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Supplemental information

**Global seroprevalence of neutralizing antibodies
against adeno-associated virus serotypes
used for human gene therapies**

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SUPPLEMENTAL MATERIAL

Table S1. Prevalence of neutralizing antibody (NAb) positivity in adult participants (n = 502) by biological sex.

Males, N = 276										
AAV vector	Serum dilution									
	1:1	1:2	1:4	1:8	1:16	1:80	1:400	1:2,000	1:10,000	1:50,000
AAV1 (n = 276)										
n ^a (%)	206 (74.6)	168 (60.9)	143 (51.8)	126 (45.7)	115 (41.7)	69 (25.0)	21 (7.6)	4 (1.4)	0	0
95% CI	(69.1–79.7)	(54.8–66.7)	(45.7–57.8)	(39.7–51.7)	(35.8–47.7)	(20.0–30.5)	(4.8–11.4)	(0.4–3.7)	(0.0–1.3)	(0.0–1.3)
AAV5 (n = 276)										
n ^a (%)	176 (63.8)	110 (39.9)	71 (25.7)	45 (16.3)	22 (8.0)	5 (1.8)	0	0	0	0
95% CI	(57.8–69.4)	(34.0–45.9)	(20.7–31.3)	(12.1–21.2)	(5.1–11.8)	(0.6–4.2)	(0.0–1.3)	(0.0–1.3)	(0.0–1.3)	(0.0–1.3)
AAV6 (n = 276)										
n ^a (%)	195 (70.7)	147 (53.3)	123 (44.6)	110 (39.9)	87 (31.5)	37 (13.4)	10 (3.6)	0	0	0
95% CI	(64.9–76.0)	(47.2–59.3)	(38.6–50.6)	(34.0–45.9)	(26.1–37.4)	(9.6–18.0)	(1.8–6.6)	(0.0–1.3)	(0.0–1.3)	(0.0–1.3)
AAV8 (n = 276)										
n ^a (%)	163 (59.1)	142 (51.4)	130 (47.1)	107 (38.8)	92 (33.3)	42 (15.2)	11 (4.0)	1 (0.4)	0	0
95% CI	(53.0–64.9)	(45.4–57.5)	(41.1–53.2)	(33.0–44.8)	(27.8–39.2)	(11.2–20.0)	(2.0–7.0)	(0.0–2.0)	(0.0–1.3)	(0.0–1.3)
AAV9 (n = 276)										
n ^a (%)	159 (57.6)	138 (50.0)	126 (45.7)	106 (38.4)	93 (33.7)	47 (17.0)	10 (3.6)	1 (0.4)	0	0
95% CI	(51.5–63.5)	(43.9–56.1)	(39.7–51.7)	(32.6–44.4)	(28.1–39.6)	(12.8–22.0)	(1.8–6.6)	(0.0–2.0)	(0.0–1.3)	(0.0–1.3)
AAVRh74var (n = 276)										
n ^a (%)	157 (56.9)	137 (49.6)	120 (43.5)	107 (38.8)	92 (33.3)	42 (15.2)	10 (3.6)	1 (0.4)	0	0
95% CI	(50.8–62.8)	(43.6–55.7)	(37.5–49.6)	(33.0–44.8)	(27.8–39.2)	(11.2–20.0)	(1.8–6.6)	(0.0–2.0)	(0.0–1.3)	(0.0–1.3)

continued

Table S1 continued: Prevalence of neutralizing antibody (NAb) positivity in adult participants (n = 502) by biological sex

Females (N=226)										
AAV vector	Serum dilution									
	1:1	1:2	1:4	1:8	1:16	1:80	1:400	1:2,000	1:10,000	1:50,000
AAV1 (n = 226)										
n ^a (%)	170 (75.2)	137 (60.6)	125 (55.3)	110 (48.7)	94 (41.6)	66 (29.2)	26 (11.5)	5 (2.2)	0	0
95% CI	(69.1, 80.7)	(53.9, 67.0)	(48.6, 61.9)	(42.0, 55.4)	(35.1, 48.3)	(23.4, 35.6)	(7.7, 16.4)	(0.7, 5.1)	(0.0, 1.6)	(0.0, 1.6)
AAV5 (n = 226)										
n ^a (%)	145 (64.2)	95 (42.0)	65 (28.8)	45 (19.9)	27 (11.9)	8 (3.5)	0	0	0	0
95% CI	(57.5, 70.4)	(35.5, 48.8)	(23.0, 35.1)	(14.9, 25.7)	(8.0, 16.9)	(1.5, 6.9)	(0.0, 1.6)	(0.0, 1.6)	(0.0, 1.6)	(0.0, 1.6)
AAV6 (n = 226)										
n ^a (%)	157 (69.5)	131 (58.0)	109 (48.2)	93 (41.2)	78 (34.5)	38 (16.8)	11 (4.9)	1 (0.4)	0	0
95% CI	(63.0, 75.4)	(51.2, 64.5)	(41.6, 55.0)	(34.7, 47.9)	(28.3, 41.1)	(12.2, 22.3)	(2.5, 8.5)	(0.0, 2.4)	(0.0, 1.6)	(0.0, 1.6)
AAV8 (n = 226)										
n ^a (%)	140 (61.9)	122 (54.0)	109 (48.2)	95 (42.0)	86 (38.1)	45 (19.9)	15 (6.6)	2 (0.9)	0	0
95% CI	(55.3, 68.3)	(47.2, 60.6)	(41.6, 55.0)	(35.5, 48.8)	(31.7, 44.7)	(14.9, 25.7)	(3.8, 10.7)	(0.1, 3.2)	(0.0, 1.6)	(0.0, 1.6)
AAV9 (n = 226)										
n ^a (%)	131 (58.0)	121 (53.5)	105 (46.5)	92 (40.7)	85 (37.6)	53 (23.5)	18 (8.0)	4 (1.8)	0	0
95% CI	(51.2, 64.5)	(46.8, 60.2)	(39.8, 53.2)	(34.2, 47.4)	(31.3, 44.3)	(18.1, 29.5)	(4.8, 12.3)	(0.5, 4.5)	(0.0, 1.6)	(0.0, 1.6)
AAVRh74var (n = 226)										
n ^a (%)	136 (60.2)	123 (54.4)	109 (48.2)	96 (42.5)	85 (37.6)	49 (21.7)	13 (5.8)	1 (0.4)	0	0
95% CI	(53.5, 66.6)	(47.7, 61.0)	(41.6, 55.0)	(35.9, 49.2)	(31.3, 44.3)	(16.5, 27.6)	(3.1, 9.6)	(0.0, 2.4)	(0.0, 1.6)	(0.0, 1.6)

N=number of sampled adult participants in the subgroup; n=number of evaluable adult participants.

n^a=number of adult participants who were NAb positive out of the total number of evaluable adult participants.

Percentages are based on the number of evaluable participants.

95% CI for binomial proportion were calculated using the Clopper-Pearson (exact) method.

AAV, adeno-associated virus; CI, confidence interval; NAb, neutralizing antibody.

Table S2. Prevalence of neutralizing antibody (NAb) positivity in adult participants (n = 502) by age group.

Aged 16-40 years (N = 95)										
AAV vector	Serum dilution									
	1:1	1:2	1:4	1:8	1:16	1:80	1:400	1:2,000	1:10,000	1:50,000
AAV1 (n = 95)										
n ^a (%)	61 (64.2)	41 (43.2)	34 (35.8)	29 (30.5)	24 (25.3)	16 (16.8)	4 (4.2)	1 (1.1)	0	0
95% CI	(53.7, 73.8)	(33.0, 53.7)	(26.2, 46.3)	(21.5, 40.8)	(16.9, 35.2)	(9.9, 25.9)	(1.2, 10.4)	(0.0, 5.7)	(0.0, 3.8)	(0.0, 3.8)
AAV5 (n = 95)										
n ^a (%)	47 (49.5)	25 (26.3)	19 (20.0)	12 (12.6)	5 (5.3)	1 (1.1)	0	0	0	0
95% CI	(39.1, 59.9)	(17.8, 36.4)	(12.5, 29.5)	(6.7, 21.0)	(1.7, 11.9)	(0.0, 5.7)	(0.0, 3.8)	(0.0, 3.8)	(0.0, 3.8)	(0.0, 3.8)
AAV6 (n = 95)										
n ^a (%)	54 (56.8)	37 (38.9)	28 (29.5)	25 (26.3)	19 (20.0)	10 (10.5)	2 (2.1)	0	0	0
95% CI	(46.3, 67.0)	(29.1, 49.5)	(20.6, 39.7)	(17.8, 36.4)	(12.5, 29.5)	(5.2, 18.5)	(0.3, 7.4)	(0.0, 3.8)	(0.0, 3.8)	(0.0, 3.8)
AAV8 (n = 95)										
n ^a (%)	43 (45.3)	39 (41.1)	31 (32.6)	27 (28.4)	25 (26.3)	12 (12.6)	3 (3.2)	0	0	0
95% CI	(35.0, 55.8)	(31.1, 51.6)	(23.4, 43.0)	(19.6, 38.6)	(17.8, 36.4)	(6.7, 21.0)	(0.7, 9.0)	(0.0, 3.8)	(0.0, 3.8)	(0.0, 3.8)
AAV9 (n = 95)										
n ^a (%)	43 (45.3)	37 (38.9)	29 (30.5)	25 (26.3)	22 (23.2)	12 (12.6)	3 (3.2)	0	0	0
95% CI	(35.0, 55.8)	(29.1, 49.5)	(21.5, 40.8)	(17.8, 36.4)	(15.1, 32.9)	(6.7, 21.0)	(0.7, 9.0)	(0.0, 3.8)	(0.0, 3.8)	(0.0, 3.8)
AAVRh74var (n = 95)										
n ^a (%)	41 (43.2)	35 (36.8)	30 (31.6)	25 (26.3)	20 (21.1)	11 (11.6)	3 (3.2)	0	0	0
95% CI	(33.0, 53.7)	(27.2, 47.4)	(22.4, 41.9)	(17.8, 36.4)	(13.4, 30.6)	(5.9, 19.8)	(0.7, 9.0)	(0.0, 3.8)	(0.0, 3.8)	(0.0, 3.8)

continued

Table S2 continued: Prevalence of neutralizing antibody (NAb) positivity in adult participants (n = 502) by age group

Aged 41-60 years (N = 407)										
AAV vector	Serum dilution									
	1:1	1:2	1:4	1:8	1:16	1:80	1:400	1:2,000	1:10,000	1:50,000
AAV1 (n=407)										
n ^a (%)	315 (77.4)	264 (64.9)	234 (57.5)	207 (50.9)	185 (45.5)	119 (29.2)	43 (10.6)	8 (2.0)	0	0
95% CI	(73.0, 81.4)	(60.0, 69.5)	(52.5, 62.3)	(45.9, 55.8)	(40.5, 50.4)	(24.9, 33.9)	(7.8, 14.0)	(0.9, 3.8)	(0.0, 0.9)	(0.0, 0.9)
AAV5 (n = 407)										
n ^a (%)	274 (67.3)	180 (44.2)	117 (28.7)	78 (19.2)	44 (10.8)	12 (2.9)	0	0	0	0
95% CI	(62.5, 71.9)	(39.3, 49.2)	(24.4, 33.4)	(15.5, 23.3)	(8.0, 14.2)	(1.5, 5.1)	(0.0, 0.9)	(0.0, 0.9)	(0.0, 0.9)	(0.0, 0.9)
AAV6 (n=407)										
n ^a (%)	298 (73.2)	241 (59.2)	204 (50.1)	178 (43.7)	146 (35.9)	65 (16.0)	19 (4.7)	1 (0.2)	0	0
95% CI	(68.6, 77.5)	(54.3, 64.0)	(45.2, 55.1)	(38.9, 48.7)	(31.2, 40.7)	(12.5, 19.9)	(2.8, 7.2)	(0.0, 1.4)	(0.0, 0.9)	(0.0, 0.9)
AAV8 (n = 407)										
n ^a (%)	260 (63.9)	225 (55.3)	208 (51.1)	175 (43.0)	153 (37.6)	75 (18.4)	23 (5.7)	3 (0.7)	0	0
95% CI	(59.0, 68.6)	(50.3, 60.2)	(46.1, 56.1)	(38.1, 48.0)	(32.9, 42.5)	(14.8, 22.5)	(3.6, 8.4)	(0.2, 2.1)	(0.0, 0.9)	(0.0, 0.9)
AAV9 (n = 407)										
n ^a (%)	247 (60.7)	222 (54.5)	202 (49.6)	173 (42.5)	156 (38.3)	88 (21.6)	25 (6.1)	5 (1.2)	0	0
95% CI	(55.8, 65.5)	(49.6, 59.5)	(44.7, 54.6)	(37.7, 47.5)	(33.6, 43.2)	(17.7, 25.9)	(4.0, 8.9)	(0.4, 2.8)	(0.0, 0.9)	(0.0, 0.9)
AAVRh74var (n = 407)										
n ^a (%)	252 (61.9)	225 (55.3)	199 (48.9)	178 (43.7)	157 (38.6)	80 (19.7)	20 (4.9)	2 (0.5)	0	0
95% CI	(57.0, 66.7)	(50.3, 60.2)	(43.9, 53.9)	(38.9, 48.7)	(33.8, 43.5)	(15.9, 23.9)	(3.0, 7.5)	(0.1, 1.8)	(0.0, 0.9)	(0.0, 0.9)

N=number of sampled adult participants in the subgroup; n=number of evaluable adult participants.

n^a=number of adult participants who were NAb positive out of the total number of evaluable adult participants.

Percentages are based on the number of evaluable participants.

95% CI for binomial proportion were calculated using the Clopper-Pearson (exact) method.

AAV, adeno-associated virus; CI, confidence interval; NAb, neutralizing antibody.

Table S3. Prevalence of neutralizing antibody (NAb) positivity in adult participants (n = 502) by disease area.

Cancer (N = 340)										
AAV vector	Serum dilution									
	1:1	1:2	1:4	1:8	1:16	1:80	1:400	1:2,000	1:10,000	1:50,000
AAV1 (n = 340)										
n ^a (%)	263 (77.4)	226 (66.5)	203 (59.7)	181 (53.2)	163 (47.9)	100 (29.4)	34 (10.0)	7 (2.1)	0	0
95% CI	(72.5, 81.7)	(61.2, 71.5)	(54.3, 65.0)	(47.8, 58.6)	(42.5, 53.4)	(24.6, 34.6)	(7.0, 13.7)	(0.8, 4.2)	(0.0, 1.1)	(0.0, 1.1)
AAV5 (n = 340)										
n ^a (%)	232 (68.2)	153 (45.0)	101 (29.7)	65 (19.1)	36 (10.6)	10 (2.9)	0	0	0	0
95% CI	(63.0, 73.2)	(39.6, 50.5)	(24.9, 34.9)	(15.1, 23.7)	(7.5, 14.4)	(1.4, 5.3)	(0.0, 1.1)	(0.0, 1.1)	(0.0, 1.1)	(0.0, 1.1)
AAV6 (n = 340)										
n ^a (%)	254 (74.7)	204 (60.0)	179 (52.6)	158 (46.5)	127 (37.4)	56 (16.5)	16 (4.7)	1 (0.3)	0	0
95% CI	(69.7, 79.2)	(54.6, 65.2)	(47.2, 58.1)	(41.1, 51.9)	(32.2, 42.7)	(12.7, 20.8)	(2.7, 7.5)	(0.0, 1.6)	(0.0, 1.1)	(0.0, 1.1)
AAV8 (n = 340)										
n ^a (%)	222 (65.3)	196 (57.6)	181 (53.2)	154 (45.3)	138 (40.6)	62 (18.2)	18 (5.3)	3 (0.9)	0	0
95% CI	(60.0, 70.3)	(52.2, 63.0)	(47.8, 58.6)	(39.9, 50.8)	(35.3, 46.0)	(14.3, 22.8)	(3.2, 8.2)	(0.2, 2.6)	(0.0, 1.1)	(0.0, 1.1)
AAV9 (n = 340)										
n ^a (%)	215 (63.2)	190 (55.9)	172 (50.6)	153 (45.0)	139 (40.9)	73 (21.5)	21 (6.2)	5 (1.5)	0	0
95% CI	(57.9, 68.4)	(50.4, 61.2)	(45.1, 56.0)	(39.6, 50.5)	(35.6, 46.3)	(17.2, 26.2)	(3.9, 9.3)	(0.5, 3.4)	(0.0, 1.1)	(0.0, 1.1)
AAVRh74var (n = 340)										
n ^a (%)	217 (63.8)	192 (56.5)	176 (51.8)	160 (47.1)	136 (40.0)	67 (19.7)	17 (5.0)	2 (0.6)	0	0
95% CI	(58.5, 68.9)	(51.0, 61.8)	(46.3, 57.2)	(41.7, 52.5)	(34.8, 45.4)	(15.6, 24.3)	(2.9, 7.9)	(0.1, 2.1)	(0.0, 1.1)	(0.0, 1.1)

continued

Table S3 continued: Prevalence of neutralizing antibody (NAb) positivity in adult participants (n = 502) by disease area

Other (N = 162)										
AAV vector	Serum dilution									
	1:1	1:2	1:4	1:8	1:16	1:80	1:400	1:2,000	1:10,000	1:50,000
AAV1 (n = 162)										
n ^a (%)	113 (69.8)	79 (48.8)	65 (40.1)	55 (34.0)	46 (28.4)	35 (21.6)	13 (8.0)	2 (1.2)	0	0
95% CI	(62.1, 76.7)	(40.8, 56.7)	(32.5, 48.1)	(26.7, 41.8)	(21.6, 36.0)	(15.5, 28.7)	(4.3, 13.3)	(0.1, 4.4)	(0.0, 2.3)	(0.0, 2.3)
AAV5 (n = 162)										
n ^a (%)	89 (54.9)	52 (32.1)	35 (21.6)	25 (15.4)	13 (8.0)	3 (1.9)	0	0	0	0
95% CI	(46.9, 62.8)	(25.0, 39.9)	(15.5, 28.7)	(10.2, 21.9)	(4.3, 13.3)	(0.4, 5.3)	(0.0, 2.3)	(0.0, 2.3)	(0.0, 2.3)	(0.0, 2.3)
AAV6 (n = 162)										
n ^a (%)	98 (60.5)	74 (45.7)	53 (32.7)	45 (27.8)	38 (23.5)	19 (11.7)	5 (3.1)	0	0	0
95% CI	(52.5, 68.1)	(37.8, 53.7)	(25.6, 40.5)	(21.0, 35.3)	(17.2, 30.7)	(7.2, 17.7)	(1.0, 7.1)	(0.0, 2.3)	(0.0, 2.3)	(0.0, 2.3)
AAV8 (n = 162)										
n ^a (%)	81 (50.0)	68 (42.0)	58 (35.8)	48 (29.6)	40 (24.7)	25 (15.4)	8 (4.9)	0	0	0
95% CI	(42.1, 57.9)	(34.3, 50.0)	(28.4, 43.7)	(22.7, 37.3)	(18.3, 32.1)	(10.2, 21.9)	(2.2, 9.5)	(0.0, 2.3)	(0.0, 2.3)	(0.0, 2.3)
AAV9 (n = 162)										
n ^a (%)	75 (46.3)	69 (42.6)	59 (36.4)	45 (27.8)	39 (24.1)	27 (16.7)	7 (4.3)	0	0	0
95% CI	(38.4, 54.3)	(34.9, 50.6)	(29.0, 44.3)	(21.0, 35.3)	(17.7, 31.4)	(11.3, 23.3)	(1.8, 8.7)	(0.0, 2.3)	(0.0, 2.3)	(0.0, 2.3)
AAVRh74var (n = 162)										
n ^a (%)	76 (46.9)	68 (42.0)	53 (32.7)	43 (26.5)	41 (25.3)	24 (14.8)	6 (3.7)	0	0	0
95% CI	(39.0, 54.9)	(34.3, 50.0)	(25.6, 40.5)	(19.9, 34.0)	(18.8, 32.7)	(9.7, 21.2)	(1.4, 7.9)	(0.0, 2.3)	(0.0, 2.3)	(0.0, 2.3)

N=number of sampled adult participants in the subgroup; n=number of evaluable adult participants.

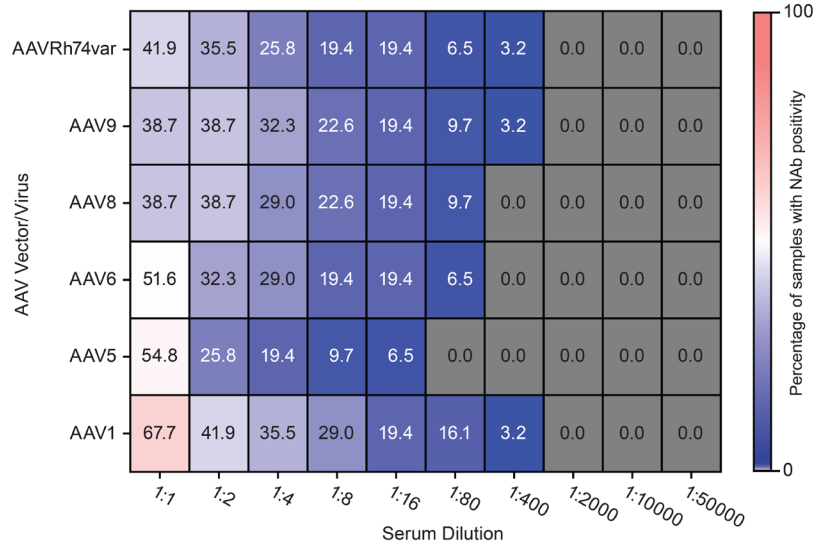
n^a=number of adult participants who were NAb positive out of the total number of evaluable adult participants.

Percentages are based on the number of evaluable participants.

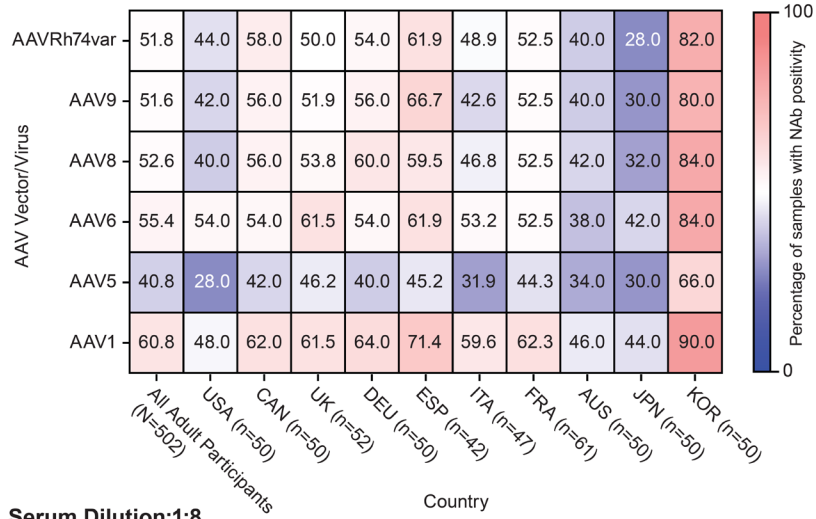
95% CI for binomial proportion were calculated using the Clopper-Pearson (exact) method.

AAV, adeno-associated virus; CI, confidence interval; NAb, neutralizing antibody

A. US-only Pediatric Participants



B. Serum Dilution:1:2



C. Serum Dilution:1:8

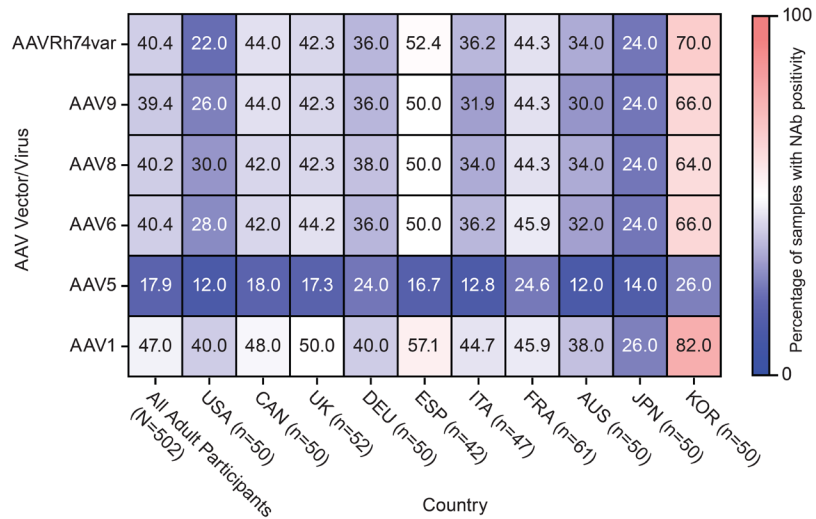
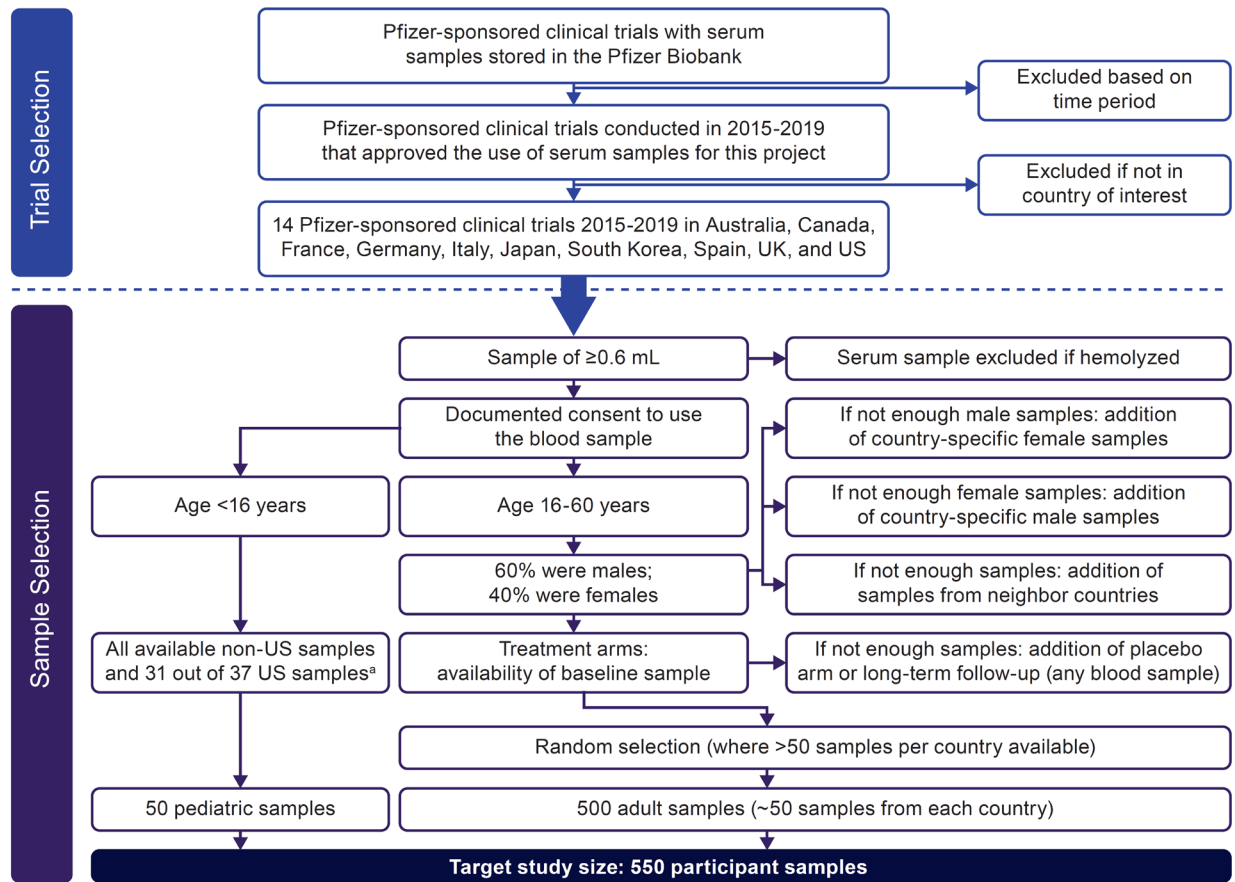
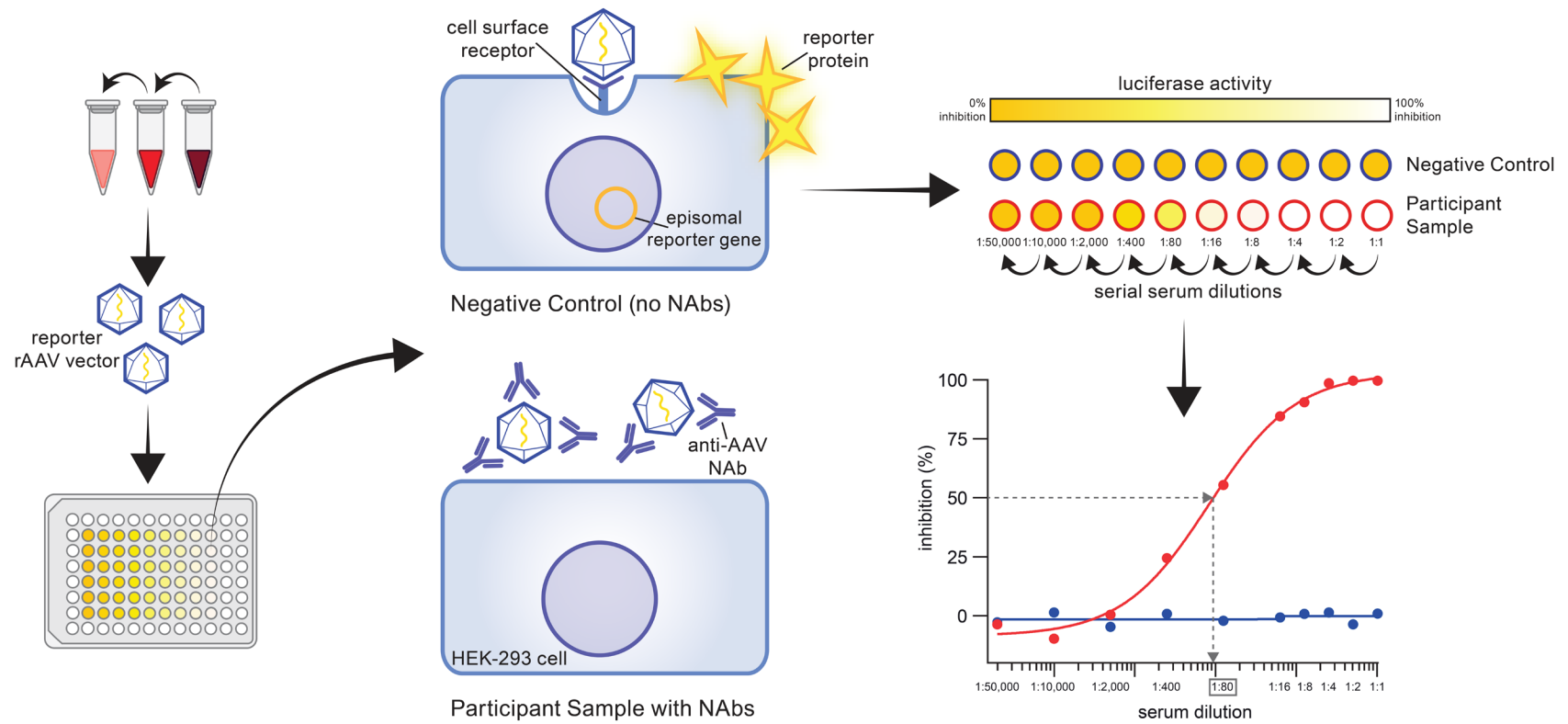


Figure S1. Prevalence of neutralizing antibody (NAb) positivity in US-only pediatric participants (n=31) by AAV serotype and serum dilution and adult participants (n=502) by AAV serotype and country at 1:2 and 1:8 serum dilutions. (A) Heatmap of the prevalence of NAb activity for US-only pediatric participants against 6 AAV serotypes across serum dilutions. Numbers inside squares indicate the percentage of serum samples positive for NAb activity against the designated serotype at the designated serum dilution (out of all samples tested for that AAV at that serum dilution). Gray squares indicate that all tested serum samples were negative for NAb activity against the serotype at the specified dilution. (B & C) Heatmaps of the prevalence of NAb activity against 6 AAV serotypes for all adult participants (left column) and the subset of participants from each country for the 1:2 serum dilution (B) and the 1:8 serum dilution (C). Numbers inside squares indicate the percentage of serum samples positive for NAb activity against the designated serotype in the designated country (out of all samples tested for that AAV in that country). AAV=adeno-associated virus; NAb=neutralizing antibody; USA=United States; CAN=Canada; UK=United Kingdom; DEU=Germany; ESP=Spain; ITA=Italy; FRA=France; AUS=Australia; JPN=Japan; KOR=South Korea.



^a31 pediatric samples were randomly selected from the pool of 37 US samples

Figure S2. Algorithm for trial and sample selection. Flowchart shows the inclusion and exclusion criteria that were applied to select clinical trials (top) and participant samples (bottom) for inclusion in the study. The target study size was 550 participant samples (500 adults [~50 serum samples per country] and 50 children). 552 samples (n=502 adults, n=50 children) were ultimately analyzed in this study.



1) Serum samples were serially diluted, incubated with a reporter rAAV vector, then incubated with cells

2) anti-AAV NAb inhibit transduction of the reporter gene (luciferase)

3) Luciferase activity was measured and the 50% inhibition titer (ID50) calculated

Figure S3. Cell-based transduction inhibition assay to measure anti-AAV neutralizing antibodies (NAb). (1) Serial dilutions (1:1, 1:2, 1:4, 1:8, 1:16, 1:80, 1:400, 1:2,000, 1:10,000, 1:50,000) were prepared from participant and control serum samples. The serum samples were incubated with a fixed amount of reporter recombinant AAV (rAAV) vector. The rAAV vector comprised a specific AAV capsid and encoded a firefly luciferase reporter gene. The samples were then

incubated with target cells (Human Embryonic Kidney 293 [HEK-293] cell line) in a multi-well plate. (2) The presence of anti-AAV NAb in a sample inhibits transduction of the rAAV vector (bottom), decreasing the amount of luciferase activity in target cells compared with the negative control sample that does not contain NAb (top). (3) Luciferase activity was measured as luminescence, where 100% inhibition represents complete inhibition of reporter gene transduction relative to control. Hypothetical activity from a participant sample is shown in the red circles; activity from a negative control sample is shown in the blue circles. The percent inhibition of luciferase activity was plotted against the serial dilutions of the serum sample, and an inhibition curve was calculated. The 50% inhibition titer (ID_{50}) is the highest serum dilution that achieves 50% inhibition of luciferase activity relative to the negative control. This hypothetical example represents an ID_{50} of 1:80. AAV=adeno-associated virus; rAAV=recombinant AAV; NAb=neutralizing antibody.