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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 | e S1. | HCPM | mean | conce | en | ntratior | ו (ng/ | m | IL) of a | nti-A | A | V and | anti-t | ra | ansgen | e IgG | Ì |
|---------|---------|----------------|----------|----------------------|----|-------------|---------|---|-------------|---------|----|-------------|---------------|------------|-------------|---------|---|
| and I | gM f | or AAV | 9-Cas | 9, <mark>AA</mark> \ | /9 | -Cas9- | ·FS a | n | d AAV | 9-μD\ | (S | dose | d <i>md</i> 2 | x I | mice. | | |
| Vector | Antigen | Immunogloublin | Pre | SD | Ν | Post-1 2wks | SD | Ν | Post-1 4wks | SD | Ν | Post-2 2wks | SD | Ν | Post-2 6wks | SD | Ν |
| Cas9-FS | | | 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-FS | | | 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 | | 1-0 | 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 | | ige | 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 | 4 41 /0 | | 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 | AAV9 | | 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 |
| uDYS | | IgM | 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 | | | 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 |
| uDYS | | lgG | 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-ES | AAV2 | | 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 |
| UDYS | | lgM | 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 |
| Caco ES | | | 2 795+04 | 15016 1 | 5 | 1.62E±05 | 103103.3 6 | 2.21E±05 | 90622.0 6 | 3.00E+05 | 90220.2 6 | 2.84E±05 | 74222.2 6 |
| Case | | | 2 195+04 | 5424.2 | 2 | 1.02E+05 | 94102.5 0 | 1 995+05 | 90022.0 0 | 2 16E±05 | 62761 7 5 | 2.04E+05 | 57972.2 5 |
| UDVS | | lgG | 3.10E+04 | 17529.0 | 4 | 1.54E+05 | 121210.9 6 | 2.60E+05 | 81400.0 6 | 3.10E+05 | 559777 G | 2.13E+05 | 59220.1 6 |
| Combined | | | 2.32E+04 | 14661.4 | 4 | 1.54E+05 | 00126.2 17 | 2.09E+05 | 86540.0 17 | 3.24E+05 | 64125 4 17 | 3.12E+05 | 62422 17 |
| Combined | AAV8 | | 3.15E+04 | 14651.4 | 12 | 1.51E+05 | 99130.2 17 | 2.28E+05 | 86540.9 17 | 3.13E+05 | 04135.4 17 | 2.91E+05 | 02423 17 |
| Cas9-F5 | | | 3.83E+05 | 206258.8 | 5 | 1.89E+06 | 1326187.0 6 | 5.95E+05 | 848117.5 6 | 2.00E+00 | 20//80/.9 0 | 1.17E+06 | 1586331.2 6 |
| Case | | lgM | 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 | 92/154.3 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 | | | 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 | | laG | 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 | | 0 | 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 | AAVMYO | | 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 | | | 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 | | laM. | 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 | | .9 | 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 | | | 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 | | InG. | 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 | | igo | 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 | Car0 | | 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 | Case | | 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 | | la M | 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 | | igivi | 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 | | | 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 | | IgG | 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 | μDYS | | 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 |
| UDYS | | lgM | 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 |
| Somonieu | | | | .00141.0 | 14 | | 130330.0 17 | | .0002.2 17 | 2.000.00 | 010001.0 17 | | 02200.0 17 |

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

| Phil Sole-14 S | Time point | Antigen | Immunobulin | Average (ng/mL) | SD | Ν | Tukey's multiple comparison test | Key: levels | s of statistica | l significance | | | | |
|--|----------------------|---------|-------------|-----------------|------------|----|--|---------------|-----------------|-------------------|--------------|----------------|------------|---------|
| Past Jossi, Jossi J | Pre | | | 3.05E+04 | 14334.69 | 12 | Pre vs Post-1 2wks (****), Pre vs Post-1 4wks (****), Pre vs Post-2 | p < 0.05 (* |) | | | | | |
| Dial Laws AV9 Pict 107 2882.811 17 4044 (m) Pict 2 / 2045 (") p < 0.001 (") | Post-1 2wks | | | 1.20E+05 | 21743.74 | 17 | 2wks (****), Pre vs Post-2 6wks (****), Post-1 2wks vs Post-1 | p < 0.01 (* | *) | | | | | |
| Pack2 dws 154:640 2415:03 17 Pack2 dws (n) Pack | Post-1 4wks | AAV9 | lgG | 1.57E+05 | 28828.11 | 17 | 4wks (***), Post-1 2wks vs Post-2 2wks (****), Post-1 2wks vs | p < 0.001 (| ***) | | | | | |
| Pack 2 wins | Post-2 2wks | | | 1.94E+05 | 34125.03 | 17 | Post-2 6wks (****), Post-1 4wks vs Post-2 2wks (**), Post-1 4wks | p < 0.0001 | (****) | | | | | |
| Petal Zavis AVV Ipt 1.28/2-06 TVPC106 TV Peat Zavis AVV Ipt 4.58/2-05 42057.01 TV Pave Post 2/avis Key: synchis in Figures 1 and 22 represent algorificance belower comparisons Peat Zavis 4.58/2-05 42057.01 TV Pave Post 1 2wis (") Key: synchis in Figures 1 and 22 represent algorificance belower comparisons Peat Zavis 1.48/2-06 42057.01 TV Pave Post 1 2wis (") Key: synchis in Figures 1 and 32 represent algorificance belower comparisons Peat Zavis 1.48/2-06 42057.01 TV Pave Post 2/4 (west) Key: synchis in Figures 1 and 32 represent algorificance belower comparisons Peat Zavis 1.48/2-06 42057.01 TV Pave Post 2/4 (west) Key: synchis in Figures 1 and 32 represent algorificance belower comparisons Peat Zavis 1.48/2-06 42057.01 TV Pave Post 2/2 west) Key: synchis in Figures 1 and 32 represent algorificance belower comparisons Peat Zavis 42050.01 1.72 keys Post 2/2 west ("Post 2/2 keys ("Post 2/ | Post-2 6wks | | | 1.84E+05 | 24265.1 | 17 | vs Post-2 6wks (*) | | 1 | | | | | |
| Piet 2 day Part 2 day | Pre | | | 1.27E+05 | 79770.6 | 12 | | Kev: symb | ols in Figure | s 1 and S2 re | epresent sia | nificance bet | ween comp | arisons |
| Pate 1 dots AV/9 IgAt 1 4 458 ± 00 200 ± 76 ± 71 Prove Post 1 dots (1') A time points are significantly different compared to Post 1 (a wesk) Past 2 dots 3 07 ± 05 ± 05 ± 05 ± 05 ± 05 ± 05 ± 05 ± | Post-1 2wks | | | 1.13E+06 | 1017145.6 | 17 | | #: time poir | nts are signif | icantly differe | nt compare | d to Pre | | |
| Diable 2 basis Bo7E-50 222025 17 State 4000 State 40000 State 40000 State 4000 | Post-1 4wks | AAV9 | laM. | 4.53E+05 | 420357.8 | 17 | Pre vs Post-1 2wks (**) | & time poi | nts are signi | ficantly differe | ent compare | d to Post-1 (2 | weeks) | |
| Part 144E-00 177 Part < | Post-2 2wks | | 0 | 8 07E+05 | 923925.9 | 17 | | \$: time poir | nts are signif | icantly differe | ent compare | to Post-1 (4 | weeks) | |
| Pre 321E-04 11176 12 Prev Post-1 dess (""), Prev Post-2 dess (""), Prev Post-2 Prev Post-2 | Post-2 6wks | | | 1.46E+06 | 3677581.6 | 17 | | +: time poi | nts are sign | ificantly differ | ent compare | d to Post-2 (| 2 weeks) | |
| Test: 2 data 138E:06 64082.8 if 7 2 dws (***). Post 1 dws vs Post-1 dws vs Post-1 Pest: 2 dws 228E:05 66023.8 if 7 2 dws (**). Post 2 dws (*) Post 2 dws vs 2 Pest: 2 dws 228E:05 66023.8 if 7 2 dws (**). Post 2 dws (*). Post 2 dws vs 2 2 Pest: 2 dws 2 dws (*). Post 2 dws (*). Post 2 dws (*). Post 2 dws (*). Post 2 dws (*). 2 2 Pest: 2 dws 3 dSt=0.6 de 6023.8 if 7 Post 2 dws (*). Post 2 dws (*). Post 2 dws (*). 2 | Pre | | | 3 21E+04 | 11176 | 12 | Pre ve Poet-1 2wke (***) Pre ve Poet-1 (wke (****) Pre ve Poet-2 | e i unio poi | into are orgin | incurrent y annor | on compare | | 2 1100110) | |
| Dest: 1 dats AV/2 UP Trace 1/2 dats UP Trace 1/2 dats UP | Post-1 2wks | | | 1.36E+05 | 64068.6 | 17 | 2wke (****) Pre ve Poet-2 6wke (****) Poet-1 2wke ve Poet-1 | | | | | | | |
| Desc:2 bysis Desc:2 bysis< | Post-1 4wks | AAV2 | laG | 1.76E+05 | 62247.9 | 17 | 4wks (**) Post-1 2wks vs Post-2 2wks (****) Post-1 2wks vs Post- | | | | | | | |
| Desit 2 basis 2 271:03 4 4977.6 17 Post 2 basis 0 0 0 0 Pest 1 basis 6 551:00 6 551:00 6 551:00 | Poet-2 2wke | | 190 | 2.68E+05 | 66023.8 | 17 | 2 6wks (****). Post-1 4wks vs Post-2 2wks (***). Post-1 4wks vs | | | | | | | |
| Control Addle 103 5 4500-20 12 Control Control <thcontrol< th=""> <thcontrol< th=""> <thco< th=""><td>Post-2 £wks</td><td></td><td></td><td>2.002+05</td><td>49477.6</td><td>17</td><td>Post-2 6wks (*). Post-2 2wks vs Post-2 6wks (*)</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></thco<></thcontrol<></thcontrol<> | Post-2 £wks | | | 2.002+05 | 49477.6 | 17 | Post-2 6wks (*). Post-2 2wks vs Post-2 6wks (*) | | | | | | | |
| Pash 2 with 3 bit 3 | Pre | | | 4.80E+05 | 545092.5 | 12 | | | | | | | | |
| Dist 1 with AVA2 IgAV1 SSSE*03 1000 1000 11 NS Image: NSS 1000 1000 1000 1000 1000 1000 1000 | Poet-1 2wke | | | 9.53E±05 | 861203.3 | 17 | | | | | | | | |
| Instruction | Post 1 Awks | AAV/2 | IaM. | 6 90E+05 | 10001203.5 | 17 | NS | | | | | | | |
| Index 2 bits Index 2 bits< | Post-1 4wks | 74442 | igini | 0.092+05 | 1128072.2 | 17 | 10 | | | | | | | |
| Prost-Davis Prost-Davis Post-1 davis Post-1 davis Post-1 davis Post-2 dav | Post-2 2wks | | | 9.59E+05 | 16062076 | 17 | | | | | | | | |
| Image Joint Joints Li Provide Joints Li Provide Joints Joint | POSI-2 OWKS | | | 4.07 E+00 | 14661.4 | 12 | Design Design (1) Design Design (1) (1) | | | | | | | |
| Index:14wis AVUS Igo Index:14wis AWUS Index:14wis Index:14wis <thinde< th=""><td>Pre Dest 4 Outles</td><td></td><td></td><td>3.15E+04</td><td>14651.4</td><td>12</td><td>Pre vs Post-1 2wks (*), Pre vs Post-1 4wks (*****), Pre vs Post-2</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></thinde<> | Pre Dest 4 Outles | | | 3.15E+04 | 14651.4 | 12 | Pre vs Post-1 2wks (*), Pre vs Post-1 4wks (*****), Pre vs Post-2 | | | | | | | |
| Post-1 Awis Post-2 | Post-1 2wks | A A1/0 | 1=0 | 1.51E+05 | 99136.2 | 17 | 2WKS (****), Pre VS POSt-2 6WKS (****), Post-1 2WKS VS POSt-1 Audre (****), Post-1 2udre ve Post-2 2udre (*****), Post-1 2udre ve | | | | | | | |
| Post-2 wiks 2.915-05 6.915.4 17 verbic wiks (*) 0.905-1 | Post-1 4wks | AAVO | ige | 2.28E+05 | 86540.9 | 17 | 4WKS (), POSE-1 2WKS VS POSE-2 2WKS (), POSE-1 2WKS VS Peet 2 Funks (***) Peet 1 Aurks us Peet 2 2 Junks (**) Peet 1 Aurks | | | | | | | |
| Prost-2 wrks 2.508-103 6.242.5 1 Or Out Control (1) Prost-1 wrks AVM8 Max 1.36E-06 1078257.4 17 Post-1 wrks AVM8 Max 1.36E-06 1078257.4 17 Post-2 wrks 2.75E-06 30328-07 17 Pre vs Post-1 2wks (**) 0 0 Post-2 wrks 2.75E-06 30328-10 17 Pre vs Post-1 2wks (***), Pre vs Post-2 0 </th <td>Post-2 2wks</td> <td></td> <td></td> <td>3.13E+05</td> <td>64135.4</td> <td>17</td> <td>rost-2 owks (), rost-1 4wks vs rost-2 2wks (), rost-1 4wks ve Poet-2 6wke (*)</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> | Post-2 2wks | | | 3.13E+05 | 64135.4 | 17 | rost-2 owks (), rost-1 4wks vs rost-2 2wks (), rost-1 4wks ve Poet-2 6wke (*) | | | | | | | |
| Image Zober-US Holzeb/J Image Image <thimage< th=""> Image Image</thimage<> | Post-2 6wks | | | 2.91E+05 | 62423 | 1/ | VS FOSEZ OWRS () | | | | | | | |
| Jobs 1. Awks Posk 1. Awks Posk 2. | Pre | | | 2.69E+05 | 163266.9 | 12 | | | | | | | | |
| Post-1 Aws Post-2 wis Avis (7.35±40 80/33.8 1/1 Pre vs Post-1 2wks (°) Image: Constraint of the state of | Post-1 2wks | | 1.44 | 1.36E+06 | 1078257.4 | 17 | Den un Dent 4 Andre (1) | | | | | | | |
| Post-2 dwis 1.71E+06 187/0193 17 Pre 3.08E+04 14318 12 Post-2 dwis 3.08E+04 14318 12 Post-2 dwis 3.08E+04 14318 12 Post-2 dwis 2.09E+05 43909.8 17 4wis ("). Post-2 dwis (""). Post-2 dwi | Post-1 4wks | AAV8 | IGM | 7.35E+05 | 801383.8 | 17 | Pre vs Post-1 2wks (*) | | | | | | | |
| Post-2 wks 2.75 ±r06 63062773 17 Post-1 2wks 3.08 ±r04 14318 12 Post-1 2wks 2.09 ±r05 31325.4 17 Post-2 2wks 2.09 ±r05 31325.4 17 Post-2 2wks 2.09 ±r05 31701.1 17 Post-2 2wks 2.09 ±r05 37701.1 17 Post-2 2wks 2.09 ±r05 37701.1 17 Post-2 2wks 2.09 ±r05 37701.1 17 Post-2 2wks 1.99 ±r06 197467.7 17 Post-2 2wks 1.99 ±r06 197467.7 17 Post-2 2wks 3.00 ±r04 149821.1 12 Post-2 2wks 3.00 ±r04 149821.1 12 Post-2 2wks 3.00 ±r04 149821.1 12 Post-2 2wks 3.03 ±r04 12821.1 12 Post-2 2wks 3.03 ±r04 12821.1 12 Post-2 2wks 3.03 ±r04 1768.2 17 Post-2 2wks 3.03 ±r04 1768.2 17 | Post-2 2wks | | | 1.71E+06 | 1875019.9 | 17 | | | | | | | | |
| Pre 3.08±+04 14318 12 pre vs Post-1 2wks (***), Pre vs Post-1 4wks (***), Pre vs Post-2 4/4 4 | Post-2 6wks | | | 2.75E+06 | 6306277.9 | 17 | | | | | | | | |
| Posi-1 Zwks Posi-1 Zwks Posi-1 Zwks Posi-2 Zwks 1.76E+05 31325.4 17 Z Zwks (***), Posi-1 Zwks vs Posi-1 2 dwks (***), Posi-1 Zwks vs Posi-2 Zwks (***), Posi-1 Zwks vs Posi- 2 dwks (***), Posi-1 Zwks vs Posi-2 Zwks (***), Posi-1 Zwks vs Posi- 2 dwks (***), Posi-1 Zwks vs Posi-2 Zwks (***), Posi-1 Zwks vs Posi- 2 dwks (***), Posi-1 Zwks vs Posi- 2 dwks (***) Image: Comparison of the compa | Pre | | | 3.08E+04 | 14318 | 12 | Pre vs Post-1 2wks (****), Pre vs Post-1 4wks (****), Pre vs Post-2 | | | | | | | |
| Post-1 wks AVM/VD IgG 2.09E+05 43909.8 17 wks (*), Post-1 2wks vs Post-2 wks (****) Post-1 2wks vs Post-2 wks (****) Post-2 wks 2.06E+05 32700.8 17 2 6wks (****) 2 6wks (***) 2 6wks (**) 2 | Post-1 2wks | | | 1.76E+05 | 31325.4 | 17 | 2wks (****), Pre vs Post-2 6wks (****), Post-1 2wks vs Post-1 | | | | | | | |
| Post-2 dwks 2.40E+05 37701.1 17 2 6wks (***) 0 0 0 Pre 2.85E+05 210207.9 12 0 | Post-1 4wks | AAVMYO | lgG | 2.09E+05 | 43909.8 | 17 | 4wks (*), Post-1 2wks vs Post-2 2wks (****), Post-1 2wks vs Post- | | | | | | | |
| Post-2 dwks 2.36E+05 32700.8 17 | Post-2 2wks | | | 2.40E+05 | 37701.1 | 17 | 2 6wks (****) | | | | | | | |
| Pre 2.65E+05 210207.9 12 Post-1 2wks 1.99E+06 1578767.7 17 Post-2 2wks 1.25E+06 1496317.2 17 Post-2 2wks 3.05E+06 1496317.2 17 Post-2 2wks 3.05E+06 1185584 17 Pre 3.05E+04 1023.7 17 Post-1 2wks 3.05E+04 1023.7 17 Post-2 2wks 3.05E+04 1023.7 17 Post-2 2wks 3.05E+04 12618.2 17 Post-2 2wks 3.05E+04 12618.2 17 Post-2 2wks 3.02E+04 13768.3 17 Post-2 2wks 1.02E+05 60847.1 12 Post-2 2wks 1.02E+05 60847.1 17 Post-2 2wks | Post-2 6wks | | | 2.36E+05 | 32700.8 | 17 | | | | | | | | |
| Post-1 dwks Post-1 dwks Post-2 dwks 1.99E+06 1597467.7 17 Pre vs Post-1 2wks (**) Pre vs Post-1 2wks (**) Image: Constraint of the state of | Pre | | | 2.65E+05 | 210207.9 | 12 | | | | | | | | |
| Post-1 wks AVMVO IgM 8.58E+05 801882.4 17 Pre vs Post-1 2wks (**) Image: Constraint of the state | Post-1 2wks | | | 1.99E+06 | 1597467.7 | 17 | | | | | | | | |
| Post-2 dwks 1.25E+06 1496317.2 17 Image: Constraint of the state of t | Post-1 4wks | AAVMYO | lgM | 8.58E+05 | 801882.4 | 17 | Pre vs Post-1 2wks (**) | | | | | | | |
| Post-2 dwks 3.60E+04 1185584 17 Post-1 dwks Cas9 IgG 3.08E+04 104237 17 Post-1 dwks Cas9 IgG 3.08E+04 104237 17 Post-1 dwks Cas9 IgG 3.08E+04 104237 17 Post-2 dwks 3.08E+04 12144.6 17 NS 0 0 Post-2 dwks 3.08E+04 13766.3 17 NS 0 0 0 Post-2 dwks 7.07E+05 2490410.6 17 NS 0 0 0 0 Post-1 dwks Cas9 IgG 1.02E+05 60847.1 17 NS 0< | Post-2 2wks | | | 1.25E+06 | 1496317.2 | 17 | | | | | | | | |
| Pre 3.03E+04 13822.1 12 Post-12wks 3.09E+04 1243.7 17 NS 0 | Post-2 6wks | | | 3.60E+06 | 11185584 | 17 | | | | | | | | |
| Post-1 wks Cas9 IgG 3.05E+04 1214.6 17 NS Image: Case of the sector o | Pre | | | 3.03E+04 | 13822.1 | 12 | | | | | | | | |
| Post-1 wks Cas9 IgG 3.38E+04 12144.6 17 NS Post-2 wks 3.08E+04 13765.3 17 | Post-1 2wks | | | 3.05E+04 | 10423.7 | 17 | | | | | | | | |
| Post-2 zwks 3.08E+04 12618.2 17 0 </th <td>Post-1 4wks</td> <td>Cas9</td> <td>lgG</td> <td>3.39E+04</td> <td>12144.6</td> <td>17</td> <td>NS</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> | Post-1 4wks | Cas9 | lgG | 3.39E+04 | 12144.6 | 17 | NS | | | | | | | |
| Post-2 dwks 3.25E+04 13766.3 17 Pre 1.02E+05 84877.4 12 Post-1 dwks Cas9 IgM 1.02E+05 60847.1 17 Post-1 dwks Cas9 IgM 1.02E+05 60847.1 17 NS Post-2 dwks 1.05E+05 1052486 17 NS 0 0 Post-2 dwks 7.84E+04 67598.1 17 NS 0 0 Post-1 dwks 9.054.2 3.48E+04 10384.5 17 0 0 0 Post-1 dwks µDYS IgG 3.48E+04 10384.5 17 NS 0 | Post-2 2wks | | | 3.08E+04 | 12618.2 | 17 | | | | | | | | |
| Pre 1.02E+05 84877.4 12 Post-14wks Cas9 IgM 1.02E+05 2490410.6 17 Post-22wks 1.02E+05 125486 17 NS 0 0 Post-22wks 7.84E+04 67598.1 17 NS 0 0 0 Post-12wks 7.84E+04 67598.1 17 0 0 0 0 Post-12wks 3.04E+04 10384.5 17 0 </th <td>Post-2 6wks</td> <td></td> <td></td> <td>3.23E+04</td> <td>13766.3</td> <td>17</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> | Post-2 6wks | | | 3.23E+04 | 13766.3 | 17 | | | | | | | | |
| Post-1 zwks 7.07E+05 2490410.6 17 NS Post-1 zwks Cas9 IgM 1.02E+05 60847.1 17 NS Image: Case of the state of | Pre | | | 1.02E+05 | 84877.4 | 12 | | | | | | | | |
| Post-1 wks Cas9 IgM 1.02E+05 60847.1 17 NS Post-2 dwks 1.15E+05 125486 17 | Post-1 2wks | | | 7.07E+05 | 2490410.6 | 17 | | | | | | | | |
| Post-2 zwks 1.15E+05 125486 17 Post-2 zwks 7.84E+04 67598.1 17 Pre 3.04E+04 10384.5 17 Post-1 zwks 3.14E+04 10384.5 17 Post-2 zwks 3.11E+04 12527.9 17 Post-2 zwks 3.11E+04 9709.4 17 Post-2 zwks 3.11E+04 9709.4 17 Post-2 zwks 3.11E+04 9709.4 17 | Post-1 4wks | Cas9 | lgM | 1.02E+05 | 60847.1 | 17 | NS | | | | | | | |
| Post-2 δwks 7.84E+04 67598.1 17 Pre 3.04E+04 14196.7 12 Post-1 2wks 3.14E+04 10384.5 17 Post-1 4wks µDYS IgG 3.43E+04 11398.7 Post-1 2wks 3.14E+04 10384.5 17 Post-2 2wks 3.15E+04 2227.9 17 Post-2 2wks 3.18E+04 9709.4 17 Post-16 4th 55 th 55 th 55 th 45 th 12 15 th 45 th 12 16 th 45 th 16 th 12 | Post-2 2wks | | | 1.15E+05 | 125486 | 17 | | | | | | | | |
| Pre 3.04E+04 14196.7 12 Post-12wks µDYS IgG 3.14E+04 10384.5 17 Post-22wks 3.31E+04 1139.7 17 NS Image: Control of the state of th | Post-2 6wks | | | 7.84E+04 | 67598.1 | 17 | | | | | | | | |
| Post-12wks 3.14E+04 10384.5 17 NS Post-12wks .0.14E+04 11398.7 17 NS | Pre | | | 3.04E+04 | 14196.7 | 12 | | | | | | | | |
| Post-1 4wks μDYS IgG 3.43E+04 11398.7 17 NS Post-2 2wks 3.11E+04 12527.9 17 NS Image: Constraint of the state | Post-1 2wks | | | 3.14E+04 | 10384.5 | 17 | | | | | | | | |
| Post-2 2wks 3.11E+04 12527.9 17 Post-2 5wks 3.18E+04 9709.4 17 Post-2 5wks 1.8E+05 155141.6 12 | Post-1 4wks | μDYS | lgG | 3.43E+04 | 11398.7 | 17 | NS | | | | | | | |
| Post-2 6wks 3.16E+04 9709.4 17 Pre 186E+05 15544.6 12 | Post-2 2wks | | | 3.11E+04 | 12527.9 | 17 | | | | | | | | |
| Pro 186E+05 1551416 12 | Post-2 6wks | | | 3.18E+04 | 9709.4 | 17 | | | | | | | | |
| 10 100100 1001010 12 | Pre | | | 1.86E+05 | 155141.6 | 12 | | | | | | | | |
| Post-1 2wks 1.52E+05 158995.3 17 | Post-1 2wks | | | 1.52E+05 | 158995.3 | 17 | | | | | | | | |
| Post-1 4wks µDYS lgM 1.30E+05 73932.2 17 NS | Post-1 4wks | μDYS | lgM | 1.30E+05 | 73932.2 | 17 | NS | | | | | | | |
| | Post-2 2wks | | | 2.38E+05 | 319951.9 | 17 | | | | | | | | |
| Post-2 2wks 2.38E+05 319951.9 17 | Post-2 6wks | | | 1.37E+05 | 92260.8 | 17 | | | | | | | | |
| | Post-2 2wks | | | 2.38E+05 | 319951.9 | 17 | | | | | | | | |
| Post-2 2wks 2.38E+05 319951.9 17 | Post-2 6wks | | | 1.37E+05 | 92260.8 | 17 | | | | | | | | |

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

| Vector | Complement | Pre | SD | Ν | Post-1 5hrs | SD | Ν | Post-1 2wks | SD | Ν | Post-2 5hrs | SD | Ν | Post-2 2wks | SD | Ν |
|----------|-----------------|--------|------|----|-------------|-------|----|-------------|-------|----|-------------|-------|----|-------------|-------|----|
| Cas9-FS | | 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 | C3 (mg/ml.) | 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 | C3 (IIIg/IIIE) | 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 | | 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 | C4 (ng/mL) | 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 | C4 (lig/lilL) | 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 | | 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 | C5b9 (pg/ml.) | 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 | Cobe (lig/lilc) | 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 | Ν | Tukey's multiple comparison test | Kev: levels | of statistica | l significanc | a | | | |
|-------------|--------------|---------|-------|----|--|------------------|----------------|----------------|-------------|---------------|-------------|-------------|
| Bro | oompionioni | 1 74 | 0.4 | 18 | · | ~ ~ 0.05 (*) | | | | | | |
| FIE | - | 1.74 | 0.4 | 10 | Pre vs Post-2 5hrs (****), Post-1 5hrs | 0 < 0.05 () | | | | | | |
| Post-1 5hrs | | 2.00 | 1.0 | 18 | ve Poet 2 5bre (***) Poet 1 2wke ve | p < 0.01 (** | ') | | | | | |
| Post-1 2wks | C3 (mg/mL) | 1.83 | 0.5 | 18 | Post 2 Shre (****) Post 2 Shre ve Post | o < 0.001 (* | ***) | | | | | |
| Post-2 5hrs | | 1.16 | 0.5 | 18 | 2 2 wke (****) | p < 0.0001 | (****) | | | | | |
| Post-2 2wks | | 1.59 | 0.5 | 18 | 2 2003 () | | | | | | | |
| Pre | | 146.30 | 52.7 | 9 | | Key: symbo | ols in Figure | s 2B-C and | S3A represe | nt significan | ice between | comparisons |
| Post-1 5hrs | | 102.57 | 35.6 | 9 | | t: time poin | its are signif | icantly differ | ent compare | d to Post-2 | (5 hours) | |
| Post-1 2wks | C4 (ng/mL) | 99.22 | 72.5 | 9 | Pre vs Post-2 5hrs (*) | | | | | | | |
| Post-2 5hrs | | 73.03 | 18.1 | 9 | | | | | | | | |
| Post-2 2wks | | 77.28 | 68.3 | 9 | | | | | | | | |
| Pre | | 551.62 | 86.7 | 9 | | | | | | | | |
| Post-1 5hrs | | 561.62 | 196.8 | 9 | | | | | | | | |
| Post-1 2wks | C5b9 (ng/mL) | 584.66 | 120.3 | 9 | NS | | | | | | | |
| Post-2 5hrs | | 497.41 | 120.7 | 9 | | | | | | | | |
| Post-2 2wks | | 527.13 | 126.3 | 9 | | | | | | | | |

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

| Case The 53 300.0 6 1151.27 600.3 644.70 170.5 6 3156.87 1132.4 6 444.70 170.5 6 3156.87 1132.4 6 444.70 170.5 6 3156.8 6 655.2 453.48 6 322.48 173.7 6 CaseF Mich 0.7 6 33.6 6 20.7 8 13.95.4 13.95.4 6 35.8 13.85.4 6 35.8 13.85.4 6 35.8 16 16 0.6 6 3.7 6 3.7 6 110 0.5 6 3.7 17.7 10.9 18 10.9 10. | Vector | Analyte | Pre | SD | Ν | Post-1 5hrs | SD | Ν | Post-1 2wks | SD | Ν | Post-2 5hrs | SD | Ν | Post-2 2wks | SD | Ν |
|--|----------|---------|---------|--------|----|--------------|--------|----|-------------|-------|----|-------------|--------|----|-------------|-------|----|
| Case // DVS (P+10) 71.0 98.43 3 6 802.0 802.5 110.6 6 925.43 227.7 110.6 6 925.43 110.7 6 925.43 110.6 6 93.55 110.6 6 93.55 110.6 6 110.7 100.7 <th< td=""><td>Cas9-FS</td><td></td><td>776.53</td><td>300.0</td><td>6</td><td>1151.27</td><td>660.3</td><td>6</td><td>484.70</td><td>170.5</td><td>6</td><td>3156.87</td><td>1382.4</td><td>6</td><td>544.69</td><td>187.7</td><td>6</td></th<> | Cas9-FS | | 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 |
| μDYS 594.33 247.1 6 984.98 1015.4 6 283.75 2110.1 6 285.74 1015.8 16 285.74 1015.8 16 285.74 1015.74 1015 110 0.5 6 373 5.0 6 1110 0.5 6 373 5.0 6 1110 0.5 6 0.5 0.4 6 373 5.0 6 1110 0.5 6 373 5.0 6 1110 0.5 6 0.5 0.6 0.5 0.6 0.5 0.6 0.5 0.6 0.5 0.6 0.773 0.00 0.75 0.75 0.75 0.75 0.75 0.75 0.75 0.75 0.75 0.75 0.75 <th< td=""><td>Cas9</td><td>IP-10</td><td>710.99</td><td>368.3</td><td>6</td><td>568.40</td><td>336.4</td><td>6</td><td>200.56</td><td>201.2</td><td>6</td><td>6055.32</td><td>4834.8</td><td>6</td><td>320.28</td><td>173.7</td><td>6</td></th<> | Cas9 | IP-10 | 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 |
| Combined 683.95 300.6 16 891.55 725.8 18 395.44 195.51 395.54 328.2 18 457.0 191.9 100.5 Camb 0.76 0.3 6.0 1.10 0.4 6 0.05 0.4 6 4.05 2.4 6 1.10 0.5 6 1.16 0.5 6 4.16 6 0.05 0.4 6 4.05 1.0 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 4.05 0.05 6 0.05 0.05 6 0.05 0.05 6 0.05 0.05 0.05 0.0 | 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 |
| CaseP UP-16 O.F. O.S. 6 3.7.6 0.0 6 1.10 0.0.5 0.0 CaseP O.S. O.S. C.S. C.S. <thc.s.< th=""> <thc.s.< th=""> <thc.s.< td="" th<=""><td>Combined</td><td></td><td>693.95</td><td>300.6</td><td>18</td><td>891.55</td><td>725.8</td><td>18</td><td>356.34</td><td>219.5</td><td>18</td><td>3955.49</td><td>3288.2</td><td>18</td><td>459.70</td><td>191.9</td><td>18</td></thc.s.<></thc.s.<></thc.s.<> | 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 |
| Label MIP-18 0.07 0.02 6 1.10 0.4 6 0.46 6 1.08 0.46 6 1.08 0.46 6 1.08 0.46 6 1.10 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.45 0.46 0.46 0.46 0.46 0.46 0.46 0.46 | Cas9-FS | | 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 |
| DLD 38 Description Description <thdescription< th=""> <thdescription< th=""> <thd< td=""><td>Casy</td><td>MIP-1β</td><td>0.67</td><td>0.2</td><td>6</td><td>1.10</td><td>0.4</td><td>6</td><td>0.85</td><td>0.4</td><td>6</td><td>4.08</td><td>2.4</td><td>6</td><td>1.10</td><td>0.5</td><td>6</td></thd<></thdescription<></thdescription<> | Casy | MIP-1β | 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 |
| Causer Bit P3 42.0 0 200.12 200.20 111.06 53.1 10 244.06 445.0 6 99.87 45.0 10 UpYS Combined 00.255 55.8 6 115.80 10.25 6 11.30 10.25 6 55.7 42.0 10 10 10.25 <td>Combined</td> <td></td> <td>0.20</td> <td>0.2</td> <td>10</td> <td>1.19</td> <td>1.0</td> <td>10</td> <td>0.50</td> <td>0.6</td> <td>10</td> <td>3.18</td> <td>4.7</td> <td>10</td> <td>1.15</td> <td>1.4</td> <td>10</td> | Combined | | 0.20 | 0.2 | 10 | 1.19 | 1.0 | 10 | 0.50 | 0.6 | 10 | 3.18 | 4.7 | 10 | 1.15 | 1.4 | 10 |
| Case /FS MCP-1 102.95 28.8 6 119.99 100.2 6 611.33 402.8 6 55.90 44.3 6 Cambred 128.8 55.6 161.8 142.7 6 45.97 100.7 6 45.97 142.6 6 45.97 143.6 6 45.97 143.6 6 52.0 143.6 6 52.0 14.8 6 52.0 17.6 4.89 142.7 16 143.6 16 6.23 22.0 10.6 6 53.0 4.81.5 6 2.20 10.5 6 2.20 10.5 6 2.20 11.5 6 2.20 10.5 6 2.20 10.5 6 2.20 10.5 6 2.20 10.5 6 2.20 10.5 6 2.20 10.5 6 2.20 10.5 6 2.20 10.5 6 2.20 10.5 6 2.20 10.5 10.5 10.5 10.5 10.5 | Cae9-ES | | 89.73 | 42.0 | 6 | 260.12 | 269.2 | 6 | 111.06 | 53.1 | 6 | 244.08 | 4.0 | 6 | 89.87 | 35.1 | 6 |
| mumber MCP-1 gass 55.5 6 161.89 147.7 6 669.6 6 17.73 1007.7 6 44.99 14.4 22.2 10 6 649.99 14.4 22.2 10 6 547.3 1007.7 6 44.9 14.4 10 6 547.2 22.6 6 22.0 10.6 6 22.2 22.6 22.0 10.6 6 22.2 22.6 22.0 10.6 6 22.2 6 22.0 10.6 6 22.2 6 22.0 10.6 6 22.0 10.6 6 22.0 10.6 6 22.0 10.6 10.6 10.6 10.6 10.6 10.6 12.0 71.8 10.6 12.0 71.8 10.6 11.2 73.8 10.6 12.0 73.8 10.6 12.0 73.8 10.6 12.0 73.8 10.6 12.0 73.8 10.6 12.0 73.8 10.7 10.8 <t< td=""><td>Cas9</td><td></td><td>102.95</td><td>26.8</td><td>6</td><td>118.98</td><td>203.2</td><td>6</td><td>151.80</td><td>108.2</td><td>6</td><td>611.33</td><td>402.6</td><td>6</td><td>55.90</td><td>44.3</td><td>6</td></t<> | Cas9 | | 102.95 | 26.8 | 6 | 118.98 | 203.2 | 6 | 151.80 | 108.2 | 6 | 611.33 | 402.6 | 6 | 55.90 | 44.3 | 6 |
| Combined 96.17 4.09 18 100.33 17.73 18 100.4 20.06 18 434.72 30.00 18 64.22 12.2 18 Casap-FS Combined 31.4 19 6 7.74 3.8 6 6.53 2.30 6 5.33 6 4.64 13.6 18 6 6.33.4 2.08 5.23 2.40 13.6 18 6.63 2.23 18 4.06 12.3 3.6 6 4.23 18 4.06 2.77 18 6.64 2.23 6 2.23 4.74 6 6.23 2.77 18 6.64 2.23 6.23 6.23 4.74 6 6.23 12.0 7.84 1.85 1.82 2.49 1.00 18 7.33 6 1.44 1.15 3.33 0.9 6 4.23 1.83 1.80 1.83 1.80 1.80 1.80 1.80 1.80 1.80 1.80 1.80 1.80 | UDYS | MCP-1 | 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 | | 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 |
| μργS μργB μργB <t< td=""><td>Cas9</td><td>THE «</td><td>2.99</td><td>0.7</td><td>6</td><td>3.84</td><td>1.8</td><td>6</td><td>3.63</td><td>1.6</td><td>6</td><td>6.23</td><td>2.2</td><td>6</td><td>2.30</td><td>1.0</td><td>6</td></t<> | Cas9 | THE « | 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 | IINI-u | 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 |
| Cas9 μ 11.93 15.3 6 70.32 59.9 6 22.3 6 52.32 47.4 6 6.2.1 3.3 6 μ 30.9 8.1 6 40.50 27.4 6 13.25 22.2 6 68.54 65.1 6 5.75 3.8 6 Cas9 3.30 0.4 6 4.71 2.0 6 3.18 0.6 6 3.05 0.7 6 2.36 1.0 6 Cas9 3.36 1.1 6 3.52 1.2 6 2.43 1.4 6 2.24 1.4 6 2.26 1.2.6 1.2.4 1.6 1.2.6 1.2.4 1.0 1.6 2.42 1.1 6 2.28 6 1.2.4 1.2.6 1.2.4 1.2.6 1.2.4 1.2.1 8 1.2.4 1.2.1 1.2.4 1.2.1 1.2.4 1.2.1 8 1.2.4 1.2.1 1.2.4 <td>Combined</td> <td></td> <td>2.87</td> <td>1.5</td> <td>18</td> <td>4.93</td> <td>3.4</td> <td>18</td> <td>4.13</td> <td>1.8</td> <td>18</td> <td>6.40</td> <td>2.9</td> <td>18</td> <td>4.06</td> <td>2.7</td> <td>18</td> | 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 (p)OYS Combined μP.6 (a)S 11.29 7.8 6 49.94 35.2 6 13.92 13.9 6 12.007 24.96 6 2.98 6 7.75 18 6.75 3.8 6 Cas9-FS (a)97S L.15 3.39 0.9 6 4.81 1.6 6 3.21 0.8 6 2.64 1.5 6 2.36 1.0 6 Cas9-FS (p)OYS 3.70 0.8 6.4 4.81 1.6 6 2.24 1.4 6 1.234 1.4 6 2.29 0.8 6 Cas9-FS (p)OYS 2.7 0.8 1.8 4.35 1.6 1.8 2.24 1.1 1.6 1.24 1.7 6 1.24 <t< td=""><td>Cas9-FS</td><td></td><td>19.39</td><td>15.3</td><td>6</td><td>70.32</td><td>59.9</td><td>6</td><td>24.96</td><td>22.3</td><td>6</td><td>32.32</td><td>47.4</td><td>6</td><td>6.21</td><td>3.3</td><td>6</td></t<> | Cas9-FS | | 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 |
| $ \begin{array}{ $ | Cas9 | IL-6 | 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 |
| Combined CaseP-FS (DPVS) 1.15 (L-15) 13.26 (A) 11.3 (A) 18 (A) 14.73 (A) 19.18 (A) 7.344 (A) 14.74 (A) 14.73 (A) 19.18 (A) 7.344 (A) 14.74 (A) 14.73 (A) 19.18 (A) 7.344 (A) 14.74 (A) 14.73 (A) 14.74 (A) 14.74 (A) </td <td>µDYS</td> <td></td> <td>9.09</td> <td>8.1</td> <td>6</td> <td>40.50</td> <td>27.4</td> <td>6</td> <td>13.25</td> <td>22.2</td> <td>6</td> <td>68.54</td> <td>55.1</td> <td>6</td> <td>5.75</td> <td>3.8</td> <td>6</td> | 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 |
| $ \begin{array}{c} \begin{tabular}{ c c c c c c c c c c c c c c c c c c c$ | 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 |
| Lens β.39 0.9 6 4.81 10 6 3.21 0.9 6 2.68 1.2 6 Cambined 3.27 0.8 1.6 6.52 1.2 6 2.44 1.0 18 2.67 1.2 18 2.244 1.0 18 2.67 1.2 18 2.244 1.0 18 2.67 1.2 18 2.244 1.0 18 2.67 1.2 18 2.24 1.0 18 2.67 1.1 18 2.67 1.1 18 2.67 13.0 6 12.4 1.0 18 2.67 13.0 6 12.4 18.1 18 12.11 18 17.0 18 17.0 18 12.11 18 18 12.11 18 18 12.11 18 18 11.14 18 18 11.14 18 18 11.14 18 18 12.11 18 18 12.11 18 18 12 | Cas9-FS | | 3.07 | 0.4 | 6 | 4./1 | 2.0 | 6 | 3.18 | 0.6 | 6 | 3.05 | 0.7 | 6 | 2.36 | 1.0 | 6 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Case | IL-15 | 3.39 | 0.9 | 6 | 4.81 | 1.0 | 6 | 3.21 | 0.8 | 6 | 2.04 | 1.5 | 6 | 2.08 | 1.2 | 6 |
| | Combined | | 3.30 | 1.1 | 19 | 3.52 | 1.2 | 19 | 2.43 | 1.3 | 19 | 2.34 | 1.4 | 19 | 2.29 | 1.0 | 19 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9-ES | | 6.54 | 4.5 | 6 | 14.53 | 9.4 | 6 | 12.34 | 4.1 | 6 | 13.51 | 9.6 | 6 | 12.44 | 7.7 | 6 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | 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 |
| | UDYS | MIP-2α | 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 |
| $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$ | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9-FS | | 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 |
| $ \begin{array}{ $ | Cas9 | 11_19 | 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 | IL-10 | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | 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 |
| $ \begin{array}{c} \begin{array}{c} Cas9 \\ \mu DYS \\ \hline Cas9 \\ \hline Cas9 \\ \mu DYS \\ \hline Cas9 \\ \hline Cas9 \\ \mu DYS \\ \hline Cas9 \\ \hline Cas9 \\ \mu DYS \\ \hline Cas9 \\ \hline Cas9 \\ \mu DYS \\ \hline Cas9 \\ \hline Cam$ | Cas9-FS | | 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 |
| $\begin{array}{c c c c c c c c c c c c c c c c c c c $ | Cas9 | IL-1α | 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 |
| $ \begin{array}{c} \label{combined}{Cas9-FS} \\ \hline Cas9-FS \\ \hline L-5 \\ \hline Cas9-FS \\ \hline L-7 \\ \hline MPYS \\ L-7 \\ \hline M108 \\ \hline M108 \\ L-7 \\ \hline M108 \\ \hline $ | 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 |
| $ \begin{array}{c} \begin{array}{c} \begin{array}{c} \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \$ | Combined | | 10.73 | 5.7 | 18 | 15.89 | 1.8 | 18 | 7.46 | 8.4 | 18 | 10.01 | 15.7 | 18 | 16.03 | 11.9 | 18 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9-F3 | | 9.14 | 3.2 | 6 | 6.50 | 2.5 | 6 | 8.86 | 4.9 | 6 | 10.24 | 7.9 | 6 | 6.54 | 2.0 | 6 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | UDYS | IL-5 | 4 23 | 2.0 | 6 | 4.01 | 1.6 | 6 | 3.65 | 2.7 | 6 | 10.24 | 10.8 | 6 | 3.37 | 17 | 6 |
| $ \begin{array}{c} \hline Cas9-FS \\ \hline Cas9-FS \\ \mu DYS \\ \hline Combined \\ \hline IL-7 \\ \hline IL$ | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9-FS | | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | 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 |
| 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 (as9) µDYS IL-9 44.40 15.9 6 012.33 63.9 6 78.83 45.0 6 55.60 34.6 6 77.1 37.2 6 Cas9 µDYS IL-9 44.40 15.1 6 30.3 21.3 6 98.66 123.1 6 31.34 20.5 6 39.06 22.1 6 Cas9-FS IL-10 5.06 4.1 6 8.64 5.4 6 5.71 3.9 6 5.84 1.9 6 4.53 5.0 6 Cas9 IL-10 2.53 1.0 6 1.89 0.9 6 4.40 1.8 4.63 3.2 18 3.88 4.3 18 Cas9 IL-13 IL-13 2.55 6 53.05 38.0< | μDYS | IL-/ | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9-FS | | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9 | IL-9 | 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 |
| 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 μDYS L-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-FS Gombined 3.03 2.1 6 5.21 4.7 18 4.68 4.0 18 4.69 3.2 18 3.88 4.3 18 Cas9-FS L-13 21.03 11.1 6 40.63 29.2 6 23.63 9.2 6 3.41 22.6 6 Cas9-FS L-13 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 Cas9 MIG 11.4 5.5 6 35.8 16 16.10 14.5 | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9-FS | | 3.05 | 4.1 | 0 | 8.04 5.10 | 5.4 | 6 | 5./1 | 3.9 | 6 | 5.98 | 4.4 | 0 | 5.67 | 5.0 | 6 |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | UDVS | IL-10 | 2.53 | 2.1 | 6 | 1.90 | 4.1 | 6 | 4.29 | 2.3 | 6 | 2.04 | 0.7 | 6 | 4.55 | 0.8 | 6 |
| Cas9-FS pDYS L-13 Construction (Cas9-FS) (Cas9-FS) L-13 Construction (Cas9-FS) (Cas9-FS) L-13 Construction (Cas9-FS) (Cas9-FS) L-13 Construction (Cas9-FS) L-13 L-14 L-14 <thl-14< th=""> <thl-14<< td=""><td>Combined</td><td></td><td>3.54</td><td>2.8</td><td>18</td><td>5.21</td><td>47</td><td>18</td><td>4.68</td><td>4.0</td><td>18</td><td>4.69</td><td>3.2</td><td>18</td><td>3.88</td><td>4.3</td><td>18</td></thl-14<<></thl-14<> | Combined | | 3.54 | 2.8 | 18 | 5.21 | 47 | 18 | 4.68 | 4.0 | 18 | 4.69 | 3.2 | 18 | 3.88 | 4.3 | 18 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9-FS | | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | μDYS | IL-13 | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9-FS | | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9 | MIG | 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 |
| 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 μDYS 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 MIP-1α 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 Combined 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 65.72 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 65.72 14.7 6 108.61 43.9 6 62.40 | 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 |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | Cas9-FS | | 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 |
| $ \begin{array}{ c c c c c c c c c c c c c c c c c c c$ | Cas9 | MIP-1α | 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 |
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | Combined | | 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 |
| Case 58.27 13.1 6 100.51 43.3 6 02.40 14.7 6 95.93 50.9 6 75.67 36.2 6 Loss 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 Loss 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 | Caso ES | | 65 70 | 1.4 | 6 | 108.61 | 10.2 | 10 | 62.40 | 1.5 | 6 | 2.08 | 2.3 | 6 | 2.34 | 2.0 | 10 |
| μDYS Combined 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 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 | Case | | 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 |
| 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 | UDYS | RANTES | 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.

| pono | | | , | | Isiliou vootoi uutu | | | | | | | | |
|--------------|-----------|-----------------|--------|----|---|------------|------------|---------------|-----------------|--------------|---------------|------------|-----------|
| Time point | Analyte | Average (pg/mL) | SD | Ν | Tukey's multiple comparison test | Ke | y: levels | of statistica | significance | | | | |
| Pre | | 693.95 | 300.6 | 18 | Pre vs Post-1 2wks (**), Pre vs Post-2 5hrs (**), | p < | < 0.05 (*) | | | | | | |
| Post-1 5hrs | | 891.55 | 725.8 | 18 | Pre vs Post-2 2wks (*), Post-1 5hrs vs Post-1 | D < | < 0.01 (** |) | | | | | |
| Poet-1 2wke | IP-10 | 356 34 | 219.5 | 18 | 2wks (*) Post-1 5hrs vs Post-2 5hrs (*) Post-1 | P 6 | < 0.001 (* | ***) | | | | | |
| Post 0 Ehrs | 1-10 | 2005 40 | 215.5 | 10 | 2wks (), Post-1 5hrs VS Post-2 5hrs (), Post-1 | <i>p</i> < | 0.001 | / | | | | | |
| Post-2 5hrs | | 3955.49 | 3288.Z | 18 | 2WKS VS POSC-2 SHIS (), POSC-2 SHIS VS POSC-2 | p < | < 0.0001(|) | | | | | |
| Post-2 2wks | | 459.70 | 191.9 | 18 | 2WKS (**) | | | | | | | | |
| Pre | | 0.56 | 0.3 | 18 | | Ke | y: symbo | ols in Figure | s 2D and S3B | represent si | ignificance b | etween con | nparisons |
| Post-1 5hrs | | 1.48 | 1.8 | 18 | | #: f | time poin | ts are signif | cantly differen | t compared | to Pre | | |
| Post 1 2wks | MID-18 | 0.70 | 0.4 | 19 | Pre vs Post-2 5hrs (*), Post-1 2wks vs Post-2 | 4-1 | timo poin | te are signif | contly differen | teempared | to Post 1 /5 | hours) | |
| FUSETZWKS | Will - Th | 0.70 | 0.4 | 10 | 5hrs (*) | 1.0 | unie poin | is are signin | cantiy unleren | compared | ID FOSI-1 (5 | nours) | |
| Post-2 5hrs | | 3.66 | 4.0 | 18 | | 1:0 | time poin | ts are signif | cantly differen | t compared | to Post-2 (5 | hours) | |
| Post-2 2wks | | 1.14 | 0.9 | 18 | | | | | | | | | |
| Pre | | 95.17 | 40.9 | 18 | | | | | | | | | |
| Post-1 5hrs | | 180.33 | 177.3 | 18 | | | | | | | | | |
| Post 1 Julio | MCD.1 | 100.04 | 90.6 | 10 | Pre vs Post-2 5hrs (*), Post-1 2wks vs Post-2 | | | | | | | | |
| POSET ZWKS | WICF-1 | 105.54 | 80.0 | 10 | 5hrs (**), Post-2 5hrs vs Post-2 2wks (**) | | | | | | | | |
| Post-2 5hrs | | 343.72 | 300.9 | 18 | | | | | | | | | |
| Post-2 2wks | | 64.92 | 42.2 | 18 | | | | | | | | | |
| Pre | | 2.87 | 1.5 | 18 | | | | | | | | | |
| Poet-1 5hre | | 4.93 | 3.4 | 18 | - | | | | | | | | |
| Post 1 2wks | TNE | 4.12 | 1.0 | 10 | Pre vs Post-2 5hrs (**), Post-1 2wks vs Post-2 | | | | | | | | |
| POSET ZWKS | IINF-0 | 4.13 | 1.0 | 10 | - 5hrs (*) | | | | | | | | |
| Post-2 5hrs | | 6.40 | 2.9 | 18 | | | | | | | | | |
| Post-2 2wks | | 4.06 | 2.7 | 18 | | | | | | | | | |
| Pre | | 13.26 | 11.3 | 18 | | | | | | | | | |
| Post-1 5hrs | | 53.25 | 42.5 | 18 | Pre vs Post-1 5hrs (**). Pre vs Post-1 2wks (*). | | | | | | | | |
| Post 1 2wks | 11-6 | 14 72 | 10.0 | 10 | Post-1 Ehrs us Post-1 2wks (**) Post-1 Ehrs us | | | | | | | | |
| Pust 0 Ehrs | 12-0 | 19.75 | 145.0 | 10 | Post 2 Jude (**) | | | | | | | | |
| Post-2 5hrs | | /3.64 | 145.8 | 18 | POST=2 2WKS (==) | | | | | | | | |
| Post-2 2wks | | 4.97 | 3.2 | 18 | | | | | | | | | |
| Pre | | 3.27 | 0.8 | 18 | Den un Dente d Flore (8) P. D. 1 d D. 1 d D. 1 | | | | | | | | |
| Post-1 5hrs | | 4.35 | 1.6 | 18 | Pre vs Post-1 5hrs (*), Pre vs Post-1 2wks (**), | | | | | | | | |
| Post-1 2wke | IL-15 | 2.94 | 1.0 | 18 | Post-1 5hrs vs Post-1 2wks (*), Post-1 5hrs vs | | | | | | | | |
| Poet 2 Eber | 15 | 2.57 | 1.5 | 10 | Post-2 5hrs (*), Post-1 5hrs vs Post-2 2wks | | | | | | | | |
| Post-2 Shrs | | 2.0/ | 1.2 | 10 | (***) | | | | | | | | |
| Post-2 2wks | | 2.44 | 1.0 | 18 | | | | | | | | | |
| Pre | | 7.29 | 3.7 | 18 | | | | | | | | | |
| Post-1 5hrs | | 11.90 | 6.6 | 18 | | | | | | | | | |
| Post-1 2wks | MIP-2α | 10.51 | 5.2 | 18 | Pre vs Post-1 5hrs (*) | | | | | | | | |
| Poet-2 5hre | | 11.44 | 5.9 | 18 | | | | | | | | | |
| Post-2 Jills | | 12.44 | 0.0 | 10 | | | | | | | | | |
| Post-2 2wks | | 12.41 | 8.3 | 18 | | | | | | | | | |
| Pre | | 691.91 | 977.6 | 18 | | | | | | | | | |
| Post-1 5hrs | | 615.81 | 558.5 | 18 | | | | | | | | | |
| Post-1 2wks | IL-18 | 262.60 | 165.0 | 18 | Post-1 5hrs vs Post-1 2wks (*) | | | | | | | | |
| Post-2 5hrs | | 345.02 | 283.6 | 18 | | | | | | | | | |
| Post-2 Julia | | 345.02 | 205.0 | 10 | - | | | | | | | | |
| POSt-2 ZWKS | | 200.28 | 00.3 | 10 | | | | | | | | | |
| Pre | | 10.73 | 5.7 | 18 | | | | | | | | | |
| Post-1 5hrs | | 15.89 | 7.8 | 18 | | | | | | | | | |
| Post-1 2wks | IL-1α | 11.71 | 8.4 | 18 | NS | | | | | | | | |
| Post-2 5hrs | | 16.01 | 15.7 | 18 | | | | | | | | | |
| Post-2 2wks | | 16.03 | 11.9 | 18 | - | | | | | | | | |
| 1 03C2 2WK3 | | 6.30 | 4.5 | 10 | | | | | | | | | |
| Pre | | 6.50 | 4.5 | 10 | | | | | | | | | |
| Post-1 5hrs | | 6.25 | 2.5 | 18 | | | | | | | | | |
| Post-1 2wks | IL-5 | 6.66 | 4.2 | 18 | NS | | | | | | | | |
| Post-2 5hrs | | 10.85 | 9.5 | 18 | | | | | | | | | |
| Post-2 2wks | | 5.62 | 3.6 | 18 | | | | | | | | | |
| Dre | | 12.91 | 7.9 | 10 | | | | | | | | | |
| Fie | | 12.01 | 7.0 | 10 | | | | | | | | | |
| Post-1 5hrs | | 15.65 | 11.8 | 18 | - | | | | | | | | |
| Post-1 2wks | IL-7 | 13.77 | 9.8 | 18 | NS | | | | | | | | |
| Post-2 5hrs | | 8.02 | 4.9 | 18 | | | | | | | | | |
| Post-2 2wks | | 17.22 | 14.9 | 18 | | | | | | | | | |
| Pre | | 58.78 | 37.1 | 18 | | | | | | | | | |
| Port 1 Ehro | | 65.60 | 50.9 | 10 | - | | | | | | | | |
| Post-1 onrs | | 05.00 | 70.0 | 10 | | | | | | | | | |
| Post-1 2wks | IL-9 | 80.81 | 72.9 | 18 | NS | | | | | | | | |
| Post-2 5hrs | | 42.81 | 30.5 | 18 | | | | | | | | | |
| Post-2 2wks | | 60.35 | 40.2 | 18 | | | | | | | | | |
| Pre | | 3.54 | 2.8 | 18 | | | | | | | | | |
| Post-1 5hre | | 5,21 | 47 | 18 | | | | | | | | | |
| Post 1 July | 11.10 | A 69 | 4.0 | 10 | NC | | | | | | | | |
| Post-1 ZWKS | 10-10 | 4.00 | +.0 | 10 | N3 | | | | | | | | |
| Post-2 5hrs | | 4.69 | 3.2 | 18 | | | | | | | | | |
| Post-2 2wks | | 3.88 | 4.3 | 18 | | | | | | | | | |
| Pre | | 28.00 | 17.6 | 18 | | | | | | | | | |
| Post-1 5hrs | | 36.43 | 31.3 | 18 | | | | | | | | | |
| Post 1 2wks | 11-13 | 31.26 | 22.4 | 18 | NS | | | | | | | | |
| Post 0 Et | 10-13 | 17.00 | 12.4 | 10 | 113 | | | | | | | | |
| Post-2 5hrs | | 17.89 | 13.5 | 18 | | | | | | | | | |
| Post-2 2wks | | 25.05 | 21.6 | 18 | | | | | | | | | |
| Pre | | 163.50 | 128.1 | 18 | | | | | | | | | |
| Post-1 5hrs | | 212.56 | 171.0 | 18 | | | | | | | | | |
| Post-1 2wke | MIG | 133.69 | 126.8 | 18 | NS | | | | | | | | |
| Post 2 Ehr | | 177.43 | 112.1 | 10 | | | | | | | | | |
| Post-2 onrs | | 1/7.42 | 113.1 | 10 | | | | | | | | | |
| Post-2 2wks | | 118.69 | 121.1 | 18 | | | | | | | | | |
| Pre | | 1.54 | 1.4 | 18 | | | | | | | | | |
| Post-1 5hrs | | 5.67 | 10.2 | 18 | | | | | | | | | |
| Post-1 2wke | MIP-10 | 1.77 | 15 | 18 | NS | | | | | | | | |
| Deat 0 Ehr | | 2.0 | 2.5 | 10 | | | | | | | | | |
| Post-2 Shrs | | 2.08 | 2.3 | 18 | | | | | | | | | |
| Post-2 2wks | | 2.34 | 2.8 | 18 | | | | | | | | | |
| Pre | | 58.79 | 17.0 | 18 | | | | | | | | | |
| Post-1 5hrs | | 79.08 | 37.8 | 18 | | | | | | | | | |
| Post-1 2wke | RANTES | 54.84 | 24.5 | 18 | NS | | | | | | | | |
| Post 2 Ehre | | 74.22 | 25.0 | 19 | | | | | | | | | |
| Post 2 Onis | | 17.22 | 33.0 | 10 | | | | | | | | | |
| Post-2 2wks | | 60.46 | 28.3 | 18 | | | | | | | | | |

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-A | b1, H2-DM | a, H2-T23, H | 12-Q4, H2-T | 22, Cd40 | | | | | | | | |
| mmu05416 | Viral myocarditis | -1.9869 | 0.005362 | 0.097804 | H2-Q6, Dm | id, H2-Q7, H | 12-K1, H2-Eb1, | H2-Ab1, H | 2-DMa, H2- | T23, H2-Q4 | , H2-T22 | | | | | | | | |
| mmu00900 | Terpenoid backbone biosynthesis | -1.9622 | <2.2e-16 | 0.077823 | Hmgcr, Zm | pste24, Mvk | , Fdps, Acat1 | | | | | | | | | | | | |
| mmu04962 | Vasopressin-regulated water reabsorption | -1.8845 | 0.004739 | 0.11042 | Arhgdib, D | ynll2, Rab5l | b, Dctn1, Dync1 | h1, Dync1 | 2, Prkacb, | Creb1, Dync | 1li1, Rab5c | | | | | | | | |
| mmu04514 | Cell adhesion molecules (CAMs) | -1.8411 | 0.002725 | 0.12494 | H2-Q6, H2 | Q7, H2-K1, | H2-Eb1, Selple | g, H2-Ab1, | Cadm3, Se | I, H2-DMa, | ltgb1, H2-T2 | 3, H2-Q4, H | 2-T22 | | | | | | |
| mmu04672 | Intestinal immune network for IgA production | -1.7852 | 0.013761 | 0.14842 | H2-Eb1, H | 2-Ab1, H2-D | Ma, Map3k14, | Cd40 | | | | | | | | | | | |
| mmu05412 | Arrhythmogenic right ventricular cardiomyopathy (ARVC) | -1.6911 | 0.025229 | 0.24552 | Dmd, Tcf7. | Ctnna1, Slo | 8a1. Lef1. Itab | 1. Tcf7l2. C | acnb3 | | | | | | | | | | |
| mmu04612 | Antigen processing and presentation | -1.5963 | 0.035714 | 0.2543 | H2-Q6, H2 | -Q7, H2-K1, | Tap2, Ifi30, Kir | d1. H2-Eb1 | , Tapbp, C | 174, H2-Ab1 | , H2-DMa, I | | Q4, H2-T22, | Psme3, Cre | b1 | | | | |
| mmu00310 | Lysine degradation | -1.5956 | 0.041463 | 0.23565 | Setd1a, Kn | nt2b, Ash1l, | Acat1, Ehmt2, I | Nsd2, Kmt2 | d, Plod3, K | mt2a, Setd1 | b | | | | | | | | |
| mmu05340 | Primary immunodeficiency | -1.5876 | 0.042222 | 0.2284 | II2rg, Tap2 | Cd40, Rfxa | ap, Tap1 | | | | | | | | | | | | |
| mmu04625 | C-type lectin receptor signaling pathway | 2.4507 | <2.2e-16 | <2.2e-16 | Ptgs2, Clea | 4d, Clec4e | Nfkbia, Tnfl1b | , Nirp3, Itp | 2, Clec4n, | Fcer1g, Map | kapk2 | | | | | | | | |
| mmu04621 | NOD-like receptor signaling pathway | 2.4526 | <2.2e-16 | <2.2e-16 | Cxcl2, Cxc | 13, Nfkbia, T | nf, li1b, Nirp3, l | tpr2, Ccl2, | Ctsb, Erbin, | Tnfaip3, Ca | isp4, Jun, G | abarap, Vda | c2, Tank, Tx | n1 | | | | | |
| mmu04064 | NF-kappa B signaling pathway | 2.4686 | <2.2e-16 | <2.2e-16 | Cxcl2, Ccl4 | , Ptgs2, Cd | 14, Nfkbia, Tnf, | Gadd45b, | ll1b, Bcl2a1 | b, Tnfaip3, I | Bcl2a1a, Cf | ar, Icam1 | | | | | | | |
| mmu04657 | IL-17 signaling pathway | 2.4712 | <2.2e-16 | <2.2e-16 | Cxcl2, Cxc | 3, Ptgs2, N | fkbia, Tnf, II1b, 4 | Ccl2, Cebp | b, Fos, Tnfa | ip3 | | | | | | | | | |
| mmu04620 | Toll-like receptor signaling pathway | 2.5526 | <2.2e-16 | <2.2e-16 | Ccl3, Spp1 | , Ccl4, Cd14 | 4, Nfkbia, Tnf, II | 1b, Fos, Ma | p2k3, Jun, | Tlr2, Myd88 | , Irak1, Tab | 2 | | | | | | | |
| mmu04142 | Lysosome | 2.5714 | <2.2e-16 | <2.2e-16 | Atp6v0c, C | tsd, Ctsb, Li | taf, Ctsl, Psap, | Hgsnat, Cd | 68, Ap3s1, | Lamp2, Gns | , Atp6v0d2, | Ctsa, Cita, I | gf2r, Slc11a | 1, Neu1, Gla | , Lgmn, Hex | a, Npc1, Tp | p1, Atp6v0d | 1, Lamp1, A | 4tp6v0b |
| mmu05134 | Legionellosis | 2.6797 | <2.2e-16 | <2.2e-16 | Cxcl2, Cxc | 13, Cd14, Nf | kbia, Tnf, II1b | | | | | | | | | | | | |
| mmu05132 | Salmonella infection | 2.7148 | <2.2e-16 | <2.2e-16 | Cxcl2, Ccl3 | 8, Cxcl3, Ccl | 14, Cd14. II1a, II | 1b, Rab7 | | | | | | | | | | | |
| mmu04668 | TNF signaling pathway | 2.8446 | <2.2e-16 | <2.2e-16 | Cxcl2, Cxc | 3, Ptgs2, N | fkbia, Tnf, II1b, 6 | Ccl2, Csf1, | Cebpb, Tnf | rsf1b, Junb, | Socs3, Fos | Tnfaip3, Atf | 4, Cflar, Bcl3 | , Icam1, Ma | p2k3, Jun | | | | |
| mmu04060 | Cytokine-cytokine receptor interaction | 2,9309 | <2.2e-16 | <2.2e-16 | Cxcl2, Ccl3 | 8. II1m, Cxcl | 3. Ccl4. Tnf. II1 | a, II1b, Ccle | . Ccl2, II1fs | . Csf1. Inhb | a. Tnfrsf1b. | 11r2. Ccr1. 0 | Gdf15, II1rap | | | | | | |

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

| mmud390 Hippo signaling pathway -1.9161 < 22.e-16 0.26287 Cond2, Lind1, Cond3, Smad7, Tgh1, id2, Tgh12, Lats1, Mob1a, Itb2, Apc, Mob1b, Ywhaz, Bmp2, Pp2c1a, Tgh2 mmud3182 Hippo signaling pathway -1.8776 0.00687 0.0078 0.0088 0.007825 Akap13, Met24, Ace, Met2, Me |
|---|
| mmu0318 RNA degradation -1.8778 0.006873 0.18065 Viriefs (c.not3, Otx6, Zeche7, Cont1, Cnot2, Dhx36, Tie37, Xm1, Pan3, Exoes3, PNI |
| mmu01252 Endocrine resistance -1.7216 0.012324 0.43833 Nort, Repskh1, Bd2, Zolkn1b, Map2k1, Adoy7, Raf1, Mapk14, Bd2, Napk14, Adoy2, Raf1, Kl3cb mmu04261 Anternetic signaling in cardiomyocytes -1.8614 0.023850 0.024852 Nickl2, Pikk3c, Bd2; Nickl2, Raf1, Klack, Nich2, Bdax, Hark1, Mick3cb Mickab4 Add225 Pikk3cp, Riks7, Pikk3cb Mickab4 Add22 Nickl2, Raf1, Kira2, Kirk1, PH1, Jack2, Add23, Raf1, Kira2, Kirk1, Mickab4 Add225 Pikk3cp, Riks7, Bd2; Add43 Mick1, Fikk2, Fikk3cb Mickab2 Nickl2, Raf1, Kira2, Kirk1, PH1, Jack2, Add24, Raf1, Kira2, Kirk1, Add27, Raf1, Mapk14, Age, Bmp/2 Mickab2 Nickl2, Raf1, Kira2, Kirk1, PH1, Jack2, Add24, Raf1, Crdl Mickab2 Mickab2 Mickab2 Nickl2, Raf1, Kira2, Kirk1, PH1, Jack2, Raf1, Crdl Mickab2 Mickab2 Mickab2 Nickl2, Raf1, Kira2, Kirk1, PH1, Jack2, Raf1, Crdl Mickab2 Mickab2 Mickab2 Nickl2, Raf1, Kira2, Kirk1, PH1, Jack2, Raf1, Crdl Mickab2 Mickab2 Mickab2 Nickl2, Raf1, Kira2, Kirk1, PH1, Jack2, Raf1, Crdl Mickab2 Mickab2 Mickab2 Nickl2, Raf1, Kira2, Kirk1, PH1, Jack2, Raf1, Crdl Mickab2 Mickab2 Mickab2 Nickl2, Raf1, Kira2, Kirk1, PH1, Jack2, Raf1, Crdl Mickab2 Mickab2 |
| mmud4281 Adtenergic signaling in cardiomycoytes -1.8814 0.022885 0.44225 Pik3cg, pik3c5, Pik3c6, Bcl2, Camk2d, Canal, SicBa1, Adcy7, Mapk14, Adn22, Creb1, Ppp2ca, Tpm3, Ppp211, Abt2, Abp2a2, Gnaq mmud4550 Signaling pathways regulating pluripotency of stem cells -1.622 0.02727 0.44225 Pik1cH, Pit1K, Pit1, KPit, CAR4, Blaj, Kita9, Kita7, Tilma2, Pikkh, Kita8, Map2k1, Qamb, Hast, Raft, H2-D1, Hgb2, Pipos, Malat, Nort mmud4550 Signaling pathways regulating pluripotency of stem cells -1.622 0.02727 0.44761 (Atc), Kita1, Kita7, Kita7, Hina2, Pikkh, Sita8, Map2k1, Qamb, Caka, Raft, H2-D1, Hgb2, Pipos, Malat, Nort mmud0420 Valine, leucine and isoleucine adgradation -1.582 0.04751 0.0 |
| mmud4650 Natural killer cell mediated cytobaxicity 1.6221 0.024284 0.50073 Kirk1, Prit, Fyn, Cd48, Itgal, Kira9, Kira7, K |
| mmud4550 Signaling patways regulating pluripotency of stem cells 1.622 0.027.273 0.43463 Meins, IRIT, TG3, Skil, Map2k1, Liz, R4T, Map2k1, Age, Ramp2 Imag2 (1) mmud0412 ENB signaling pathway 1.586 0.04751 0.04751 0.01812 0.04751 0.04751 0.01812 0.04751 0.04751 0.01812 0.04751 0.04751 0.01815 0.04751 0.04751 0.01812 0.04751 0.01812 0.04751 0.01782 Ake3, Meich, Back, Meich, Back, Meich, Back, Meich, Back, Map2k1, Cank2, Raid, Crkk, Ang2k1, Cank2, Raid, Crkk Map2k1, Cank2, Raid, |
| mmu0412 ErbB signaling pathway -1.5862 0.04112 0.04750 EvbB, hps8kb1, Cdkn1b, Pkcb, Mag2k1, Cank2d, Raf1, Crkl Immu0426 mmu04282 Parathyroid hormone synthesis, secretion and action -1.516 0.030380 0.04709 Hadhb, Aaco, Hibdah, Hingdah Immu0428 Parathyroid hormone synthesis, secretion and action -1.1361 0.030380 0.047409 Hadhb, Aaco, Hibdah, Hingdah Immu04392 Hippo signaling pathway -1.1288 0.057492 Linnu, Last1, Mob1a, Mob1b Immu04392 Hippo signaling pathway -1.1288 0.057492 Linnu, Last1, Mob1a, Mob1b Immu04517 Immu04513 African trypanosomiasis 2.5754 <2.2e-16 |
| mmu02820 Valine, leucine and isoleucine degradation -1.5715 0.03308 0.44709 Handha, Aacs, Hibadh, Hingest Imagest mmu04282 Valine, leucine and isoleucine degradation -1.1361 0.03319 0.57325 Kap13, Met2G, |
| mmu04282 Parathyroid hormone synthesis, secretion and action -1.1381 0.03725 Akey31, Me224, Me2c, Bd2, Prickb, Mag2k1, Gnai3, Adey7, Lrp6, Raf1, Mef2a, Arbgef11 |
| mmud4392 Hippo signaling pathway -1.128 0.547493 Unity Lasts, Mob1a, Mob1b Impose Signaling pathway -1.288 0.547493 Unity Lasts, Mob1a, Mob1b Impose Signaling pathway -2.578 -2.26-16 Cx202, NKbia, Hpg2a, Cx23, Cx21, Tri, Triajp3, I11b Impose Signaling pathway -2.562 -2.2e-16 Cx21, NKbia, Hpg2a, Cx23, Cx21, Tri, Triajp3, I11b Impose Signaling pathway -2.662 -2.2e-16 Cx21, NKbia, Hpg2a, Cx23, Cx21, Tri, Triajp3, I11b Impose Signaling pathway 2.6624 -2.2e-16 Cx21, NKbia, Hpg2, Cx23, Cx21, Tri, Triajp3, I11b Impose Signaling pathway 2.6624 -2.2e-16 Cx21, NKbia, Hpg2, Cx23, Cx21, Tri, Triajp3, I11b, Nampt, NKbib, Gabarap, Tank, Hsp90ab1, Psp0ab1, Psp1a |
| mmud657 IL-17 signaling pathway 2.5785 < 22e-16 |
| mmu05133 African trypanosomiasis 26533 <22e-16 i>26243 I>26333 <22e-16 I>26243 I>26333 I>263333 I>263333 I>2633 |
| mmu04621 NDD-like receptor signaling pathway 2.6624 < 2.2e-16 cxd2, NKbia, tpr2, Cxd3, NIrp3, Cd2, Txn1, Tnf, Tnfaip3, I1b, Nampt, NKbib, Gabarap, Tank, Hsp90ab1, Pstpip1 mmu04620 Imatopoietic cell lineage 2.8752 < 2.2e-16 |
| mmu0640 Hematopoietic cell lineage 2.8752 c.22e-16 I.2.E.b.1 H2-A.b.1 Tnf, I1b, Igb3, Cd24a, Cd9 mmu05140 Leishmaniasis 2.7002 c.2e-16 Ntbia, Pgs2, Markstl, H2-Eb1, H2-Ab, Tnf, I1b, Ntbib Immu5312 mmu05132 Salmonella infection 2.7653 c.2e-16 Cxd2, Cd3, Cxd3, I1a, Cd14, ACA, I1sh, R42Ab, Tnf, I1b, Ntbib Immu5132 |
| mmu05140 Leishmanlasis 2.7002 <2.2e-16 Nikbia, Pigs2, Marcks11, H2-Eb1, H2-Aa, II1a, H2-Ab1, Tnf, II1b, Nikbia mmu05132 Salmonella infection 2.7635 <2.2e-16 |
| mmu05132 Salmonella infection 2.7635 <2.2e-16 <2.2e-16 <cxcl2, actg1<="" ccl3,="" ccl4,="" cd14,="" cxcl3,="" il1a,="" il1b,="" plekhm2,="" rac1,="" td=""></cxcl2,> |
| |
| mmu05134 Legionellosis 2.8207 <2.2e-16 <2.2e-16 Cxcl2, Nfkbia, Cxcl3, Cd14, Tnf, II1b, Arf1 |
| mmu04668 TNF signaling pathway 2.865 <2.2e-16 <2.2e-16 <cxcl2, atf4,="" ccl2,="" cxcl3,="" icam1<="" ii1b,="" junb,="" nfkbia,="" ptgs2,="" td="" tnf,="" tnfaip3,=""></cxcl2,> |
| mmu04060 Cytokine-cytokine receptor interaction 2.893 <2.2e-16 Cxcl2, Ccl3, Cxcl3, II1a, Ccl2, II19, Ccl6, II1rn, Tnf, Ccl4, Ccr1, II1b, Ppbp, Ccr7, Cxcl16, Tnfrsf1b, P/4, Fas, Gdf15, Cxcr4 |
| mmu04064 NF-kappa B signaling pathway 3.1212 <2.2e-16 Cxcl2, Nfkbia, Ptgs2, Gadd45b, Bcl2a1b, Cd14, Tnf, Ccl4, Bcl2a1a, Tnfaip3, II1b, 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 10¹⁴ 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. 1.0×10^{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.



Post-1 Post-2

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.



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).



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

A) Stacked violin plot of $Fc\gamma Rs$, complement receptors, TLRs, canonical and noncanonical 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 upand down-regulated genes contain a Log₂(Fold Change) > 0.5 or Log₂(Fold Change) < 0.5 and -Log₁₀(*p*-value) > 2.0.