
Suppl Fig. S1
Suppl Fig. S2

(a) AAV2

(b) AAV6

Transduction efficiency (% control)
| Cat ID | Gender | Age (months) | Breed | Source | No. of times of FPV Vaccinations | Days from the last Vaccination | Cluster ID (k-means) | Cluster ID (t-SNE) |
|--------|--------|-------------|-------|--------|--------------------------------|-------------------------------|----------------------|-------------------|
| G1-1   |        |             |       | Cornell Univ., NY |                            |                               | Ek                   | Et                |
| G1-2   |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-3   |        |             |       | Cornell Univ., NY |                            |                               | Dk                   | Et                |
| G1-4   |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-5   |        |             |       | Cornell Univ., NY |                            |                               | Dk                   | Dt                |
| G1-6   |        |             |       | Cornell Univ., NY |                            |                               | Ek                   | Ct                |
| G1-7   |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-8   |        |             |       | Cornell Univ., NY |                            |                               | Ek                   | Ct                |
| G1-9   |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-10  |        |             |       | Cornell Univ., NY |                            |                               | Dk                   | Et                |
| G1-11  |        |             |       | Cornell Univ., NY |                            |                               | Dk                   | Dt                |
| G1-12  |        |             |       | Cornell Univ., NY |                            |                               | Dk                   | Et                |
| G1-13  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-14  |        |             |       | Cornell Univ., NY |                            |                               | Dk                   | Et                |
| G1-15  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-16  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-17  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-18  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-19  |        |             |       | Cornell Univ., NY |                            |                               | Dk                   | Dt                |
| G1-20  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-21  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-22  |        |             |       | Cornell Univ., NY |                            |                               | Ak                   | Ct                |
| G1-23  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-24  |        |             |       | Cornell Univ., NY |                            |                               | Dk                   | Et                |
| G1-25  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-26  |        |             |       | Cornell Univ., NY |                            |                               | Ak                   | At                |
| G1-27  |        |             |       | Cornell Univ., NY |                            |                               | Ak                   | At                |
| G1-28  |        |             |       | Cornell Univ., NY |                            |                               | Ak                   | At                |
| G1-29  |        |             |       | Cornell Univ., NY |                            |                               | Ak                   | At                |
| G1-30  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-31  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-32  |        |             |       | Cornell Univ., NY |                            |                               | Dk                   | Et                |
| G1-33  |        |             |       | Cornell Univ., NY |                            |                               | Dk                   | Ct                |
| G1-34  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G1-35  |        |             |       | Cornell Univ., NY |                            |                               | Ck                   | Ct                |
| G2-1   |        |             |       | Biochem Serv., PA |                            |                               | Dk                   | Et                |
| G2-2   |        |             |       | Biochem Serv., PA |                            |                               | Dk                   | Et                |
| G2-3   |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-4   |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-5   |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-6   |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-7   |        |             |       | Biochem Serv., PA |                            |                               | Ak                   | Ct                |
| G2-8   |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-9   |        |             |       | Biochem Serv., PA |                            |                               | Dk                   | Dt                |
| G2-10  |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-11  |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-12  |        |             |       | Biochem Serv., PA |                            |                               | Dk                   | Dt                |
| G2-13  |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-14  |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-15  |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-16  |        |             |       | Biochem Serv., PA |                            |                               | Dk                   | Dt                |
| G2-17  |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-18  |        |             |       | Biochem Serv., PA |                            |                               | Dk                   | Dt                |
| G2-19  |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G2-20  |        |             |       | Biochem Serv., PA |                            |                               | Ck                   | Ct                |
| G3-1   |        |             |       | Liberty Res., NY  |                            |                               | Bk                   | Ct                |
| G3-2   |        |             |       | Liberty Res., NY  |                            |                               | Bk                   | Dt                |
| G3-3   |        |             |       | Liberty Res., NY  |                            |                               | Bk                   | Ct                |
| G3-4   |        |             |       | Liberty Res., NY  |                            |                               | Bk                   | Dt                |
| G3-5   |        |             |       | Liberty Res., NY  |                            |                               | Bk                   | Ct                |
| G3-6   |        |             |       | Liberty Res., NY  |                            |                               | Bk                   | Ct                |
| G3-7   |        |             |       | Liberty Res., NY  |                            |                               | Bk                   | Ct                |
|     | Cat ID | Sex | Age | Color | Group | Cluster ID | Location | Weight (lbs) | Breed |
|-----|--------|-----|-----|-------|-------|------------|----------|--------------|-------|
| G3-8| F      | 4.3 | DSH | Liberty Res., NY | 2     | 8          | Dk       | Bt           |
| G3-9| F      | 5.9 | DSH | Liberty Res., NY | 2     | 8          | Bk       | Bt           |
| G3-10| F     | 6.0 | DSH | Liberty Res., NY | 2     | 8          | Bk       | Bt           |
| G3-11| F     | 4.4 | DSH | Liberty Res., NY | 2     | 8          | Bk       | Bt           |
| G3-12| F     | 5.7 | DSH | Liberty Res., NY | 2     | 8          | Bk       | Bt           |
| G3-13| F     | 5.1 | DSH | Liberty Res., NY | 2     | 8          | Dk       | Bt           |
| G3-14| F     | 7.5 | DSH | Liberty Res., NY | 2     | 8          | Dk       | Bt           |
| G3-15| M     | 8.4 | DSH | Liberty Res., NY | 2     | 152        | Bk       | Dk           |
| G3-16| M     | 8.7 | DSH | Liberty Res., NY | 2     | 152        | Bk       | Bt           |
| G3-17| M     | 8.2 | DSH | Liberty Res., NY | 3     | 99         | Bk       | Bt           |
| G3-18| M     | 3.6 | DSH | Liberty Res., NY | 1     | 33         | Ak       | At           |
| G3-19| M     | 9.6 | DSH | Liberty Res., NY | 2     | 153        | Bk       | Bt           |
| G3-20| M     | 8.0 | DSH | Liberty Res., NY | 3     | 99         | Ak       | At           |
| G3-21| M     | 7.9 | DSH | Liberty Res., NY | 3     | 95         | Bk       | Bt           |
| G3-22| M     | 8.4 | DSH | Liberty Res., NY | 2     | 144        | Ak       | At           |
| G3-23| M     | 8.3 | DSH | Liberty Res., NY | 2     | 144        | Ak       | At           |
| G3-24| M     | 8.7 | DSH | Liberty Res., NY | 2     | 153        | Bk       | Bt           |
| G3-25| M     | 8.0 | DSH | Liberty Res., NY | 3     | 99         | Bk       | C1           |
| G3-26| M     | 3.6 | DSH | Liberty Res., NY | 1     | 33         | Ak       | At           |
| G3-27| M     | 8.4 | DSH | Liberty Res., NY | 2     | 154        | Ak       | At           |
| G3-28| M     | 4.5 | DSH | Liberty Res., NY | 2     | 68         | Ak       | At           |
| G3-29| M     | 8.3 | DSH | Liberty Res., NY | 3     | 99         | Bk       | Bt           |
| G3-30| F     | 4.9 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-1 | F     | 4.9 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-2 | F     | 4.9 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-3 | M     | 4.9 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-4 | M     | 4.9 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-5 | F     | 4.8 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-6 | M     | 4.8 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-7 | M     | 4.8 | DSH | Liberty Res., NY | 2     | 14         | Ak       | C1           |
| G4-8 | F     | 4.8 | DSH | Liberty Res., NY | 2     | 14         | Ak       | C1           |
| G4-9 | F     | 4.8 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-10| M    | 4.8 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-11| M    | 4.8 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-12| M    | 4.7 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-13| F    | 4.7 | DSH | Liberty Res., NY | 2     | 14         | Ak       | At           |
| G4-14| M    | 4.7 | DSH | Liberty Res., NY | 2     | 14         | Ek       | At           |

**Supplementary Table S1. Information of the cats used in this study.** Cat IDs are composed of the group names followed by each cat-specific identification numbers (IDs) in each group, linked with a hyphen between the group names and cat-specific IDs. G1, Group 1; G2, Group 2; G3, Group 3 and G4, Group 4. DSH, domestic shorthair mixed breed cats. For Cluster IDs, please refer to the main text. Blank cells indicate that no information was available. NY, New York State; PA, Pennsylvania State.
Supplementary Table S2. A comparison between clusters of the 99 cat serum samples identified by k-means clustering and those visualized in a two-dimensional space by t-SNE. For the definition of Cluster IDs (i.e., At, Ak, etc.), please refer to the main text.

| Cluster ID (t-SNE) | Cluster ID (k-means clustering) |
|--------------------|---------------------------------|
|                    | Ak    | Bk    | Ck    | Dk    | Ek    | Total |
| At                 | 24    | 0     | 0     | 1     | 0     | 25    |
| Bt                 | 1     | 17    | 1     | 1     | 0     | 20    |
| Ct                 | 2     | 2     | 22    | 1     | 0     | 27    |
| Dt                 | 0     | 1     | 1     | 9     | 0     | 11    |
| Et                 | 0     | 0     | 0     | 11    | 5     | 16    |
| **Total**          | **27**| **20**| **24**| **23**| **5** | **99**|

Supplementary Table S2. A comparison between clusters of the 99 cat serum samples identified by k-means clustering and those visualized in a two-dimensional space by t-SNE. For the definition of Cluster IDs (i.e., At, Ak, etc.), please refer to the main text.
| No. of Ab-reacting AAV serotypes | Client-owned cats with AAV-binding Abs (n=32) | Feral cats with AAV-binding Abs (n=20) | P value (* statistically significant) |
|---------------------------------|-----------------------------------------------|----------------------------------------|--------------------------------------|
|                                 | No. of cats | Percentage | No. of cats | Percentage |                                    |
| 1                               | 3           | 9          | 1           | 5          |                                    |
| 2 or less                       | 3           | 9          | 5           | 25         | 0.5028                              |
| 3 or less                       | 4           | 13         | 9           | 45         | 0.03621*                            |
| 4 or less                       | 7           | 22         | 11          | 55         | 0.05181                             |
| 5 or less                       | 10          | 31         | 12          | 60         | 0.14352                             |
| 6 or less                       | 10          | 31         | 14          | 70         | 0.022314*                           |
| 7 or less                       | 11          | 34         | 15          | 75         | 0.016134*                           |
| 8 or less                       | 12          | 38         | 15          | 75         | 0.026613*                           |
| 9 or less                       | 14          | 44         | 15          | 75         | 0.08784                             |
| 10 or less                      | 25          | 78         | 17          | 85         | 1                                    |
| 11 or less                      | 32          | 100        | 20          | 100        | 1                                    |

Supplementary Table S3. The degree of broad reactivity of AAV-binding antibodies (Abs) in client-owned and feral cat populations. The extent of broad reactivity of AAV-binding antibodies was assessed by the number of antibody-reacting AAV serotypes in each cat carrying AAV binding antibodies. A statistical comparison of the degree of broad reactivity between client-owned cats and feral cats was done using a two-sided Boschloo’s exact unconditional test. Please note that the top and bottom comparisons are reciprocal to each other, providing exactly same P values for each corresponding comparison. Bonferroni-corrected P values are shown.
| No. of Ab-reacting AAV serotypes | Client-owned cats with AAV-binding Abs (n=32) | SPF cats with AAV-binding Abs (n=27) | P value (* statistically significant) |
|---------------------------------|-----------------------------------------------|--------------------------------------|--------------------------------------|
|                                 | No. of cats | Percentage | No. of cats | Percentage |                                     |
| 1                               | 3           | 9          | 4           | 15         | 1                                    |
| 2 or less                       | 3           | 9          | 9           | 33         | 0.08274                              |
| 3 or less                       | 4           | 13         | 14          | 52         | 0.00339*                             |
| 4 or less                       | 7           | 22         | 15          | 56         | 0.03279*                             |
| 5 or less                       | 10          | 31         | 19          | 70         | 0.00607*                             |
| 6 or less                       | 10          | 31         | 22          | 81         | 0.0002874*                           |
| 7 or less                       | 11          | 34         | 23          | 85         | 0.0002481*                           |
| 8 or less                       | 12          | 38         | 25          | 93         | 0.00001971*                          |
| 9 or less                       | 14          | 44         | 26          | 96         | 0.00002265*                          |
| 10 or less                      | 25          | 78         | 26          | 96         | 0.16884                              |
| 11 or less                      | 32          | 100        | 27          | 100        | 1                                    |

| No. of Ab-reacting AAV serotypes | Client-owned cats with AAV-binding Abs (n=32) | SPF cats with AAV-binding Abs (n=27) | P value (* statistically significant) |
|---------------------------------|-----------------------------------------------|--------------------------------------|--------------------------------------|
|                                 | No. of cats | Percentage | No. of cats | Percentage |                                     |
| 1 or more                       | 32          | 100        | 27          | 100        | 1                                    |
| 2 or more                       | 29          | 91         | 23          | 85         | 1                                    |
| 3 or more                       | 29          | 91         | 18          | 67         | 0.08274                              |
| 4 or more                       | 28          | 88         | 13          | 48         | 0.00339*                             |
| 5 or more                       | 25          | 78         | 12          | 44         | 0.03279*                             |
| 6 or more                       | 22          | 69         | 8           | 30         | 0.00607*                             |
| 7 or more                       | 22          | 69         | 5           | 19         | 0.0002874*                           |
| 8 or more                       | 21          | 66         | 4           | 15         | 0.0002481*                           |
| 9 or more                       | 20          | 63         | 2           | 7          | 0.00001971*                          |
| 10 or more                      | 18          | 56         | 1           | 4          | 0.00002265*                          |
| 11 or more                      | 7           | 22         | 1           | 4          | 0.16884                              |

Supplementary Table S4. The degree of broad reactivity of AAV-binding antibodies (Abs) in client-owned and SPF cat populations. The extent of broad reactivity of AAV-binding antibodies was assessed by the number of antibody-reacting AAV serotypes in each cat carrying AAV binding antibodies. A statistical comparison of the degree of broad reactivity between client-owned cats and SPF cats was done using a two-sided Boschloo's exact unconditional test. Please note that the top and bottom comparisons are reciprocal to each other, providing exactly same P values for each corresponding comparison. Bonferroni-corrected P values are shown.
| No. of Ab-reacting AAV serotypes | Feral cats with AAV-binding Abs (n=20) | SPF cats with AAV-binding Abs (n=27) | P value (* statistically significant) |
|---------------------------------|----------------------------------------|---------------------------------------|--------------------------------------|
|                                 | No. of cats | Percentage | No. of cats | Percentage |
| 1                               | 1           | 5          | 4           | 15         | 1          |
| 2 or less                       | 5           | 25         | 9           | 33         | 1          |
| 3 or less                       | 9           | 45         | 14          | 52         | 1          |
| 4 or less                       | 11          | 55         | 15          | 56         | 1          |
| 5 or less                       | 12          | 60         | 19          | 70         | 1          |
| 6 or less                       | 14          | 70         | 22          | 81         | 1          |
| 7 or less                       | 15          | 75         | 23          | 85         | 1          |
| 8 or less                       | 15          | 75         | 25          | 93         | 0.4086     |
| 9 or less                       | 15          | 75         | 26          | 96         | 0.14511    |
| 10 or less                      | 17          | 85         | 26          | 96         | 0.8247     |
| 11 or less                      | 20          | 100        | 27          | 100        | 1          |

Supplementary Table S5. The degree of broad reactivity of AAV-binding antibodies (Abs) in feral and SPF cat populations. The extent of broad reactivity of AAV-binding antibodies was assessed by the number of antibody-reacting AAV serotypes in each cat carrying AAV binding antibodies. A statistical comparison of the degree of broad reactivity between feral cats and SPF cats was done using a two-sided Boschloo's exact unconditional test. Please note that the top and bottom comparisons are reciprocal to each other, providing exactly same P values for each corresponding comparison. Bonferroni-corrected P values are shown.
| Groups of cats       | No. of serotype-serotype combinations showing linkage values of seropositivity for each category |
|---------------------|-------------------------------------------------------------------------------------------------|
|                     | Linkage values ≤ 3 (low to moderate)                                                            |
|                     | Linkage values > 3 (high)                                                                       |
| Group 1 (client-owned) | 49                                                                                | 61          |
| Group 2 (feral)      | 63                                                                                | 47          |
| Group 3 (SPF)        | 75                                                                                | 35          |

Supplementary Table S6. Overall degree of seroreactivity linkage of AAV-binding antibodies between different serotypes in each cat group. A total of 110 seroreactivity linkage values in each animal group (Fig. 2) are categorized into two groups, one with values of 3 and less and the other with values of 3 or more. The number of each category between two groups was statistically compared between two groups. P=0.020 for Group 1 vs. Group 2, P=0.30 for Group 2 vs. Group 3, and P=0.0012 for Group 1 vs. Group 3. P values were determined by a two-sided Boschloo's exact unconditional test followed by Bonferroni correction.
Supplementary Table S7. Comparisons of the prevalence and levels of AAV-binding antibodies to each serotype between the groups. Multiple pair-wise comparisons were performed for each serotypes between the three groups. A two-sided Boschloo's exact unconditional test and a two-sided Mann-Whitney U-test were used as indicated. Statistically significant P values are indicated with asterisks (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, *****P<0.00001). G1, Group 1 (client-owned cats); G2, Group 2 (feral cats); and G3, Group 3 (FPV-vaccinated SPF cats).

| Serotypes | Bonferroni-corrected P values in each comparison |
|-----------|--------------------------------------------------|
|           | Comparison in the prevalence of antibodies (Boschloo) | Comparison in the levels of antibodies (Mann-Whitney) |
|           | G1 vs. G2 | G1 vs. G3 | G2 vs. G3 | G1 vs. G2 | G1 vs. G3 | G2 vs. G3 |
| AAV1      | 0.50      | 0.10      | 1         | 0.014*    | 1.8 x 10^{-3} | 0.77 |
| AAV2      | 0.86      | 1.3 x 10^{-3} | 2.1 x 10^{-4} | 0.017*    | 1.1 x 10^{-4} | 7.9 x 10^{-5} |
| AAV3      | 0.03*     | 1.1 x 10^{-5} | 0.15      | 0.049*    | 2.2 x 10^{-5} | 1 |
| AAV4      | 0.25      | 4.0 x 10^{-3} | 0.82      | 0.06      | 0.14      | 8.8 x 10^{-5} |
| AAV5      | 0.15      | 4.3 x 10^{-6} | 0.045*   | 0.11      | 1.1 x 10^{-3} | 0.61 |
| AAV6      | 0.24      | 1         | 0.044*   | 0.13      | 2.0 x 10^{-5} | 9.2 x 10^{-3} |
| AAV7      | 0.15      | 1.6 x 10^{-3} | 0.82      | 0.19      | 1.3 x 10^{-4} | 1.5 x 10^{-4} |
| AAV8      | 1         | 0.26      | 0.12      | 0.33      | 0.029*    | 0.87 |
| AAV9      | 1         | 0.014*    | 0.10      | 1         | 4.0 x 10^{-4} | 8.8 x 10^{-5} |
| AAV10     | 0.59      | 1         | 0.34      | 1         | 0.37      | 0.36 |
| AAV11     | 0.13      | 0.033*    | 1         | 1         | 0.15      | 4.8 x 10^{-3} |
| Serotype comparison | P values corrected for multiple comparisons (Bonferroni) | Antibody prevalence (n=30) | AAV-binding antibody levels (n=30) |
|---------------------|--------------------------------------------------------|---------------------------|----------------------------------|
| AAV6 vs. AAV1       | 0.0041                                                 | 9.0 x 10^{-6}             |                                  |
| AAV6 vs. AAV2       | 0.0041                                                 | 2.0 x 10^{-5}             |                                  |
| AAV6 vs. AAV3       | 2.2 x 10^{-7}                                          | 2.0 x 10^{-7}             |                                  |
| AAV6 vs. AAV4       | 4.2 x 10^{-9}                                          | 3.1 x 10^{-7}             |                                  |
| AAV6 vs. AAV5       | 2.2 x 10^{-6}                                          | 1.0 x 10^{-7}             |                                  |
| AAV6 vs. AAV7       | 4.2 x 10^{-8}                                          | 2.0 x 10^{-7}             |                                  |
| AAV6 vs. AAV8       | 1                                                      | 1.0 x 10^{-7}             |                                  |
| AAV6 vs. AAV9       | 4.2 x 10^{-9}                                          | 1.0 x 10^{-7}             |                                  |
| AAV6 vs. AAV10      | 0.75                                                   | 1.1 x 10^{-5}             |                                  |
| AAV6 vs. AAV11      | 0.011                                                  | 2.0 x 10^{-7}             |                                  |

Supplementary Table S8. Statistical comparisons of the prevalence and the levels of AAV-binding antibodies between AAV6 and other serotypes in Group 3 cats. A two-sided Boschloo's exact unconditional test and a Wilcoxon signed-rank test were used to statistically compare the prevalence and the quantities of antibody levels, respectively, between two serotype pairs. P values were determined by 55 multiple comparisons and adjusted by the Bonferroni correction.
| Aligned amino acid residues | Distance between Ca positions | Outer surface-exposed? |
|-----------------------------|-----------------------------|------------------------|
| AAV6 (Distance from K531*, Å) | FPV                         |                        |
| K531 (0)                    | D367                        | 2.22                   | Yes                     |
| D530 (3.77)                 | T366                        | 6.22                   | Yes                     |
| D532 (3.84)                 | E368                        | 5.19                   | Yes                     |
| D529 (4.81)                 | Q365                        | 5.41                   | Yes                     |
| K528 (5.78)                 | A364                        | 3.56                   | Yes                     |
| H527 (6.11)                 | G363                        | 5.05                   | Yes                     |
| K533 (6.68)                 | N369                        | 8.97                   | Yes                     |
| F534 (6.68)                 | Q370                        | 4.63                   | Yes                     |
| S526 (9.35)                 | G362                        | 5.49                   | Yes                     |
| F535 (9.8)                  | A371                        | 6.04                   | Yes                     |
| G513 (9.89)**               | Q350                        | 6.14                   | Yes                     |
| S490 (9.9)                  |                             |                        |                         |
| N512 (10.53)**              | T349                        | 1.85                   | Yes                     |
| R488 (11.2)                 |                             |                        | Yes                     |
| T574 (11.46)                | P410                        | 4.71                   | Yes                     |
| V489 (11.51)                |                             |                        | No                      |
| K491 (11.51)                |                             |                        |                         |
| E575 (11.51)                | E411                        | 3.96                   | Yes                     |
| V572 (11.52)                | R408                        | 1.62                   | Yes                     |
| N383 (12.01)**              | D215                        | 1.86                   | Yes                     |
| E564 (12.05)                | H403                        | 3.35                   | Yes                     |
| A525 (12.07)                | G360                        | 3.41                   | No                      |

RMSD (surface-exposed amino acids only) = 4.87 Å

**Supplementary Table S9. Differences in topology around the AAV6 K531 and FPV D367 regions.** *Distances from AAV6 K531 in Ca positions.** They are from neighboring subunits.
| Aligned amino acid residues | Distance between Ca positions | Outer surface-exposed? |
|-----------------------------|------------------------------|------------------------|
| AAV6 (Distance from L584*, Å) | FPV                          |                        |
| L584 (0)                    | I418                         | 6.08                   | Yes                    |
| Q585 (3.81)                 | N419                         | 5.37                   | No                     |
| N583 (3.87)                 | N417                         | 6.67                   | Yes                    |
| R488 (5.46)**               | Y342                         | 9.30                   | Yes                    |
| S586 (5.76)                 | F420                         | 12.54                  | Yes                    |
| Q487 (5.98)**               | P341                         | 9.41                   | No                     |
| D590 (6.45)                 | G441                         | 4.01                   | Yes                    |
| S588 (7.12)                 | K439                         | 11.05                  | Yes                    |
| P591 (7.28)                 | I442                         | 5.10                   | Yes                    |
| V582 (7.49)                 | Q416                         | 7.64                   | Yes                    |
| T574 (7.64)**               | P410                         | 4.71                   | Yes                    |
| V489 (7.97)**               |                              |                        |                        |
| E575 (8.27)**               | E411                         | 3.96                   | Yes                    |
| A581 (8.34)                 |                              |                        | No                     |
| T589 (8.54)                 | T440                         | 5.35                   | Yes                    |
| S587 (8.72)                 | G438                         | 16.24                  | Yes                    |
| A592 (8.83)                 | N443                         | 5.50                   | Yes                    |
| Q486 (8.93)**               | Y342                         | 3.00                   | No                     |
| N497 (9.36)**               |                              |                        | Yes                    |
| N496 (9.87)**               |                              |                        | Yes                    |
| S490 (10.06)**              |                              |                        | Yes                    |
| N498 (10.27)**              |                              |                        | Yes                    |
| F534 (10.32)**              | E368                         | 4.30                   | Yes                    |
| P536 (10.37)**              | Q370                         | 2.20                   | Yes                    |
| R576 (10.59)**              | G412                         | 8.56                   | Yes                    |
| R485 (10.67)**              | P341                         | 3.82                   | Yes                    |
| G505 (10.69)**              |                              |                        | No                     |
| M537 (11.08)**              | A371                         | 4.58                   | Yes                    |
| F535 (11.11)**              | N369                         | 4.35                   | Yes                    |
| T593 (11.14)                | Y444                         | 9.35                   | Yes                    |
| A573 (11.34)**              | Y409                         | 6.69                   | No                     |
| D495 (11.53)**              |                              |                        | Yes                    |
| F501 (11.71)**              |                              |                        | Yes                    |
| A506 (11.99)**              | Y343                         | 3.83                   | No                     |
| V580 (12.10)                | Q416                         | 6.66                   | Yes                    |
| H597 (12.27)**              | F448                         | 2.19                   | Yes                    |

RMSD (surface-exposed amino acids only) = 7.37 Å

Supplementary Table S10. Differences in topology around the AAV6 L584 and FPV I418 regions. *Distances from AAV6 L584 in Ca positions. **They are from neighboring subunits.