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Supplemental information

Innate and adaptive AAV-mediated immune responses in a mouse model of Duchenne muscular dystrophy

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Table S1. HCPM mean concentration (ng/mL) of anti-AAV and anti-transgene IgG and IgM for AAV9-Cas9, AAV9-Cas9-FS and AAV9-µDYS dosed *mdx* mice.

Vector	Antigen	Immunogloublin	Pre	SD	N	Post-1 2wks	SD	N	Post-1 4wks	SD	N	Post-2 2wks	SD	N	Post-2 6wks	SD	N
Cas9-FS	AAV9	IgG	3.55E+04	15216.6	5	1.24E+05	17843.5	6	1.49E+05	31186.4	6	1.99E+05	35224.7	6	1.85E+05	19260.2	6
Cas9			3.16E+04	5768.0	3	1.20E+05	29618.6	5	1.51E+05	33866.2	5	1.94E+05	42554.4	5	1.82E+05	37596.3	5
µDYS			2.33E+04	17608.4	4	1.16E+05	21340.4	6	1.71E+05	20931.4	6	1.90E+05	31503.8	6	1.86E+05	19356.2	6
Combined			3.05E+04	14334.7	12	1.20E+05	21743.7	17	1.57E+05	28828.1	17	1.94E+05	34125	17	1.84E+05	24265.1	17
Cas9-FS		IgM	9.28E+04	74479.3	5	1.59E+06	1483783.8	6	3.46E+05	410199.8	6	1.33E+06	1332001.4	6	4.35E+05	330423.7	6
Cas9			1.15E+05	68414.9	3	9.97E+05	792940.9	5	4.75E+05	337235.5	5	5.02E+05	283709.0	5	5.88E+05	250517.3	5
µDYS			1.79E+05	84396.0	4	7.83E+05	441980.4	6	5.40E+05	531889.9	6	5.34E+05	613854.7	6	3.22E+06	6114362.7	6
Combined			1.27E+05	79770.6	12	1.13E+06	1017145.6	17	4.53E+05	420357.76	17	8.07E+05	923925.86	17	1.46E+06	3677581.57	17
Cas9-FS	AAV2	IgG	3.53E+04	15064.2	5	1.34E+05	62356.8	6	1.77E+05	42459.9	6	2.81E+05	68983.4	6	2.32E+05	46160.6	6
Cas9			3.18E+04	5638.0	3	1.36E+05	68404.1	5	1.69E+05	66167.9	5	2.70E+05	62558.2	5	2.27E+05	53849.1	5
µDYS			2.83E+04	9891.3	4	1.39E+05	74101.1	6	1.83E+05	83647.9	6	2.54E+05	75051.0	6	2.22E+05	57682.4	6
Combined			3.21E+04	11176	12	1.36E+05	64068.6	17	1.76E+05	62247.9	17	2.68E+05	66023.8	17	2.27E+05	49477.57	17
Cas9-FS		IgM	8.97E+05	652717.9	5	8.87E+05	503360.8	6	3.70E+05	376646.9	6	1.50E+06	1506907.9	6	4.89E+05	341335.7	6
Cas9			2.34E+05	112934.7	3	1.17E+06	1402548.1	5	1.21E+06	1758233.4	5	9.52E+05	1116181.1	5	1.23E+06	1002817.2	5
µDYS			1.43E+05	103086.0	4	5.57E+05	583816.1	6	5.71E+05	413113.8	6	4.28E+05	439868.8	6	1.23E+07	28591416.1	6
Combined			4.80E+05	545092.5	12	8.53E+05	861203.3	17	6.89E+05	1000190.8	17	9.59E+05	1138073.3	17	4.87E+06	16963075.8	17
Cas9-FS	AAV8	IgG	3.79E+04	15016.1	5	1.62E+05	103102.3	6	2.21E+05	90622.0	6	3.00E+05	80229.3	6	2.84E+05	74333.3	6
Cas9			3.18E+04	5434.3	3	1.34E+05	84193.8	5	1.88E+05	82500.8	5	3.16E+05	63761.7	5	2.75E+05	57873.2	5
µDYS			2.32E+04	17538.0	4	1.54E+05	121210.8	6	2.69E+05	81400.0	6	3.24E+05	55877.7	6	3.12E+05	58230.1	6
Combined			3.15E+04	14651.4	12	1.51E+05	99136.2	17	2.28E+05	86540.9	17	3.13E+05	64135.4	17	2.91E+05	62423	17
Cas9-FS		IgM	3.83E+05	206258.8	5	1.89E+06	1326187.0	6	5.95E+05	848117.5	6	2.66E+06	2677867.9	6	1.17E+06	1586331.2	6
Cas9			1.74E+05	48389.4	3	1.03E+06	843659.1	5	6.62E+05	760626.2	5	1.21E+06	1156726.7	5	1.22E+06	756198.1	5
µDYS			2.00E+05	49800.8	4	1.09E+06	927154.3	6	9.36E+05	892042.2	6	1.16E+06	1154644.8	6	5.61E+06	10446274.2	6
Combined			2.69E+05	163266.9	12	1.36E+06	1078257.4	17	7.35E+05	801383.8	17	1.71E+06	1875019.9	17	2.75E+06	6306277.9	17
Cas9-FS	AAVMYO	IgG	3.63E+04	14961.5	5	1.74E+05	32714.5	6	1.88E+05	44831.3	6	2.32E+05	39692.5	6	2.27E+05	27347.5	6
Cas9			3.17E+04	5547.8	3	1.71E+05	35275.6	5	2.03E+05	44063.7	5	2.43E+05	46704.1	5	2.30E+05	44779.4	5
µDYS			2.32E+04	17479.2	4	1.82E+05	31621.3	6	2.37E+05	33275.7	6	2.46E+05	33380.4	6	2.49E+05	27136.4	6
Combined			3.08E+04	14318	12	1.76E+05	31325.4	17	2.09E+05	43909.8	17	2.40E+05	37701.1	17	2.36E+05	32700.8	17
Cas9-FS		IgM	3.70E+05	309935.1	5	2.63E+06	1926935.2	6	7.43E+05	977233.5	6	2.16E+06	2140910.0	6	7.77E+05	569107.0	6
Cas9			1.93E+05	32293.8	3	1.71E+06	1308016.3	5	7.85E+05	591121.0	5	8.68E+05	620971.4	5	9.07E+05	429841.0	5
µDYS			1.86E+05	37133.8	4	1.60E+06	1526662.3	6	1.03E+06	874334.5	6	6.53E+05	845407.0	6	8.67E+06	18768056.8	6
Combined			2.65E+05	210207.9	12	1.99E+06	1597467.7	17	8.58E+05	801882.4	17	1.25E+06	1496317.2	17	3.60E+06	11185583.5	17
Cas9-FS	Cas9	IgG	3.49E+04	15326.9	5	3.63E+04	13803.9	6	3.95E+04	15384.2	6	3.77E+04	13651.1	6	3.93E+04	17438.7	6
Cas9			3.17E+04	5719.7	3	2.73E+04	9139.8	5	3.09E+04	9324.3	5	2.44E+04	15646.1	5	2.95E+04	10184.3	5
µDYS			2.37E+04	16457.2	4	2.75E+04	5337.6	6	3.08E+04	10357.6	6	2.92E+04	5128.1	6	2.76E+04	11223.5	6
Combined			3.03E+04	13822.1	12	3.05E+04	10423.7	17	3.39E+04	12144.6	17	3.08E+04	12618.2	17	3.23E+04	13766.3	17
Cas9-FS		IgM	4.27E+04	24078.8	5	1.46E+05	114064.2	6	1.18E+05	59499.9	6	9.40E+04	55873.0	6	9.77E+04	62313.9	6
Cas9			9.56E+04	81337.3	3	7.33E+04	66150.9	5	9.89E+04	81978.1	5	1.34E+05	220285.6	5	6.36E+04	43320.0	5
µDYS			1.80E+05	85147.8	4	1.80E+06	4198394.4	6	8.79E+04	48783.1	6	1.19E+05	86754.5	6	7.13E+04	92261.8	6
Combined			1.02E+05	84877.4	12	7.07E+05	2490410.6	17	1.02E+05	60847.1	17	1.15E+05	125486	17	7.84E+04	67598.1	17
Cas9-FS	µDYS	IgG	3.52E+04	15244.7	5	3.76E+04	13283.5	6	4.02E+04	15233.8	6	3.87E+04	12504.6	6	3.59E+04	10146.3	6
Cas9			3.20E+04	5380.8	3	2.78E+04	9717.0	5	3.07E+04	8817.2	5	2.43E+04	15441.3	5	2.96E+04	9172.0	5
µDYS			2.34E+04	17421.8	4	2.82E+04	4715.5	6	3.15E+04	7554.5	6	2.92E+04	5956.6	6	2.95E+04	9990.6	6
Combined			3.04E+04	14196.7	12	3.14E+04	10384.5	17	3.43E+04	11398.7	17	3.11E+04	12527.9	17	3.18E+04	9709.4	17
Cas9-FS		IgM	2.25E+05	242845.3	5	1.58E+05	78693.3	6	1.22E+05	51700.4	6	4.14E+05	507931.3	6	1.66E+05	86891.6	6
Cas9			1.39E+05	33281.7	3	1.06E+05	91715.7	5	1.00E+05	80598.0	5	1.48E+05	82102.2	5	9.28E+04	57229.9	5
µDYS			1.73E+05	63036.1	4	1.85E+05	253983.5	6	1.62E+05	86055.9	6	1.36E+05	79596.8	6	1.44E+05	118387.6	6
Combined			1.86E+05	155141.6	12	1.52E+05	158995.3	17	1.30E+05	73932.2	17	2.38E+05	319951.9	17	1.37E+05	92260.8	17

Table S2. HCPM IgM and IgG multiple comparison statistics performed on the combined vector data.

Time point	Antigen	Immunobulin	Average (ng/mL)	SD	N	Tukey's multiple comparison test
Pre	AAV9	IgG	3.05E+04	14334.69	12	Pre vs Post-1 2wks (****), Pre vs Post-1 4wks (****), Pre vs Post-2 2wks (****), Pre vs Post-2 6wks (****), Post-1 2wks vs Post-1 4wks (***), Post-1 2wks vs Post-2 2wks (****), Post-1 2wks vs Post-2 6wks (****), Post-1 4wks vs Post-2 2wks (**), Post-1 4wks vs Post-2 6wks (*)
Post-1 2wks			1.20E+05	21743.74	17	
Post-1 4wks			1.57E+05	28828.11	17	
Post-2 2wks			1.94E+05	34125.03	17	
Post-2 6wks			1.84E+05	24265.1	17	
Pre	AAV9	IgM	1.27E+05	79770.6	12	Pre vs Post-1 2wks (**)
Post-1 2wks			1.13E+06	1017145.6	17	
Post-1 4wks			4.53E+05	420357.8	17	
Post-2 2wks			8.07E+05	923925.9	17	
Post-2 6wks			1.46E+06	3677581.6	17	
Pre	AAV2	IgG	3.21E+04	11176	12	Pre vs Post-1 2wks (***), Pre vs Post-1 4wks (****), Pre vs Post-2 2wks (****), Pre vs Post-2 6wks (****), Post-1 2wks vs Post-1 4wks (**), Post-1 2wks vs Post-2 2wks (****), Post-1 2wks vs Post-2 6wks (****), Post-1 4wks vs Post-2 2wks (***), Post-1 4wks vs Post-2 6wks (*), Post-2 2wks vs Post-2 6wks (*)
Post-1 2wks			1.36E+05	64068.6	17	
Post-1 4wks			1.76E+05	62247.9	17	
Post-2 2wks			2.68E+05	66023.8	17	
Post-2 6wks			2.27E+05	49477.6	17	
Pre	AAV2	IgM	4.80E+05	545092.5	12	NS
Post-1 2wks			8.53E+05	861203.3	17	
Post-1 4wks			6.89E+05	1000190.8	17	
Post-2 2wks			9.59E+05	1138073.3	17	
Post-2 6wks			4.87E+06	16963076	17	
Pre	AAV8	IgG	3.15E+04	14651.4	12	Pre vs Post-1 2wks (*), Pre vs Post-1 4wks (****), Pre vs Post-2 2wks (****), Pre vs Post-2 6wks (****), Post-1 2wks vs Post-1 4wks (***), Post-1 2wks vs Post-2 2wks (****), Post-1 2wks vs Post-2 6wks (****), Post-1 4wks vs Post-2 2wks (**), Post-1 4wks vs Post-2 6wks (*)
Post-1 2wks			1.51E+05	99136.2	17	
Post-1 4wks			2.28E+05	86540.9	17	
Post-2 2wks			3.13E+05	64135.4	17	
Post-2 6wks			2.91E+05	62423	17	
Pre	AAV8	IgM	2.69E+05	163266.9	12	Pre vs Post-1 2wks (*)
Post-1 2wks			1.36E+06	1078257.4	17	
Post-1 4wks			7.35E+05	801383.8	17	
Post-2 2wks			1.71E+06	1875019.9	17	
Post-2 6wks			2.75E+06	6306277.9	17	
Pre	AAVMYO	IgG	3.08E+04	14318	12	Pre vs Post-1 2wks (****), Pre vs Post-1 4wks (****), Pre vs Post-2 2wks (****), Pre vs Post-2 6wks (****), Post-1 2wks vs Post-1 4wks (*), Post-1 2wks vs Post-2 2wks (****), Post-1 2wks vs Post-2 6wks (****)
Post-1 2wks			1.76E+05	31325.4	17	
Post-1 4wks			2.09E+05	43909.8	17	
Post-2 2wks			2.40E+05	37701.1	17	
Post-2 6wks			2.36E+05	32700.8	17	
Pre	AAVMYO	IgM	2.65E+05	210207.9	12	Pre vs Post-1 2wks (**)
Post-1 2wks			1.99E+06	1597467.7	17	
Post-1 4wks			8.58E+05	801882.4	17	
Post-2 2wks			1.25E+06	1496317.2	17	
Post-2 6wks			3.60E+06	11185584	17	
Pre	Cas9	IgG	3.03E+04	13822.1	12	NS
Post-1 2wks			3.05E+04	10423.7	17	
Post-1 4wks			3.39E+04	12144.6	17	
Post-2 2wks			3.08E+04	12618.2	17	
Post-2 6wks			3.23E+04	13766.3	17	
Pre	Cas9	IgM	1.02E+05	84877.4	12	NS
Post-1 2wks			7.07E+05	2490410.6	17	
Post-1 4wks			1.02E+05	60847.1	17	
Post-2 2wks			1.15E+05	125486	17	
Post-2 6wks			7.84E+04	67598.1	17	
Pre	µDYS	IgG	3.04E+04	14196.7	12	NS
Post-1 2wks			3.14E+04	10384.5	17	
Post-1 4wks			3.43E+04	11398.7	17	
Post-2 2wks			3.11E+04	12527.9	17	
Post-2 6wks			3.18E+04	9709.4	17	
Pre	µDYS	IgM	1.86E+05	155141.6	12	NS
Post-1 2wks			1.52E+05	158995.3	17	
Post-1 4wks			1.30E+05	73932.2	17	
Post-2 2wks			2.38E+05	319951.9	17	
Post-2 6wks			1.37E+05	92260.8	17	

Key: levels of statistical significance

$p < 0.05$ (*)

$p < 0.01$ (**)

$p < 0.001$ (***)

$p < 0.0001$(****)

Key: symbols in Figures 1 and S2 represent significance between comparisons

#: time points are significantly different compared to Pre

&: time points are significantly different compared to Post-1 (2 weeks)

$: time points are significantly different compared to Post-1 (4 weeks)

✣: time points are significantly different compared to Post-2 (2 weeks)

Table S3. Mean concentration of complement components in AAV9-Cas9, AAV9-Cas9-FS and AAV9-µDYS dosed *mdx* mice.

Vector	Complement	Pre	SD	N	Post-1 5hrs	SD	N	Post-1 2wks	SD	N	Post-2 5hrs	SD	N	Post-2 2wks	SD	N
Cas9-FS	C3 (mg/mL)	1.30	0.5	6	1.69	0.4	6	1.89	0.5	6	1.08	0.3	6	1.67	0.3	6
Cas9		1.29	0.4	6	2.02	1.0	6	1.77	0.5	6	1.27	0.7	6	1.70	0.6	6
µDYS		1.79	0.4	6	2.28	1.4	6	1.83	0.5	6	1.14	0.4	6	1.41	0.5	6
Combined		1.74	0.4	18	2.00	1.0	18	1.83	0.5	18	1.16	0.5	18	1.59	0.5	18
Cas9-FS	C4 (ng/mL)	146.03	33.1	3	95.70	7.3	3	94.25	13.0	3	54.85	14.6	3	68.48	21.3	3
Cas9		152.81	98.8	3	85.11	46.1	3	166.32	88.2	3	91.97	3.2	3	120.51	113.1	3
µDYS		140.06	11.7	3	126.88	38.3	3	37.10	22.0	3	72.27	7.5	3	42.85	26.2	3
Combined		146.30	52.7	9	102.57	35.6	9	99.22	72.5	9	73.03	18.1	9	77.28	68.3	9
Cas9-FS	C5b9 (ng/mL)	563.74	84.1	3	668.02	106.4	3	713.74	111.0	3	594.83	102.8	3	530.21	177.8	3
Cas9		484.20	68.3	3	552.42	162.5	3	542.70	77.3	3	358.20	27.5	3	559.47	63.4	3
µDYS		606.91	82.1	3	464.41	293.2	3	497.54	24.0	3	539.20	32.2	3	491.70	157.3	3
Combined		551.62	86.7	9	561.62	196.8	9	584.66	120.3	9	497.41	120.7	9	527.13	126.3	9

Table S4. Complement C3, C4 and C5b9 multiple comparison statistics performed on the combined vector data.

Time point	Complement	Average	SD	N	Tukey's multiple comparison test
Pre	C3 (mg/mL)	1.74	0.4	18	Pre vs Post-2 5hrs (****), Post-1 5hrs vs Post-2 5hrs (***), Post-1 2wks vs Post-2 5hrs (****), Post-2 5hrs vs Post-2 2wks (****)
Post-1 5hrs		2.00	1.0	18	
Post-1 2wks		1.83	0.5	18	
Post-2 5hrs		1.16	0.5	18	
Post-2 2wks		1.59	0.5	18	
Pre	C4 (ng/mL)	146.30	52.7	9	Pre vs Post-2 5hrs (*)
Post-1 5hrs		102.57	35.6	9	
Post-1 2wks		99.22	72.5	9	
Post-2 5hrs		73.03	18.1	9	
Post-2 2wks		77.28	68.3	9	
Pre	C5b9 (ng/mL)	551.62	86.7	9	NS
Post-1 5hrs		561.62	196.8	9	
Post-1 2wks		584.66	120.3	9	
Post-2 5hrs		497.41	120.7	9	
Post-2 2wks		527.13	126.3	9	

Key: levels of statistical significance

$p < 0.05$ (*)

$p < 0.01$ (**)

$p < 0.001$ (***)

$p < 0.0001$(****)

Key: symbols in Figures 2B-C and S3A represent significance between comparisons

†: time points are significantly different compared to Post-2 (5 hours)

Table S5. Mean concentration (pg/mL) of analytes in plasma from AAV9-Cas9, AAV9-Cas9-FS and AAV9-μDYS dosed *mdx* mice.

Vector	Analyte	Pre	SD	N	Post-1 5hrs	SD	N	Post-1 2wks	SD	N	Post-2 5hrs	SD	N	Post-2 2wks	SD	N
Cas9-FS	IP-10	776.53	300.0	6	1151.27	660.3	6	484.70	170.5	6	3156.87	1382.4	6	544.69	187.7	6
Cas9		710.99	368.3	6	568.40	336.4	6	200.56	201.2	6	6055.32	4834.8	6	320.28	173.7	6
μDYS		594.33	247.1	6	954.98	1015.4	6	383.75	211.0	6	2654.30	1840.6	6	514.14	155.7	6
Combined		693.95	300.6	18	891.55	725.8	18	356.34	219.5	18	3955.49	3288.2	18	459.70	191.9	18
Cas9-FS	MIP-1β	0.76	0.3	6	2.14	2.6	6	0.76	0.3	6	3.73	5.0	6	1.16	0.6	6
Cas9		0.67	0.2	6	1.10	0.4	6	0.85	0.4	6	4.08	2.4	6	1.10	0.5	6
μDYS		0.26	0.2	6	1.19	1.8	6	0.50	0.6	6	3.18	4.7	6	1.15	1.4	6
Combined		0.56	0.3	18	1.48	1.8	18	0.70	0.4	18	3.66	4.0	18	1.14	0.9	18
Cas9-FS	MCP-1	89.73	42.0	6	260.12	269.2	6	111.06	53.1	6	244.08	45.6	6	89.87	35.1	6
Cas9		102.95	26.8	6	118.98	24.4	6	151.80	108.2	6	611.33	402.6	6	55.90	44.3	6
μDYS		92.83	55.5	6	161.89	145.7	6	66.96	56.9	6	175.73	109.7	6	48.99	41.4	6
Combined		95.17	40.9	18	180.33	177.3	18	109.94	80.6	18	343.72	300.9	18	64.92	42.2	18
Cas9-FS	TNF-α	3.14	1.9	6	7.21	4.2	6	5.42	1.1	6	7.13	3.4	6	5.27	2.6	6
Cas9		2.99	0.7	6	3.84	1.8	6	3.63	1.6	6	6.23	2.2	6	2.30	1.0	6
μDYS		2.48	1.9	6	3.74	3.0	6	3.34	2.0	6	5.83	3.3	6	4.61	3.5	6
Combined		2.87	1.5	18	4.93	3.4	18	4.13	1.8	18	6.40	2.9	18	4.06	2.7	18
Cas9-FS	IL-6	19.39	15.3	6	70.32	59.9	6	24.96	22.3	6	32.32	47.4	6	6.21	3.3	6
Cas9		11.29	7.8	6	48.94	35.2	6	5.99	3.9	6	120.07	249.6	6	2.95	0.7	6
μDYS		9.09	8.1	6	40.50	27.4	6	13.25	22.2	6	68.54	55.1	6	5.75	3.8	6
Combined		13.26	11.3	18	53.25	42.5	18	14.73	19.0	18	73.64	145.8	18	4.97	3.2	18
Cas9-FS	IL-15	3.07	0.4	6	4.71	2.0	6	3.18	0.6	6	3.05	0.7	6	2.36	1.0	6
Cas9		3.39	0.9	6	4.81	1.6	6	3.21	0.8	6	2.64	1.5	6	2.68	1.2	6
μDYS		3.36	1.1	6	3.52	1.2	6	2.43	1.3	6	2.34	1.4	6	2.29	0.8	6
Combined		3.27	0.8	18	4.35	1.6	18	2.94	1.0	18	2.67	1.2	18	2.44	1.0	18
Cas9-FS	MIP-2α	6.54	4.5	6	14.53	9.4	6	12.44	4.1	6	13.51	9.6	6	12.34	7.7	6
Cas9		7.88	3.5	6	11.53	4.0	6	9.88	2.9	6	11.52	2.8	6	12.05	8.1	6
μDYS		7.44	3.7	6	9.63	5.5	6	9.22	7.8	6	9.30	2.9	6	12.83	10.5	6
Combined		7.29	3.7	18	11.90	6.6	18	10.51	5.2	18	11.44	5.9	18	12.41	8.3	18
Cas9-FS	IL-18	1421.36	1497.9	6	990.88	777.9	6	308.98	224.3	6	372.14	212.1	6	179.18	42.6	6
Cas9		372.02	174.6	6	530.80	400.2	6	267.97	112.0	6	393.12	457.4	6	145.83	79.3	6
μDYS		282.34	112.7	6	325.73	130.2	6	210.87	154.6	6	269.82	93.7	6	293.83	62.6	6
Combined		691.91	977.6	18	615.81	558.5	18	262.60	165.0	18	345.02	283.6	18	206.28	88.3	18
Cas9-FS	IL-1α	12.10	6.7	6	19.76	10.4	6	10.70	6.2	6	24.00	25.3	6	23.62	16.9	6
Cas9		7.71	5.4	6	12.15	4.9	6	10.02	4.4	6	11.90	7.2	6	11.14	6.1	6
μDYS		12.39	4.3	6	15.77	6.0	6	14.39	13.0	6	12.12	5.9	6	13.33	7.0	6
Combined		10.73	5.7	18	15.89	7.8	18	11.71	8.4	18	16.01	15.7	18	16.03	11.9	18
Cas9-FS	IL-5	9.14	6.2	6	8.25	1.5	6	7.46	4.9	6	12.00	11.3	6	6.94	2.6	6
Cas9		5.53	3.2	6	6.50	2.5	6	8.86	3.4	6	10.24	7.9	6	6.54	5.0	6
μDYS		4.23	2.0	6	4.01	1.6	6	3.65	2.7	6	10.29	10.8	6	3.37	1.7	6
Combined		6.30	4.5	18	6.25	2.5	18	6.66	4.2	18	10.85	9.5	18	5.62	3.6	18
Cas9-FS	IL-7	17.80	10.6	6	18.05	14.2	6	14.08	10.8	6	12.50	6.1	6	21.38	17.7	6
Cas9		10.77	4.5	6	20.22	12.8	6	9.96	4.9	6	6.57	2.5	6	17.74	19.1	6
μDYS		9.88	5.4	6	8.62	4.0	6	17.28	12.4	6	5.00	1.5	6	12.56	5.4	6
Combined		12.81	7.8	18	15.63	11.8	18	13.77	9.8	18	8.02	4.9	18	17.22	14.9	18
Cas9-FS	IL-9	90.85	48.4	6	102.33	63.9	6	78.83	45.0	6	55.60	34.6	6	79.71	37.2	6
Cas9		44.40	15.9	6	61.44	36.3	6	64.96	13.1	6	41.49	34.6	6	62.28	51.3	6
μDYS		41.08	15.1	6	33.03	21.3	6	98.66	123.1	6	31.34	20.5	6	39.06	22.1	6
Combined		58.78	37.1	18	65.60	50.8	18	80.81	72.9	18	42.81	30.5	18	60.35	40.2	18
Cas9-FS	IL-10	5.06	4.1	6	8.64	5.4	6	5.71	3.9	6	5.98	4.4	6	5.67	5.0	6
Cas9		3.03	2.1	6	5.10	4.1	6	4.29	2.3	6	5.84	1.9	6	4.53	5.0	6
μDYS		2.53	1.0	6	1.89	0.9	6	4.04	5.6	6	2.26	0.7	6	1.45	0.8	6
Combined		3.54	2.8	18	5.21	4.7	18	4.68	4.0	18	4.69	3.2	18	3.88	4.3	18
Cas9-FS	IL-13	40.83	24.5	6	53.05	38.0	6	44.30	31.1	6	27.26	18.2	6	34.11	22.6	6
Cas9		21.03	11.1	6	40.63	29.2	6	23.63	9.2	6	15.35	8.5	6	25.09	26.2	6
μDYS		22.14	5.5	6	15.60	12.9	6	25.86	18.5	6	11.04	6.7	6	15.97	13.9	6
Combined		28.00	17.6	18	36.43	31.3	18	31.26	22.4	18	17.89	13.5	18	25.05	21.6	18
Cas9-FS	MIG	191.39	150.5	6	305.89	169.5	6	146.10	145.9	6	224.58	100.7	6	100.81	86.5	6
Cas9		126.81	102.0	6	145.83	80.1	6	160.31	150.4	6	167.04	128.9	6	151.85	190.8	6
μDYS		172.32	141.8	6	185.95	218.1	6	94.67	88.9	6	140.63	110.8	6	103.40	62.9	6
Combined		163.50	128.1	18	212.56	171.0	18	133.69	126.8	18	177.42	113.1	18	118.69	121.1	18
Cas9-FS	MIP-1α	2.73	1.9	6	5.91	6.6	6	2.34	1.9	6	4.01	3.3	6	2.65	2.2	6
Cas9		0.93	0.6	6	2.95	1.7	6	1.25	0.6	6	1.79	0.8	6	1.61	1.4	6
μDYS		0.97	0.5	6	8.14	17.0	6	1.70	1.8	6	2.25	1.7	6	2.74	4.4	6
Combined		1.54	1.4	18	5.67	10.2	18	1.77	1.5	18	2.68	2.3	18	2.34	2.8	18
Cas9-FS	RANTES	65.72	14.7	6	108.61	43.9	6	62.40	14.7	6	95.93	50.9	6	75.67	36.2	6
Cas9		58.27	13.1	6	69.58	27.7	6	45.51	20.5	6	74.34	13.1	6	45.68	25.6	6
μDYS		52.39	22.1	6	59.05	22.9	6	56.60	35.0	6	52.40	15.9	6	60.03	14.8	6
Combined		58.79	17.0	18	79.08	37.8	18	54.84	24.5	18	74.22	35.0	18	60.46	28.3	18

Table S6. Multiple comparison statistics of analytes detected in plasma performed on the combined vector data.

Time point	Analyte	Average (pg/mL)	SD	N	Tukey's multiple comparison test
Pre	IP-10	693.95	300.6	18	Pre vs Post-1 2wks (**), Pre vs Post-2 5hrs (**), Pre vs Post-2 2wks (*), Post-1 5hrs vs Post-1 2wks (*), Post-1 5hrs vs Post-2 5hrs (*), Post-1 2wks vs Post-2 5hrs (**), Post-2 5hrs vs Post-2 2wks (**)
Post-1 5hrs		891.55	725.8	18	
Post-1 2wks		356.34	219.5	18	
Post-2 5hrs		3955.49	3288.2	18	
Post-2 2wks		459.70	191.9	18	
Pre	MIP-1β	0.56	0.3	18	Pre vs Post-2 5hrs (*), Post-1 2wks vs Post-2 5hrs (*)
Post-1 5hrs		1.48	1.8	18	
Post-1 2wks		0.70	0.4	18	
Post-2 5hrs		3.66	4.0	18	
Post-2 2wks		1.14	0.9	18	
Pre	MCP-1	95.17	40.9	18	Pre vs Post-2 5hrs (*), Post-1 2wks vs Post-2 5hrs (**), Post-2 5hrs vs Post-2 2wks (**)
Post-1 5hrs		180.33	177.3	18	
Post-1 2wks		109.94	80.6	18	
Post-2 5hrs		343.72	300.9	18	
Post-2 2wks		64.92	42.2	18	
Pre	TNF-α	2.87	1.5	18	Pre vs Post-2 5hrs (**), Post-1 2wks vs Post-2 5hrs (*)
Post-1 5hrs		4.93	3.4	18	
Post-1 2wks		4.13	1.8	18	
Post-2 5hrs		6.40	2.9	18	
Post-2 2wks		4.06	2.7	18	
Pre	IL-6	13.26	11.3	18	Pre vs Post-1 5hrs (**), Pre vs Post-1 2wks (*), Post-1 5hrs vs Post-1 2wks (**), Post-1 5hrs vs Post-2 2wks (**)
Post-1 5hrs		53.25	42.5	18	
Post-1 2wks		14.73	19.0	18	
Post-2 5hrs		73.64	145.8	18	
Post-2 2wks		4.97	3.2	18	
Pre	IL-15	3.27	0.8	18	Pre vs Post-1 5hrs (*), Pre vs Post-1 2wks (**), Post-1 5hrs vs Post-1 2wks (*), Post-1 5hrs vs Post-2 5hrs (*), Post-1 5hrs vs Post-2 2wks (***)
Post-1 5hrs		4.35	1.6	18	
Post-1 2wks		2.94	1.0	18	
Post-2 5hrs		2.67	1.2	18	
Post-2 2wks		2.44	1.0	18	
Pre	MIP-2α	7.29	3.7	18	Pre vs Post-1 5hrs (*)
Post-1 5hrs		11.90	6.6	18	
Post-1 2wks		10.51	5.2	18	
Post-2 5hrs		11.44	5.9	18	
Post-2 2wks		12.41	8.3	18	
Pre	IL-18	691.91	977.6	18	Post-1 5hrs vs Post-1 2wks (*)
Post-1 5hrs		615.81	558.5	18	
Post-1 2wks		262.60	165.0	18	
Post-2 5hrs		345.02	283.6	18	
Post-2 2wks		206.28	88.3	18	
Pre	IL-1α	10.73	5.7	18	NS
Post-1 5hrs		15.89	7.8	18	
Post-1 2wks		11.71	8.4	18	
Post-2 5hrs		16.01	15.7	18	
Post-2 2wks		16.03	11.9	18	
Pre	IL-5	6.30	4.5	18	NS
Post-1 5hrs		6.25	2.5	18	
Post-1 2wks		6.66	4.2	18	
Post-2 5hrs		10.85	9.5	18	
Post-2 2wks		5.62	3.6	18	
Pre	IL-7	12.81	7.8	18	NS
Post-1 5hrs		15.63	11.8	18	
Post-1 2wks		13.77	9.8	18	
Post-2 5hrs		8.02	4.9	18	
Post-2 2wks		17.22	14.9	18	
Pre	IL-9	58.78	37.1	18	NS
Post-1 5hrs		65.60	50.8	18	
Post-1 2wks		80.81	72.9	18	
Post-2 5hrs		42.81	30.5	18	
Post-2 2wks		60.35	40.2	18	
Pre	IL-10	3.54	2.8	18	NS
Post-1 5hrs		5.21	4.7	18	
Post-1 2wks		4.68	4.0	18	
Post-2 5hrs		4.69	3.2	18	
Post-2 2wks		3.88	4.3	18	
Pre	IL-13	28.00	17.6	18	NS
Post-1 5hrs		36.43	31.3	18	
Post-1 2wks		31.26	22.4	18	
Post-2 5hrs		17.89	13.5	18	
Post-2 2wks		25.05	21.6	18	
Pre	MIG	163.50	128.1	18	NS
Post-1 5hrs		212.56	171.0	18	
Post-1 2wks		133.69	126.8	18	
Post-2 5hrs		177.42	113.1	18	
Post-2 2wks		118.69	121.1	18	
Pre	MIP-1α	1.54	1.4	18	NS
Post-1 5hrs		5.67	10.2	18	
Post-1 2wks		1.77	1.5	18	
Post-2 5hrs		2.68	2.3	18	
Post-2 2wks		2.34	2.8	18	
Pre	RANTES	58.79	17.0	18	NS
Post-1 5hrs		79.08	37.8	18	
Post-1 2wks		54.84	24.5	18	
Post-2 5hrs		74.22	35.0	18	
Post-2 2wks		60.46	28.3	18	

Key: levels of statistical significance

$p < 0.05$ (*)

$p < 0.01$ (**)

$p < 0.001$ (***)

$p < 0.0001$(****)

Key: symbols in Figures 2D and S3B represent significance between comparisons

#: time points are significantly different compared to Pre

†: time points are significantly different compared to Post-1 (5 hours)

‡: time points are significantly different compared to Post-2 (5 hours)

Table S7. GSEA analysis using differentially expressed genes in *Clec4d*$^{+}$*Clec4e*$^{+}$ monocytes comparing Post-2 and Pre time points.

Gene Set	Description	NES	P Value	FDR	Genes
mmu05320	Autoimmune thyroid disease	-2.1396	0.002451	0.048902	H2-Q6, H2-Q7, H2-K1, H2-Eb1, H2-Ab1, H2-DMa, H2-T23, H2-Q4, H2-T22, Cd40
mmu05416	Viral myocarditis	-1.9869	0.005362	0.097804	H2-Q6, Dmd, H2-Q7, H2-K1, H2-Eb1, H2-Ab1, H2-DMa, H2-T23, H2-Q4, H2-T22
mmu00900	Terpenoid backbone biosynthesis	-1.9622	<2.2e-16	0.077823	Hmgcr, Zmpste24, Mvk, Fdps, Acat1
mmu04962	Vasopressin-regulated water reabsorption	-1.8845	0.004739	0.11042	Arhgdib, Dynll2, Rab5b, Dctn1, Dync1h1, Dync1i2, Prkacb, Creb1, Dync1li1, Rab5c
mmu04514	Cell adhesion molecules (CAMs)	-1.8411	0.002725	0.12494	H2-Q6, H2-Q7, H2-K1, H2-Eb1, Selplg, H2-Ab1, Cadm3, Sell, H2-DMa, Itgb1, H2-T23, H2-Q4, H2-T22
mmu04672	Intestinal immune network for IgA production	-1.7852	0.013761	0.14842	H2-Eb1, H2-Ab1, H2-DMa, Map3k14, Cd40
mmu05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	-1.6911	0.025229	0.24552	Dmd, Tcf7, Ctnna1, Slc8a1, Lef1, Itgb1, Tcf7l2, Cacnb3
mmu04612	Antigen processing and presentation	-1.5963	0.035714	0.2543	H2-Q6, H2-Q7, H2-K1, Tap2, Ifi30, Klrd1, H2-Eb1, Tapbp, Cd74, H2-Ab1, H2-DMa, H2-T23, H2-Q4, H2-T22, Psme3, Creb1
mmu00310	Lysine degradation	-1.5956	0.041463	0.23565	Setd1a, Kmt2b, Ash1l, Acat1, Ehmt2, Nsd2, Kmt2d, Plod3, Kmt2a, Setd1b
mmu05340	Primary immunodeficiency	-1.5876	0.042222	0.2284	Il2rg, Tap2, Cd40, Rfxap, Tap1
mmu04625	C-type lectin receptor signaling pathway	2.4507	<2.2e-16	<2.2e-16	Ptgs2, Clec4d, Clec4e, Nfkbia, Tnfll1b, Nlrp3, Itpr2, Clec4n, Fcer1g, Mapkapk2
mmu04621	NOD-like receptor signaling pathway	2.4526	<2.2e-16	<2.2e-16	Cxcl2, Cxcl3, Nfkbia, Tnf, Il1b, Nlrp3, Itpr2, Ccl2, Ctsb, Erbin, Tnfaip3, Casp4, Jun, Gabarap, Vdac2, Tank, Txn1
mmu04064	NF-kappa B signaling pathway	2.4686	<2.2e-16	<2.2e-16	Cxcl2, Ccl4, Ptgs2, Cd14, Nfkbia, Tnf, Gadd45b, Il1b, Bcl2a1b, Tnfaip3, Bcl2a1a, Cflar, Icam1
mmu04657	IL-17 signaling pathway	2.4712	<2.2e-16	<2.2e-16	Cxcl2, Cxcl3, Ptgs2, Nfkbia, Tnf, Il1b, Ccl2, Cebpb, Fos, Tnfaip3
mmu04620	Toll-like receptor signaling pathway	2.5526	<2.2e-16	<2.2e-16	Ccl3, Spp1, Ccl4, Cd14, Nfkbia, Tnf, Il1b, Fos, Map2k3, Jun, Tlr2, Myd88, Irak1, Tab2
mmu04142	Lysosome	2.5714	<2.2e-16	<2.2e-16	Atp6v0c, Ctsd, Ctsb, Litaf, Ctsl, Psap, Hgsnat, Cd68, Ap3s1, Lamp2, Gns, Atp6v0d2, Ctsa, Cita, Igf2r, Slc11a1, Neu1, Gla, Lgmn, Hexa, Npc1, Tpp1, Atp6v0d1, Lamp1, Atp6v0b
mmu05134	Legionellosis	2.6797	<2.2e-16	<2.2e-16	Cxcl2, Cxcl3, Cd14, Nfkbia, Tnf, Il1b
mmu05132	Salmonella infection	2.7148	<2.2e-16	<2.2e-16	Cxcl2, Ccl3, Cxcl3, Ccl4, Cd14, Il1a, Il1b, Rab7
mmu04668	TNF signaling pathway	2.8446	<2.2e-16	<2.2e-16	Cxcl2, Cxcl3, Ptgs2, Nfkbia, Tnf, Il1b, Ccl2, Csf1, Cebpb, Tnfrsf1b, Junb, Socs3, Fos, Tnfaip3, Atf4, Cflar, Bcl3, Icam1, Map2k3, Jun
mmu04060	Cytokine-cytokine receptor interaction	2.9309	<2.2e-16	<2.2e-16	Cxcl2, Ccl3, Il1rn, Cxcl3, Ccl4, Tnf, Il1a, Il1b, Ccl6, Ccl2, Il1f9, Csf1, Inhba, Tnfrsf1b, Il1r2, Ccr1, Gdf15, Il1rap

Table S8. GSEA analysis using differentially expressed genes in *Clec4d*$^{+}$*Clec4e*$^{+}$ monocytes comparing Post-2 and Post-1 time points.

Gene Set	Description	NES	P Value	FDR	Genes
mmu04390	Hippo signaling pathway	-1.9161	<2.2e-16	0.26287	Ccnd2, Limd1, Ccnd3, Smad7, Tgfb1, Id2, Tcf7l2, Lats1, Mob1a, Itgb2, Apc, Mob1b, Ywhaz, Bmpr2, Ppp2ca, Ppp2r1a, Tgfbr2
mmu03018	RNA degradation	-1.8778	0.006873	0.18065	Wdr61, Cnot3, Ddx6, Zcchc7, Cnot1, Cnot2, Dhx36, Ttc37, Xrn1, Pan3, Exosc3, Pfkl
mmu01522	Endocrine resistance	-1.7216	0.012324	0.43833	Ncor1, Rps6kb1, Bcl2, Cdkn1b, Map2k1, Adcy7, Raf1, Mapk14, Bad, Notch2, Bax, Braf, Pik3cb
mmu04261	Adrenergic signaling in cardiomyocytes	-1.6814	0.023853	0.44225	Pik3cg, Pik3r5, Pik3r6, Bcl2, Camk2d, Gnai3, Slc8a1, Adcy7, Mapk14, Adrb2, Creb1, Ppp2ca, Tpm3, Ppp2r1a, Atp1b1, Atf2, Atp2a2, Gnaq
mmu04650	Natural killer cell mediated cytotoxicity	-1.6291	0.024254	0.50073	Klrd1, Klrc2, Klrk1, Prf1, Fyn, Cd48, Itgal, Klra9, Klra7, Ifnar2, Prkcb, Klra8, Map2k1, Gzmb, Hcst, Raf1, H2-D1, Itgb2, Ptpn6, Nfatc1, Ncr1
mmu04550	Signaling pathways regulating pluripotency of stem cells	-1.622	0.027273	0.43463	Meis1, Rif1, Tcf3, Skil, Map2k1, Id2, Raf1, Mapk14, Apc, Bmpr2
mmu04012	ErbB signaling pathway	-1.5862	0.041812	0.46751	Cbl, Nck1, Stat5b, Rps6kb1, Cdkn1b, Prkcb, Map2k1, Camk2d, Raf1, Crkl
mmu00280	Valine, leucine and isoleucine degradation	-1.5715	0.033088	0.44709	Hadhb, Aacs, Hibadh, Hmgcs1
mmu04928	Parathyroid hormone synthesis, secretion and action	-1.1361	0.30319	0.67325	Akap13, Mef2d, Mef2c, Bcl2, Prkcb, Map2k1, Gnai3, Adcy7, Lrp6, Raf1, Mef2a, Arhgef11
mmu04392	Hippo signaling pathway	-1.1288	0.34862	0.67493	Limd1, Lats1, Mob1a, Mob1b
mmu04657	IL-17 signaling pathway	2.5785	<2.2e-16	<2.2e-16	Cxcl2, Nfkbia, Ptgs2, Cxcl3, Ccl2, Tnf, Tnfaip3, Il1b
mmu05143	African trypanosomiasis	2.6533	<2.2e-16	<2.2e-16	Hba-a1, Hbb-bs, Hba-a2, Tnf, Hbb-bt, Il1b, Icam1, Fas
mmu04621	NOD-like receptor signaling pathway	2.6624	<2.2e-16	<2.2e-16	Cxcl2, Nfkbia, Itpr2, Cxcl3, Nlrp3, Ccl2, Txn1, Tnf, Tnfaip3, Il1b, Nampt, Nfkbib, Gabarap, Tank, Hsp90ab1, Pstpip1
mmu04640	Hematopoietic cell lineage	2.6752	<2.2e-16	<2.2e-16	H2-Eb1, H2-Aa, Il1a, Cd14, H2-Ab1, Tnf, Il1b, Itgb3, Cd24a, Cd9
mmu05140	Leishmaniasis	2.7002	<2.2e-16	<2.2e-16	Nfkbia, Ptgs2, Marcksl1, H2-Eb1, H2-Aa, Il1a, H2-Ab1, Tnf, Il1b, Nfkbib
mmu05132	Salmonella infection	2.7635	<2.2e-16	<2.2e-16	Cxcl2, Ccl3, Cxcl3, Il1a, Cd14, Ccl4, Il1b, Rac1, Plekhm2, Actg1
mmu05134	Legionellosis	2.8207	<2.2e-16	<2.2e-16	Cxcl2, Nfkbia, Cxcl3, Cd14, Tnf, Il1b, Arf1
mmu04668	TNF signaling pathway	2.865	<2.2e-16	<2.2e-16	Cxcl2, Nfkbia, Ptgs2, Cxcl3, Ccl2, Tnf, Tnfaip3, Il1b, Junb, Atf4, Icam1
mmu04060	Cytokine-cytokine receptor interaction	2.893	<2.2e-16	<2.2e-16	Cxcl2, Ccl3, Cxcl3, Il1a, Ccl2, Il1f9, Ccl6, Il1rn, Tnf, Ccl4, Ccr1, Il1b, Ppbp, Ccr7, Cxcl16, Tnfrsf1b, Pf4, Fas, Gdf15, Cxcr4
mmu04064	NF-kappa B signaling pathway	3.1212	<2.2e-16	<2.2e-16	Cxcl2, Nfkbia, Ptgs2, Gadd45b, Bcl2a1b, Cd14, Tnf, Ccl4, Bcl2a1a, Tnfaip3, Il1b, Icam1, Ube2i, Bcl2a1d

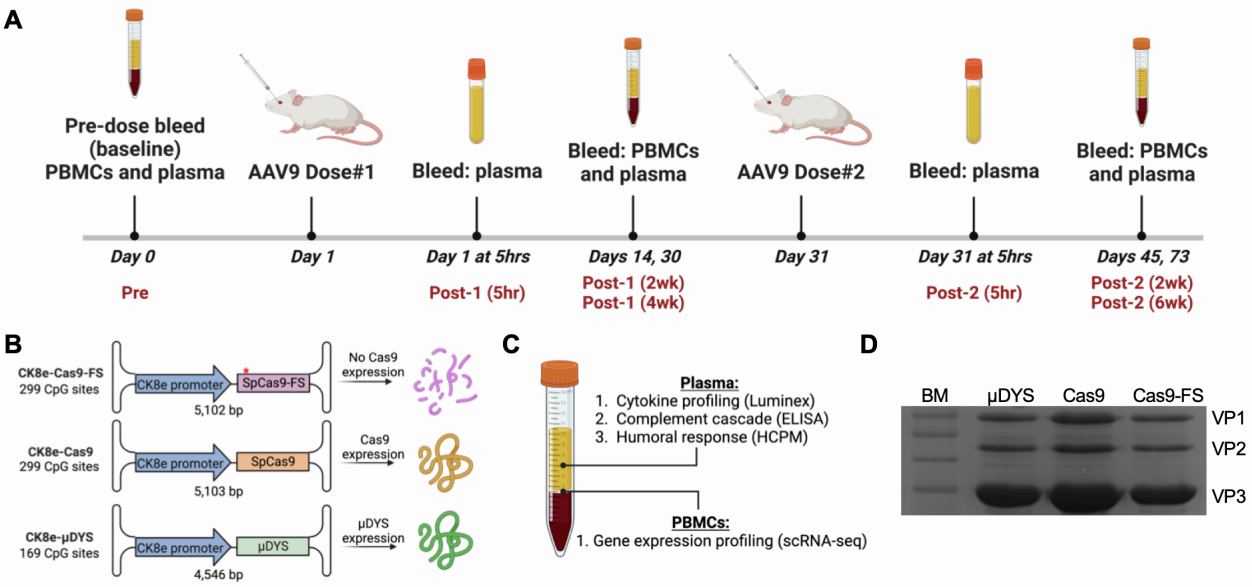

Figure S1. Experimental timeline to characterize AAV9-mediated immune responses in dual AAV dosed *mdx* mice.

A) Schematic timeline to study AAV9-induced immune responses in *mdx* mice. n=9 male and n=9 female mice (3 mice per AAV9 vector) dosed at ~1.16 x 10^{14} vg/kg. Created with BioRender.com.

B) Schematic of AAV vectors used to deliver Cas9-FS, Cas9, and µDYS. The asterisks (*) for Cas9-FS indicates a frameshift mutation located near the N-terminus of the Cas9 coding sequence, generating a pre-mature stop codon. Created with BioRender.com.

C) Plasma samples were used to assess cytokine responses (Luminex), complement levels (ELISAs), and antibody responses via high content protein microarray (HCPM). PBMCs were characterized by scRNA-seq. Created with BioRender.com.

D) Coomassie stained SDS-PAGE of the recombinant AAV9-µDYS, AAV9-Cas9, and AAV9-Cas9-FS vectors used in the study. 1.0 x 10^{12} vg of AAV was loaded per lane.

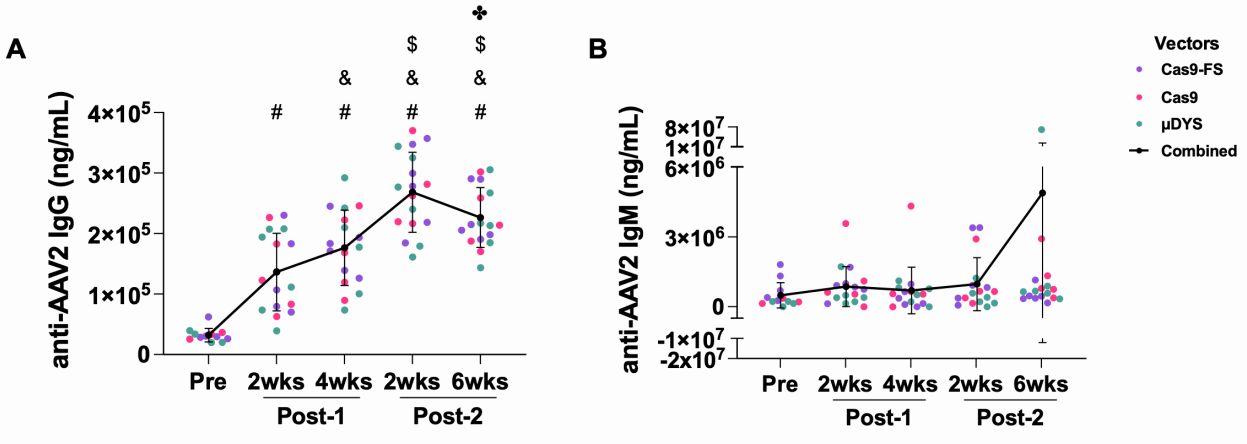

Figure S2. Assessment of anti-AAV2 antibody responses using a high content protein microarray (HCPM).

A-B) anti-AAV2 IgG and IgM responses were measured via high content protein microarray (HCPM) from male (n=9) and female (n=8) *mdx* mice at indicated time points. The key indicates the vector that was dosed and the black line represents AAV9-treated groups combined by time point, which was used for multiple comparison statistical analysis. Error bars for all HCPM graphs represent standard deviation. Symbols above time points are used to represent statistical significance, in which $p < 0.05$, for time points compared to: (#) Pre, (&) Post-1 (2 weeks), ($) Post-1 (4 weeks), and (✤) Post-2 (2 weeks). The data and levels of statistical significance between time point comparisons are available in Tables S1 and S2.

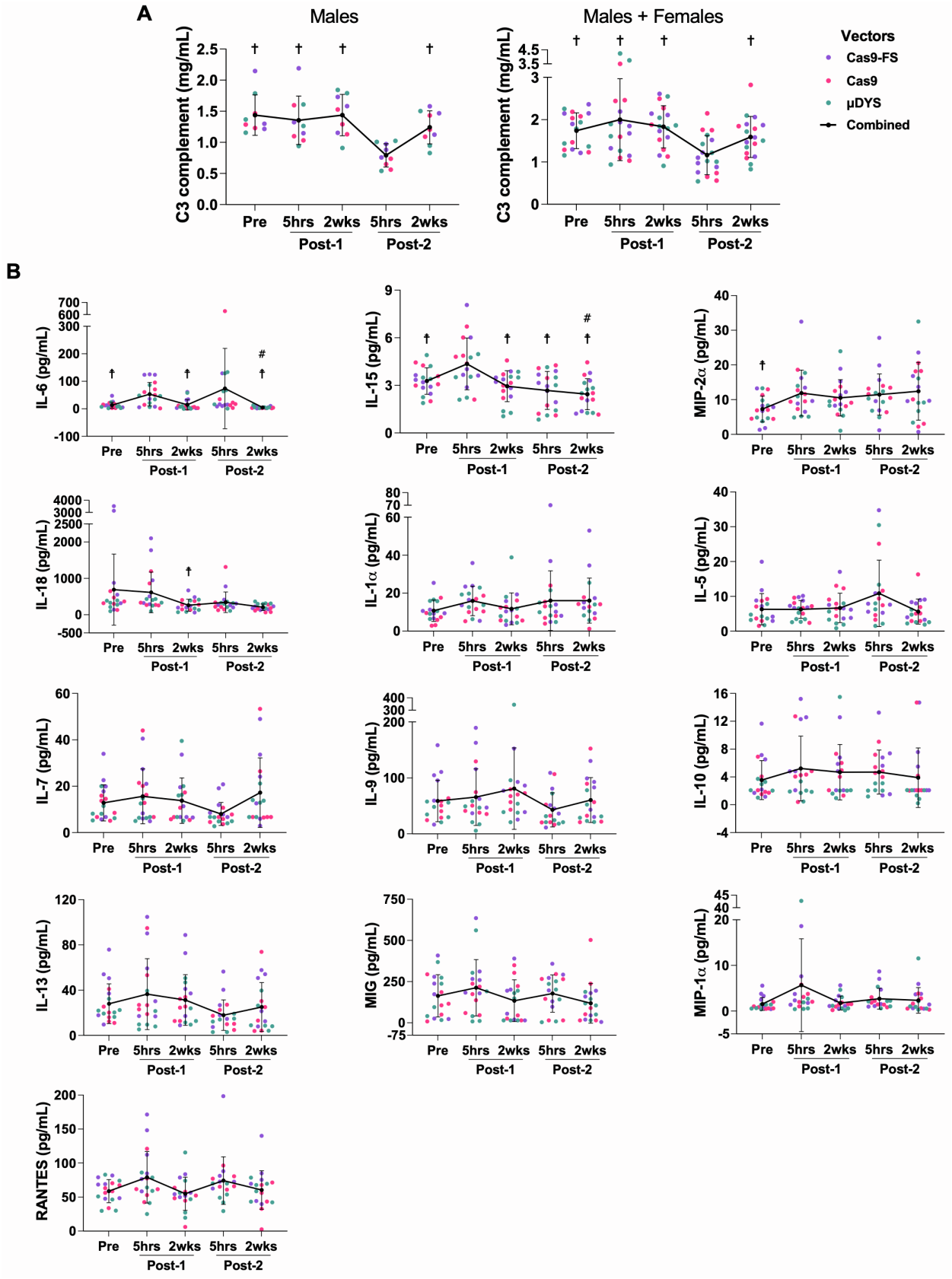
A
Males
Males + Females
C3 complement (mg/mL)
Pre
5hrs
2wks
5hrs
2wks
Post-1
Post-2
Vectors
Cas9-FS
Cas9
µDYS
Combined
B
IL-6 (pg/mL)
IL-15 (pg/mL)
MIP-2α (pg/mL)
IL-18 (pg/mL)
IL-1α (pg/mL)
IL-5 (pg/mL)
IL-7 (pg/mL)
IL-9 (pg/mL)
IL-10 (pg/mL)
IL-13 (pg/mL)
MIG (pg/mL)
MIP-1α (pg/mL)
RANTES (pg/mL)

Figure S3. Complement C3 levels in male and female *mdx* mice and remaining cytokine responses in AAV double-dosed mice.

A) Plasma from *mdx* mice was evaluated by ELISA for levels of complement C3. Left graph shows (n=9 males) and right graph shows n=9 females and n=9 males. C3 graph of females only (n=9) is shown in Figure 2A. The key indicates the vector that was dosed and the black line represents AAV9-treated groups combined by time point, which was used for multiple comparison statistical analysis. Error bars for all graphs represent standard deviation. Symbols above time points are used to represent statistical significance, in which $p < 0.05$, for time points compared to (†) Post-2 (5 hours). The data and levels of statistical significance between time point comparisons are available in Tables S3 and S4.

B) Levels of immunomodulatory analytes measured by Luminex ProcartaPlex, n=9 males and n=9 females. Symbols above time points are used to represent statistical significance, in which $p < 0.05$, for time points compared to: (#) Pre and (†) Post-1 (5 hours). The data and levels of statistical significance between time point comparisons are available in Tables S5 and S6.

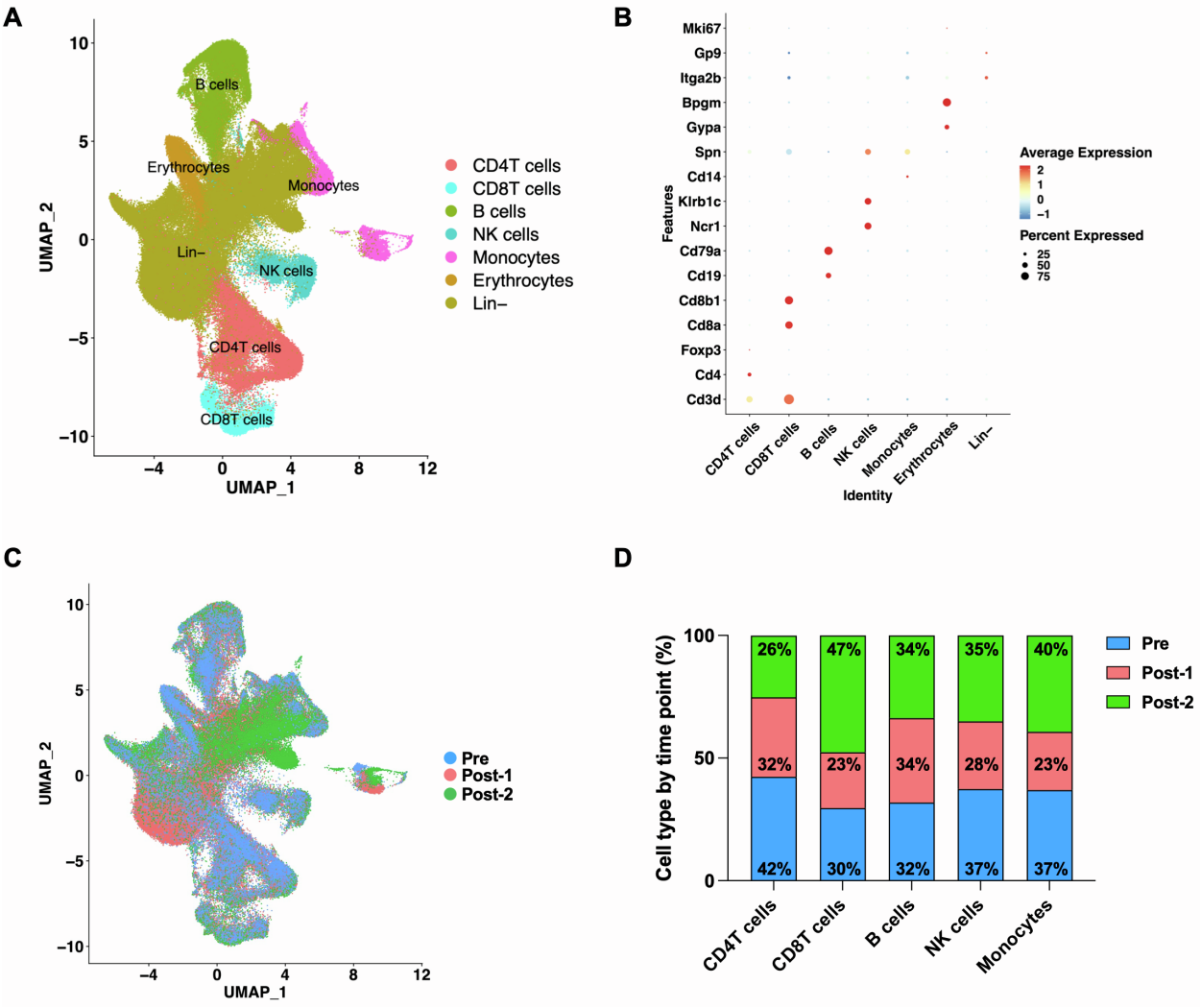
A
B cells
Erythrocytes
Monocytes
Lin–
NK cells
CD4T cells
CD8T cells
UMAP_2
UMAP_1
CD4T cells
CD8T cells
B cells
NK cells
Monocytes
Erythrocytes
Lin–
B
Mki67
Gp9
Itga2b
Bpgm
Gypa
Spn
Cd14
Klrb1c
Ncr1
Cd79a
Cd19
Cd8b1
Cd8a
Foxp3
Cd4
Cd3d
Features
Identity
Average Expression
Percent Expressed
C
Pre
Post-1
Post-2
D
Cell type by time point (%)
26%
47%
34%
35%
40%
32%
23%
34%
28%
23%
42%
30%
32%
37%
37%

Figure S4. PBMC single-cell classification for AAV9 double-dosed *mdx* mice.

A) Uniform Manifold Approximation and Projection (UMAP) of main immune cell types: T cells, B cells, NK cells, and Monocytes.

B) Dot plot of gene expression for canonical immune cell populations. Color is scaled by average expression and the dot size is proportional to the percent of cells expressing the respective gene.

C) Uniform Manifold Approximation and Projection (UMAP) color coded by time point before and after AAV dosing. Pre (blue), Post-1 (pink) and Post-2 (green).

D) Bar graph shows the percentage of T cells, B cells, NK cells and monocytes by time point. Pre (blue), Post-1 (pink) and Post-2 (green).

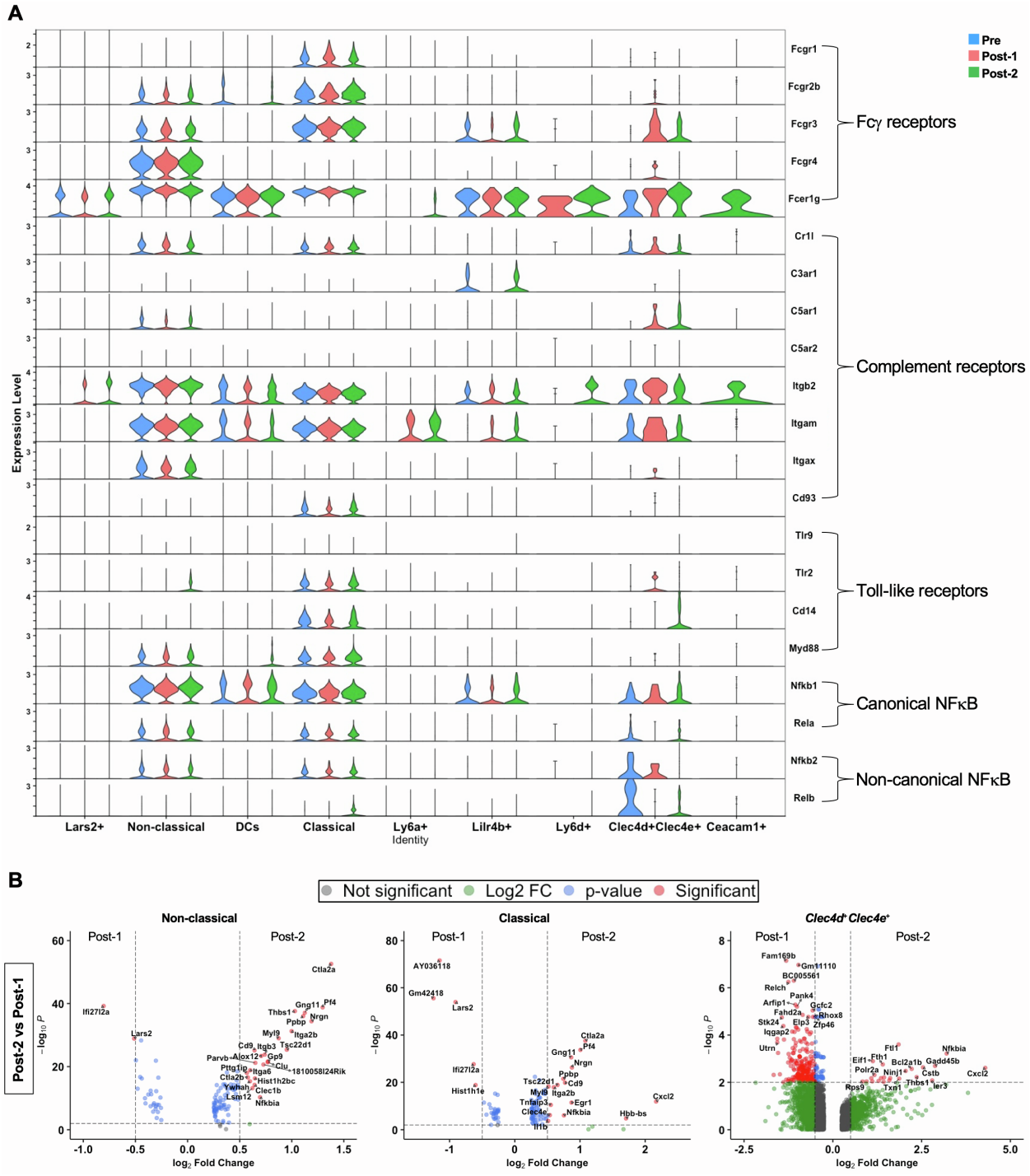
A
Pre
Post-1
Post-2
Fcgr1
Fcgr2b
Fcgr3
Fcgr4
Fcer1g
Fcγ receptors
Cr1l
C3ar1
C5ar1
C5ar2
Itgb2
Itgam
Itgax
Cd93
Complement receptors
Tlr9
Tlr2
Cd14
Myd88
Toll-like receptors
Nfkb1
Rela
Canonical NFκB
Nfkb2
Relb
Non-canonical NFκB
Expression Level
Lars2+
Non-classical
DCs
Classical
Ly6a+
Lilr4b+
Ly6d+
Clec4d+Clec4e+
Ceacam1+
Identity
B
Not significant
Log2 FC
p-value
Significant
Non-classical
Classical
Clec4d+ Clec4e+
Post-1
Post-2
Post-2 vs Post-1
−log10 P
log2 Fold Change

Figure S5. Differential gene expression among monocyte sub-populations.

A) Stacked violin plot of FcγRs, complement receptors, TLRs, canonical and non-canonical NF-κB gene expression in all monocyte sub-populations.

B) Volcano plots of classical, non-classical and *Clec4d*$^{+}$*Clec4e*$^{+}$ monocytes showing differentially expressed genes between Post-2 (2wk) and Post-1 (2wk). Significantly up- and down-regulated genes contain a Log_2(Fold Change) > 0.5 or Log_2(Fold Change) < 0.5 and -Log_{10}(*p*-value) > 2.0.