

**Supplemental information**

**A first-in-human phase I/IIa gene transfer  
clinical trial for Duchenne muscular  
dystrophy using rAAVrh74.MCK.GALGT2**

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## SUPPLEMENTARY DATA

**Table S1. Adverse Events.**

| Adverse Events                                      |           |                |                        |            |                        |                              |                    |            |
|---|-----------|----------------|------------------------|------------|------------------------|------------------------------|--------------------|------------|
| Adverse Event                                       | Onset Day | Resolution Day | Unexpected or Expected | Serious AE | Severity (CTCAE grade) | Action Taken                 | Relationship       | Treatment  |
| <b>Subject 1</b>                                    |           |                |                        |            |                        |                              |                    |            |
| Bruising (groin)                                    | Day 0     | Day 29         | Expected               | No         | 2                      | None                         | Definitely Related | None       |
| Lymphocyte count decreased                          | Day 1     | Day 377        | Unexpected             | No         | 1                      | None                         | Probably Related   | None       |
| Minimally cushingoid face                           | Day 57    | -              | Expected               | No         | 1                      | None                         | Unrelated          | None       |
| Weight gain   | Day 61    | -              | Expected               | No         | 3                      | None                         | Unrelated          | None       |
| Pharyngitis and Adenitis                            | Day 93    | Day 103        | Expected               | No         | 1                      | Non-study treatment required | Unrelated          | Medication |
| Decrease in Ejection fraction (16.5% from baseline) | Day 559   | Day 727        | Expected               | No         | 2                      | Non-study treatment required | Unrelated          | Non-drug   |
| Tachycardia   | Day 591   | Day 727        | Expected               | No         | 2                      | Non-study treatment required | Unrelated          | Medication |
| <b>Subject 2</b>                                    |           |                |                        |            |                        |                              |                    |            |
| Bleeding at the femoral catheterization site        | Day 0     | Day 0          | Expected               | No         | 2                      | Non-study treatment required | Definitely related | Non-drug   |
| Vomiting  | Day 1     | Day 1          | Unexpected             | No         | 1                      | None                         | Possibly related   | None       |
| Bruising (groin)                                    | Day 0     | Day 63         | Expected               | No         | 2                      | None                         | Definitely related | None       |
| Low hemoglobin                                      | Day 1     | Day 12         | Expected               | No         | 1                      | None                         | Unlikely related   | None       |
| Lymphocyte count decreased                          | Day 26    | Day 90         | Unexpected             | No         | 1                      | None                         | Probably Related   | None       |
| Lymphocyte count decreased                          | Day 600   | Day 733        | Unexpected             | No         | 1                      | None                         | Probably Related   | None       |

**Table S2A. Serum Chemistry Part 1.** Normal serum chemistry values are shown in parentheses in the headers. Values falling outside the normal range are bolded and italicized. (BUN, blood urea nitrogen)

| Serum Chemistry Part 1 |                |                               |                                  |                                |  |                        |   |                              |                              |                     |
|------------------------|----------------|-------------------------------|----------------------------------|--------------------------------|--|------------------------|---|------------------------------|------------------------------|---------------------|
| Nominal Visit          | Collection Day | Sodium<br>(135-145<br>mmol/L) | Potassium<br>(3.7-5.6<br>mmol/L) | Chloride<br>(95-106<br>mmol/L) | Carbon<br>Dioxide<br>(18-27<br>mmol/L) | BUN<br>(5-18<br>mg/dL) | Creatinine<br>(0.30-0.60<br>(5-12y)<br>mg/dL) | Glucose<br>(60-115<br>mg/dL) | Calcium<br>(8-10.5<br>mg/dL) | Comments<br>/Notes: |
| <b>Subject 1</b>       |                |                               |                                  |                                |  |                        |   |                              |                              |                     |
| Screening              | Day -28        | 144                           | 3.9                              | 105                            | -                                      | 9                      | 0.2   | 94                           | 9.5                          |                     |
| Day 1                  | Day 1          | 144                           | <b>3.4 (L)</b>                   | <b>108 (H)</b>                 | <b>28 (H)</b>                          | 6                      | 0.25  | 86                           | 8.5                          |                     |
| Day 7                  | Day 7          | 141                           | 4.2                              | 102                            | 26                                     | 9                      | 0.22  | <b>123 (H)</b>               | 10.1                         |                     |
| Day 14                 | Day 14         | 141                           | 4.3                              | 102                            | 25                                     | 10                     | 0.22  | 92                           | 9.4                          |                     |
| Day 30                 | Day 28         | 141                           | 4.8                              | 103                            | 25                                     | 10                     | 0.2   | <b>119 (H)</b>               | 9.5                          | Hemolyzed           |
| Day 90                 | Day 118        | 144                           | 4.4                              | 102                            | <b>29 (H)</b>                          | 13                     | 0.33  | 93                           | 9.3                          | Hemolyzed           |
| Month 6                | Day 179        | <b>146 (H)</b>                | 4                                | 106                            | 26                                     | 13                     | 0.29  | 90                           | 9.4                          |                     |
| Month 12               | Day 377        | 141                           | 4.1                              | 104                            | <b>29 (H)</b>                          | 15                     | <b>0.19 (L)</b>                               | 84                           | 9.3                          |                     |
| Month 18               | Day 559        | 143                           | 4.1                              | 104                            | 26                                     | 17                     | <b>0.16 (L)</b>                               | 89                           | 9.5                          |                     |
| Month 24               | Day 727        | 139                           | 4.3                              | 102                            | <b>30 (H)</b>                          | <b>19 (H)</b>          | <b>&lt;0.15 (L)</b>                           | 103                          | 9.6                          |                     |
| <b>Subject 2</b>       |                |                               |                                  |                                |  |                        |   |                              |                              |                     |
| Screening              | Day -44        | 140                           | 4.2                              | 103                            | <b>28 (H)</b>                          | 10                     | 0.21  | 103                          | 9.7                          |                     |
| Day 1                  | Day 1          | 139                           | 4.1                              | 105                            | 27                                     | 7                      | 0.22  | 83                           | 9.1                          |                     |
| Day 7                  | Day 5          | 140                           | 3.8                              | 105                            | 25                                     | 13                     | 0.27  | 96                           | 9.2                          |                     |
| Day 14                 | Day 12         | 138                           | 4.6                              | -                              | -                                      | 14                     | 0.46  | 103                          | 10.1                         |                     |
| Day 30                 | Day 26         | 139                           | 4.4                              | 102                            | 27                                     | 14                     | 0.22  | 92                           | 9.8                          |                     |
| Day 30                 | Day 26         | 139                           | 4.4                              | 102                            | 27                                     | 14                     | 0.22  | 92                           | 9.8                          |                     |
| Day 90                 | Day 90         | 139                           | 4.2                              | 104                            | <b>29 (H)</b>                          | 14                     | <b>0.27 (L)</b>                               | 104                          | 9.4                          |                     |
| Month 6                | Day 173        | 140                           | 4.2                              | 105                            | 26                                     | 13                     | <b>0.19 (L)</b>                               | 96                           | 9.6                          |                     |
| Month 12               | Day 376        | 139                           | <b>3.6 (L)</b>                   | 103                            | <b>30 (H)</b>                          | 11                     | <b>0.18 (L)</b>                               | 101                          | 9.3                          |                     |
| Month 18               | Day 600        | 138                           | 4.5                              | 103                            | <b>29 (H)</b>                          | 13                     | <b>0.17 (L)</b>                               | 104                          | 9.2                          |                     |
| Month 24               | Day 733        | 138                           | 4.3                              | 102                            | <b>31 (H)</b>                          | 16                     | <b>0.21 (L)</b>                               | 96                           | 9.7                          |                     |

**Table S2B. Serum Chemistry Part 2.** Normal serum chemistry values are shown in parentheses in the headers. Values falling outside the normal range are bolded and italicized. (ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; CK, creatine kinase)

| Serum Chemistry Part 2   |                |                                    |                              |                                       |  |                                     |                   |                       |                         |                   |                           |                       |                     |
|--------------------------|----------------|------------------------------------|------------------------------|---------------------------------------|--|-------------------------------------|-------------------|-----------------------|-------------------------|-------------------|---------------------------|-----------------------|---------------------|
| Nominal Visit            | Collection Day | Total Protein<br>(5.8-8.7<br>g/dL) | Albumin<br>(3.4-5.1<br>g/dL) | Total Bilirubin<br>(0.1-1.0<br>mg/dL) | Indirect Bilirubin<br>(0.1-1.0<br>mg/dL) | Direct Bilirubin<br>(<0.6<br>mg/dL) | ALT<br>(< 40 U/L) | AST<br>(15-50<br>U/L) | ALP<br>(151-342<br>U/L) | GGT<br>(8-78 U/L) | Amylase<br>(< 110<br>U/L) | CK<br>(37-430<br>U/L) | Comments/<br>Notes: |
| <b>Subject 1</b>         |                |                                    |                              |                                       |  |                                     |                   |                       |                         |                   |                           |                       |                     |
| Screening                | Day-28         | 6.9                                | 4.3                          | 0.5                                   | 0.2                                      | 0.3                                 | <b>497 (H)</b>    | <b>459 (H)</b>        | <b>88 (L)</b>           | 20                | 46                        | <b>21583 (H)</b>      |                     |
| Day-1                    | Day-1          | -                                  | -                            | -                                     | -  | -                                   | <b>409 (H)</b>    | <b>302 (H)</b>        | -                       | 18                | -                         | <b>13504 (H)</b>      |                     |
| Day 1                    | Day 1          | <b>5.4 (L)</b>                     | <b>3.3 (L)</b>               | 0.9                                   | -  | -                                   | <b>314 (H)</b>    | <b>342 (H)</b>        | <b>66 (L)</b>           | 14                | -                         | <b>15901 (H)</b>      |                     |
| Day 7                    | Day 7          | 8.1                                | 5                            | 0.6                                   | 0.2                                      | 0.4                                 | <b>382 (H)</b>    | <b>244 (H)</b>        | <b>81 (L)</b>           | 23                | 52                        | <b>6767 (H)</b>       |                     |
| Day 14                   | Day 14         | 7.1                                | 4.5                          | 0.4                                   | -  | -                                   | <b>515 (H)</b>    | <b>586 (H)</b>        | <b>72 (L)</b>           | 20                | 67                        | <b>12571 (H)</b>      |                     |
| Day 30                   | Day 28         | 7.4                                | 4.8                          | -                                     | -  | -                                   | <b>280 (H)</b>    | <b>213 (H)</b>        | <b>68 (L)</b>           | 23                | 61                        | <b>6363 (H)</b>       | Hemolyzed           |
| Day 60                   | Day 57         | -                                  | -                            | -                                     | -  | -                                   | <b>466 (H)</b>    | <b>279 (H)</b>        | -                       | 22                | -                         | <b>11310 (H)</b>      |                     |
| Day 75                   | Day 72         | -                                  | -                            | -                                     | -  | -                                   | <b>425 (H)</b>    | <b>278 (H)</b>        | -                       | 24                | -                         | <b>7803 (H)</b>       |                     |
| Day 90                   | Day 118        | 6.9                                | 4                            | 0.6                                   | -  | -                                   | <b>489 (H)</b>    | <b>368 (H)</b>        | <b>59 (L)</b>           | 28                | -                         | <b>12296 (H)</b>      | Hemolyzed           |
| Month 6                  | Day 181        | 7                                  | 4.2                          | 0.4                                   | -  | -                                   | <b>423 (H)</b>    | <b>267 (H)</b>        | <b>82 (L)</b>           | 31                | 55                        | <b>12085 (H)</b>      |                     |
| Day 216                  | Day 216        | -                                  | -                            | -                                     | -  | -                                   | <b>444 (H)</b>    | <b>250 (H)</b>        | -                       | 24                | -                         | <b>6822 (H)</b>       |                     |
| Month 12                 | Day 377        | 7                                  | 4.3                          | 0.3                                   | -  | -                                   | <b>411 (H)</b>    | <b>359 (H)</b>        | <b>112 (L)</b>          | 17                | 42                        | <b>12712 (H)</b>      |                     |
| Month 18                 | Day 559        | 7.2                                | 4.4                          | 0.3                                   | -  | -                                   | <b>269 (H)</b>    | <b>143 (H)</b>        | <b>109 (L)</b>          | 34                | -                         | <b>4739 (H)</b>       |                     |
| Month 24                 | Day 727        | 7.2                                | 4.3                          | 0.4                                   | -  | -                                   | <b>207 (H)</b>    | <b>132 (H)</b>        | <b>83 (L)</b>           | 31                | -                         | <b>5930 (H)</b>       |                     |
| <b>Subject 2</b>         |                |                                    |                              |                                       |  |                                     |                   |                       |                         |                   |                           |                       |                     |
| Screening                | Day -44        | 7.1                                | 4.5                          | 0.3                                   | -  | -                                   | <b>1020 (H)</b>   | <b>733 (H)</b>        | <b>80 (L)</b>           | <10               | 55                        | <b>32819 (H)</b>      |                     |
| Screening<br>(home draw) | Day -8         | -                                  | -                            | -                                     | -  | -                                   | <b>752 (H)</b>    | <b>383 (H)</b>        | -                       | 12                | -                         | <b>15236 (H)</b>      |                     |
| Day -1                   | Day -1         | -                                  | -                            | -                                     | -  | -                                   | <b>692 (H)</b>    | <b>399 (H)</b>        | -                       | <10               | -                         | <b>21239 (H)</b>      |                     |
| Day 1                    | Day 1          | 6.1                                | 3.6                          | -                                     | -  | -                                   | <b>594 (H)</b>    | <b>272 (H)</b>        | <b>75 (L)</b>           | <10               | -                         | <b>10275 (H)</b>      |                     |
| Day 7                    | Day 5          | 6.5                                | 3.7                          | 0.3                                   | -  | -                                   | <b>503 (H)</b>    | <b>269 (H)</b>        | <b>70 (L)</b>           | <10               | -                         | <b>12976 (H)</b>      |                     |
| Day 14                   | Day 12         | 7.8                                | 4.7                          | 0.3                                   | -  | -                                   | <b>646 (H)</b>    | <b>336 (H)</b>        | <b>78 (L)</b>           | <10               | 59                        | <b>18192 (H)</b>      |                     |
| Day 30                   | Day 26         | 7                                  | 4.2                          | 0.2                                   | -  | -                                   | <b>728 (H)</b>    | <b>718 (H)</b>        | <b>72 (L)</b>           | <10               | 44                        | <b>38270 (H)</b>      |                     |
| Day 45                   | Day 47         | -                                  | -                            | -                                     | -  | -                                   | <b>845 (H)</b>    | <b>642 (H)</b>        | -                       | 10                | -                         | -                     |                     |
| Day 60                   | Day 63         | -                                  | -                            | -                                     | -  | -                                   | <b>792 (H)</b>    | <b>335 (H)</b>        | -                       | <10               | -                         | -                     |                     |
| Day 75                   | Day 75         | -                                  | -                            | -                                     | -  | -                                   | <b>1007 (H)</b>   | <b>837 (H)</b>        | -                       | <10               | -                         | <b>43793 (H)</b>      |                     |
| Day 90                   | Day 90         | 6.9                                | 4                            | 0.3                                   | -  | -                                   | <b>840 (H)</b>    | <b>550 (H)</b>        | <b>80 (L)</b>           | <10               | -                         | <b>22039 (H)</b>      |                     |
| Month 6                  | Day 173        | 6.8                                | 4                            | 0.4                                   | -  | -                                   | <b>868 (H)</b>    | <b>660 (H)</b>        | <b>73 (L)</b>           | <10               | -                         | <b>29692 (H)</b>      |                     |
| Month 12                 | Day 376        | 7.1                                | 4.2                          | 0.4                                   | -  | -                                   | <b>830 (H)</b>    | <b>616 (H)</b>        | <b>74 (L)</b>           | <10               | -                         | <b>25721 (H)</b>      |                     |
| Month 18                 | Day 600        | 7.3                                | 4.4                          | 0.3                                   | -  | -                                   | <b>853 (H)</b>    | <b>713 (H)</b>        | <b>75 (L)</b>           | <10               | -                         | <b>27192 (H)</b>      |                     |
| Month 24                 | Day 733        | 7.2                                | 4.1                          | 0.3                                   | -  | -                                   | <b>710 (H)</b>    | <b>667 (H)</b>        | <b>80 (L)</b>           | 12                | -                         | <b>27776 (H)</b>      |                     |

**Table S3. Blood WBC Differential.** Normal values are shown in parentheses in the headers. Values falling outside the normal range are bolded and italicized. Grade 1 adverse events according to CTCAE v4.2 are underlined. (ANC, absolute neutrophil count; ALC, absolute lymphocyte count)

| Blood WBC differential |           |           |                     |                         |              |                     |                 |                   |                    |                  |  |   |
|------------------------|-----------|-----------|---------------------|-------------------------|--------------|---------------------|-----------------|-------------------|--------------------|------------------|--|---|
| Nominal Visit          | Visit Day | Diff Type | Neutrophil (36-72%) | Seg Neutrophil (32-54%) | Band (5-11%) | Lymphocyte (27-57%) | Monocyte (2-8%) | Myelocytes (0.0%) | Eosinophils (1-4%) | Basophils (0-1%) | Calculated ANC (1500-8000 /mm <sup>3</sup> ) | Calculated ALC (2500-7200/mm <sup>3</sup> ) |
| <b>Subject 1</b>       |           |           |                     |                         |              |                     |                 |                   |                    |                  |  |   |
| Screening              | Day -28   | Automated | 61                  | -                       | -            | 32                  | 6               | -                 | 1                  | 0                | <b>9150 (H)</b>                              | 4800  |
| Day -1                 | Day -1    | Automated | 48                  | -                       | -            | 42                  | 8               | -                 | 2                  | 0                | 5328   | 4662  |
| Day 1 Re-draw          | Day 1     | Automated | <b>82 (H)</b>       | -                       | -            | <b>14 (L)</b>       | 4               | -                 | <b>0 (L)</b>       | 0                | <b>9020 (H)</b>                              | <b>1540 (L)</b>                             |
| Day 7                  | Day 7     | Manual    | -                   | <b>81 (H)</b>           | -            | <b>16 (L)</b>       | 4               | -                 | -                  | -                | <b>11664 (H)</b>                             | <b>2304 (L)</b>                             |
| Day 14                 | Day 14    | Manual    | -                   | <b>74 (H)</b>           | <b>1 (L)</b> | <b>19 (L)</b>       | 4               | -                 | 1                  | -                | <b>7875 (L)</b>                              | <b>1995 (L)</b>                             |
| Day 30                 | Day 28    | Automated | <b>82 (H)</b>       | -                       | -            | <b>16 (L)</b>       | 2               | -                 | <b>0 (L)</b>       | 0                | 8692   | <b>1680 (L)</b>                             |
| Day 90                 | Day 118   | Manual    | -                   | <b>71.4 (H)</b>         | -            | <b>16.2 (L)</b>     | <b>10.5 (H)</b> | <b>1.9 (H)</b>    | -                  | -                | <b>10210 (H)</b>                             | <b>2317 (L)</b>                             |
| Month 6                | Day 181   | Automated | <b>79.4 (H)</b>     | -                       | -            | <b>13.5 (L)</b>     | 5.9             | -                 | <b>0.9 (L)</b>     | 0.3              | <b>10322 (H)</b>                             | <b>1755 (L)</b>                             |
| Month 12               | Day 377   | Automated | 63.4                | -                       | -            | 27.7                | 7.3             | -                 | 1.3                | 0.3              | 6023   | 2631  |
| Month 18               | Day 559   | Automated | 55.7                | -                       | -            | 32.9                | <b>9.4 (H)</b>  | -                 | 1.6                | 0.4              | 4456   | 2632  |
| Month 24               | Day 727   | Automated | 54                  | -                       | -            | 36.3                | 7.4             | -                 | 1.8                | 0.5              | 5130   | 3449  |
| <b>Subject 2</b>       |           |           |                     |                         |              |                     |                 |                   |                    |                  |  |   |
| Screening              | Day -44   | Automated | 69.4                | -                       | -            | <b>26.7 (L)</b>     | 3.6             | -                 | <b>0.1 (L)</b>     | 0.2              | 5968   | <b>2296 (L)</b>                             |
| Day -1                 | Day -1    | Automated | 42.4                | -                       | -            | 49.6                | 6.9             | -                 | <b>0.9 (L)</b>     | 0.2              | 4070   | 4762  |
| Day 1                  | Day 1     | Automated | 55.5                | -                       | -            | 36.5                | 7.3             | -                 | <b>0.5 (L)</b>     | 0.2              | 6105   | 4015  |
| Day 7                  | Day 5     | Automated | 54.4                | -                       | -            | 37.3                | 7               | -                 | 1                  | 0.3              | 5059   | 3469  |
| Day 14                 | Day 12    | Automated | 68.4                | -                       | -            | <b>26.6 (L)</b>     | 4.1             | -                 | <b>0.5 (L)</b>     | 0.4              | 6908   | 2687  |
| Day 30                 | Day 26    | Automated | <b>77.6 (H)</b>     | -                       | -            | <b>18.1 (L)</b>     | 3.7             | -                 | <b>0.4 (L)</b>     | 0.2              | 9700   | <b>2263 (L)</b>                             |
| Day 75                 | Day 75    | Automated | <b>78.4 (H)</b>     | -                       | -            | <b>17.7 (L)</b>     | 3.7             | -                 | <b>0.1 (L)</b>     | 0.1              | 10819  | <b>2443 (L)</b>                             |
| Day 90                 | Day 90    | Automated | 63.2                | -                       | -            | 33.2                | 3               | -                 | <b>0.4 (L)</b>     | 0.2              | 5056   | 2689  |
| Month 6                | Day 173   | Automated | <b>73.2 (H)</b>     | -                       | -            | <b>23.2(L)</b>      | 3.3             | -                 | <b>0.1 (L)</b>     | 0.2              | <b>9955 (H)</b>                              | 3155  |
| Month 12               | Day 376   | Automated | 71.1                | -                       | -            | <b>24.0 (L)</b>     | 4.2             | -                 | <b>0.5 (L)</b>     | 0.2              | <b>8603 (H)</b>                              | 2904  |
| Month 18               | Day 600   | Automated | 68.8                | -                       | -            | 27.8                | 3.1             | -                 | <b>0.2 (L)</b>     | 0.1              | 5641.6                                       | <b>2280 (L)</b>                             |
| Month 24               | Day 733   | Automated | <b>76.3 (H)</b>     | -                       | -            | <b>19.8 (L)</b>     | 3.3             | -                 | <b>0.4 (L)</b>     | 0.2              | <b>10450 (H)</b>                             | 2732  |

**Table S4. Coagulation.** Normal values are shown in parentheses in the headers. Values falling outside the normal range are bolded and italicized. (PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time)

| Coagulation      |           |                     |      |                   |              |                         |
|------------------|-----------|---------------------|------|-------------------|--------------|-------------------------|
| Nominal Visit    | Visit Day | PT<br>(12.4-14.7 s) | INR  | aPTT<br>(24-36 s) | Coag Source  | Cystatin C<br>(0.5-1.3) |
| <b>Subject 1</b> |           |                     |      |                   |              |                         |
| Screening        | Day -28   | 12.8                | 1    | 29                | Venipuncture | 0.8                     |
| Day -1           | Day -1    | 13                  | 1.02 | 30                | Venipuncture | -                       |
| Day 7            | Day 7     | <b>12.3 (L)</b>     | 0.95 | 27                | Venipuncture | -                       |
| Day 14           | Day 14    | 12.7                | 0.99 | 27                | Venipuncture | -                       |
| Day 30           | Day 28    | 12.6                | 0.98 | 27                | Venipuncture | -                       |
| Day 60           | Day 57    | -                   | -    | -                 | -            | -                       |
| Day 90           | Day 118   | 12.9                | 0.95 | 28                | Venipuncture | 0.7                     |
| Month 6          | Day 181   | 13.3                | 0.99 | 28                | Venipuncture | 0.8                     |
| Month 12         | Day 377   | 13.5                | 1.01 | 28                | Venipuncture | 0.8                     |
| Month 18         | Day 559   | 13.8                | 1.06 | 30                | Venipuncture | 0.9                     |
| Month 24         | Day 727   | 13.5                | 1.03 | 29                | Venipuncture | 0.9                     |
| <b>Subject 2</b> |           |                     |      |                   |              |                         |
| Screening        | Day -44   | 14.6                | 1.11 | 29                | Venipuncture | 0.5                     |
| Day -1           | Day -1    | 14.1                | 1.07 | 30                | Venipuncture | -                       |
| Day 1            | Day 1     | -                   | -    | -                 | -            | -                       |
| Day 7            | Day 5     | 13.9                | 1.05 | 28                | Venipuncture | -                       |
| Day 14           | Day 12    | 12.9                | 0.95 | 30                | Venipuncture | -                       |
| Day 30           | Day 26    | 13.8                | 1.04 | 29                | Venipuncture | 0.6                     |
| Day 90           | Day 90    | 14.1                | 1.07 | 32                | Venipuncture | 0.6                     |
| Month 6          | Day 173   | 14.5                | 1.1  | 31                | Venipuncture | 0.6                     |
| Month 12         | Day 376   | 14.6                | 1.13 | 30                | Venipuncture | 0.6                     |
| Month 18         | Day 600   | 13.6                | 1.06 | 28                | Venipuncture | 0.6                     |
| Month 24         | Day 733   | 13.3                | 1.03 | 29                | Venipuncture | 0.7                     |

**Table S5. Urinalysis.** Normal values are shown in parentheses in the headers. Values falling outside the normal range are bolded and italicized. (SG, specific gravity; RBC, red blood cell; WBC, white blood cell)

| Urinalysis       |           |             |            |                     |                 |                  |                  |                           |         |
|------------------|-----------|-------------|------------|---------------------|-----------------|------------------|------------------|---------------------------|---------|
| Nominal Visit    | Visit Day | Urine Color | Appearance | SG<br>(1.007-1.030) | pH<br>(4.5-8.0) | RBC<br>(0-2/HPF) | WBC<br>(< 6/HPF) | Squamous Epithelial Cells | Mucus   |
| <b>Subject 1</b> |           |             |            |                     |                 |                  |                  |                           |         |
| Screening        | Day -28   | Yellow      | Hazy       | 1.028               | 6.0             | <b>3 (H)</b>     | 2                | -                         | Present |
| Day 1            | Day 1     | Yellow      | Clear      | 1.025               | 5.0             | <b>21 (H)</b>    | 1                | -                         | Present |
| Day 7            | Day 7     | Straw       | Clear      | 1.008               | 7.0             | 2                | 1                | -                         | Present |
| Day 14           | Day 14    | Yellow      | Cloudy     | 1.021               | 7.0             | -                | <1               | -                         | Present |
| Day 30           | Day 28    | Straw       | Clear      | 1.01                | 5.0             | <b>3 (H)</b>     | 1                | -                         | Present |
| Day 90           | Day 118   | Yellow      | Clear      | 1.024               | 6.0             | -                | -                | -                         | Present |
| Month 6          | Day 181   | Yellow      | Clear      | 1.019               | 5.0             | 1                | <1               | -                         | Present |
| Month 12         | Day 377   | Straw       | Clear      | 1.011               | 5.0             | -                | <1               | -                         | Present |
| Month 18         | Day 559   | Yellow      | Clear      | 1.019               | 6.0             | <1               | <1               | -                         | -       |
| Month 24         | Day 727   | Yellow      | Clear      | 1.013               | 7.0             | <1               | <1               | -                         | Present |
| <b>Subject 2</b> |           |             |            |                     |                 |                  |                  |                           |         |
| Screening        | Day -44   | Straw       | Clear      | 1.017               | 6.0             | -                | -                | -                         | -       |
| Day 1            | Day 1     | Yellow      | Cloudy     | 1.019               | 7.0             | -                | -                | -                         | -       |
| Day 2            | Day 2     | Yellow      | Hazy       | <b>1.031 (H)</b>    | 5.0             | 2                | <1               | -                         | Present |
| Day 7            | Day 5     | Straw       | Clear      | <b>1.006 (L)</b>    | 6.0             | 1                | -                | -                         | -       |
| Day 14           | Day 12    | Yellow      | Hazy       | 1.02                | 6.0             | -                | -                | -                         | Present |
| Day 30           | Day 26    | Yellow      | Hazy       | 1.026               | 6.0             | <1               | -                | -                         | Present |
| Day 60           | Day 63    | Yellow      | Clear      | 1.024               | 5.0             | <1               | <1               | -                         | Present |
| Day 90           | Day 90    | Yellow      | Cloudy     | 1.01                | 7.0             | -                | -                | -                         | Present |
| Month 6          | Day 173   | Yellow      | Hazy       | 1.024               | 6.0             | -                | -                | -                         | Present |
| Month 12         | Day 376   | Yellow      | Hazy       | 1.024               | 6.0             | 1                | -                | -                         | Present |
| Month 18         | Day 600   | Yellow      | Cloudy     | 1.021               | 8.0             | 1                | -                | -                         | Present |
| Month 24         | Day 733   | Yellow      | Hazy       | 1.029               | 6.0             | 1                | 2                | <1                        | Present |

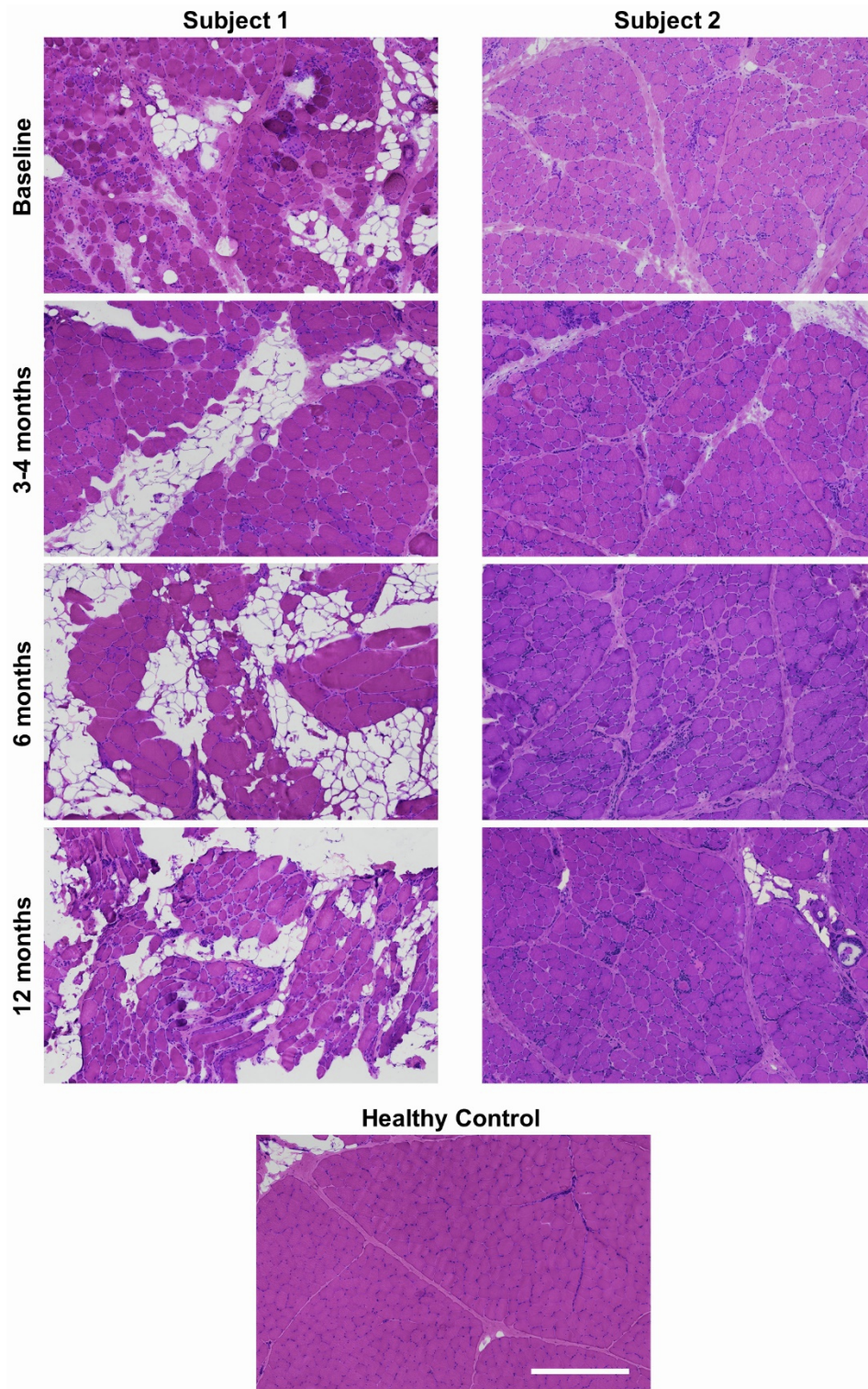
**Table S6. Urine Chemistry.** Normal values are shown in parentheses in the headers. Values falling outside the normal range are bolded and italicized.

| <b>Urine Chemistry</b> |                  |                              |                              |                              |                                |                                   |                              |   |   |
|------------------------|------------------|------------------------------|------------------------------|------------------------------|--------------------------------|-----------------------------------|------------------------------|---|---|
| <b>Nominal Visit</b>   | <b>Visit Day</b> | <b>Protein</b><br>(Negative) | <b>Glucose</b><br>(Negative) | <b>Ketones</b><br>(Negative) | <b>Bilirubin</b><br>(Negative) | <b>Occult Blood</b><br>(Negative) | <b>Nitrite</b><br>(Negative) | <b>Urobilinogen</b><br>( $< 1.1$ mg/dL) | <b>Leukocyte Esterase</b><br>(Negative) |
| <b>Subject 1</b>       |                  |                              |                              |                              |                                |                                   |                              |   |   |
| Screening              | Day -28          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Day 1                  | Day -1           | Negative                     | Negative                     | Negative                     | Negative                       | <b><i>Trace (H)</i></b>           | Negative                     | Negative                                | Negative                                |
| Day 7                  | Day 7            | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Day 14                 | Day 14           | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Day 30                 | Day 28           | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Day 90                 | Day 118          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Month 6                | Day 181          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Month 12               | Day 377          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Month 18               | Day 559          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Month 24               | Day 727          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| <b>Subject 2</b>       |                  |                              |                              |                              |                                |                                   |                              |   |   |
| Screening              | Day -44          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Day 1                  | Day 1            | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | <b><i>2 (H)</i></b>                     | Negative                                |
| Day 2                  | Day 2            | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | <b><i>2 (H)</i></b>                     | Negative                                |
| Day 7                  | Day 5            | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Day 14                 | Day 12           | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Day 30                 | Day 26           | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Day 60                 | Day 63           | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Day 90                 | Day 90           | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Month 6                | Day 173          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Month 12               | Day 376          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Month 18               | Day 600          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |
| Month 24               | Day 733          | Negative                     | Negative                     | Negative                     | Negative                       | Negative                          | Negative                     | Negative                                | Negative                                |

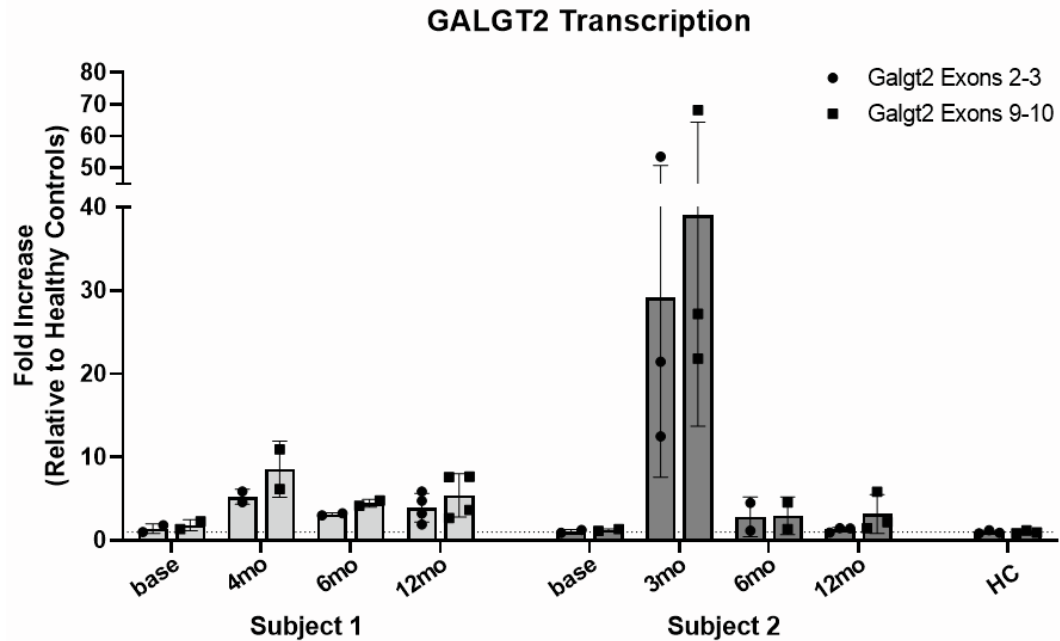


**Table S7. AAV biodistribution in muscle.** rAAVrh74.MCK.GALGT2 biodistribution was quantified by qPCR based on a standard curve from 50 to  $5 \times 10^6$  vg/ $\mu$ g. Each value (A-B or A-C) represents the average of 3 replicates from a different segment of the same muscle biopsy. Calculation for vg/diploid genome assumes  $1.67 \times 10^5$  nuclei per  $\mu$ g of human genomic DNA.

| Biodistribution  |              |                    |                   |
|------------------|--------------|--------------------|-------------------|
| Timepoint        | Biopsy Block | vg/ $\mu$ g        | vg/diploid genome |
| <b>Subject 1</b> |              |                    |                   |
| Screening        | A            | Undetermined (<50) |                   |
| Screening        | B            | Undetermined (<50) |                   |
| 4 months         | A            | 3.08E+04           | 0.185             |
| 4 months         | B            | 1.90E+04           | 0.114             |
| 6 months         | A            | 6.04E+03           | 0.036             |
| 6 months         | B            | 4.25E+03           | 0.025             |
| 12 months        | A            | 9.76E+03           | 0.059             |
| 12 months        | B            | 1.31E+04           | 0.078             |
| 12 months        | C            | 3.12E+04           | 0.187             |
| 12 months        | D            | 1.28E+04           | 0.077             |
| <b>Subject 2</b> |              |                    |                   |
| Screening        | A            | Undetermined (<50) |                   |
| Screening        | B            | Undetermined (<50) |                   |
| 3 months         | A            | 3.09E+05           | 1.854             |
| 3 months         | B            | 1.73E+05           | 1.040             |
| 3 months         | C            | 2.17E+05           | 1.301             |
| 6 months         | A            | 1.57E+04           | 0.094             |
| 6 months         | B            | 2.01E+04           | 0.121             |
| 12 months        | A            | 2.43E+04           | 0.146             |
| 12 months        | B            | 3.13E+04           | 0.188             |
| 12 months        | C            | 2.64E+04           | 0.159             |

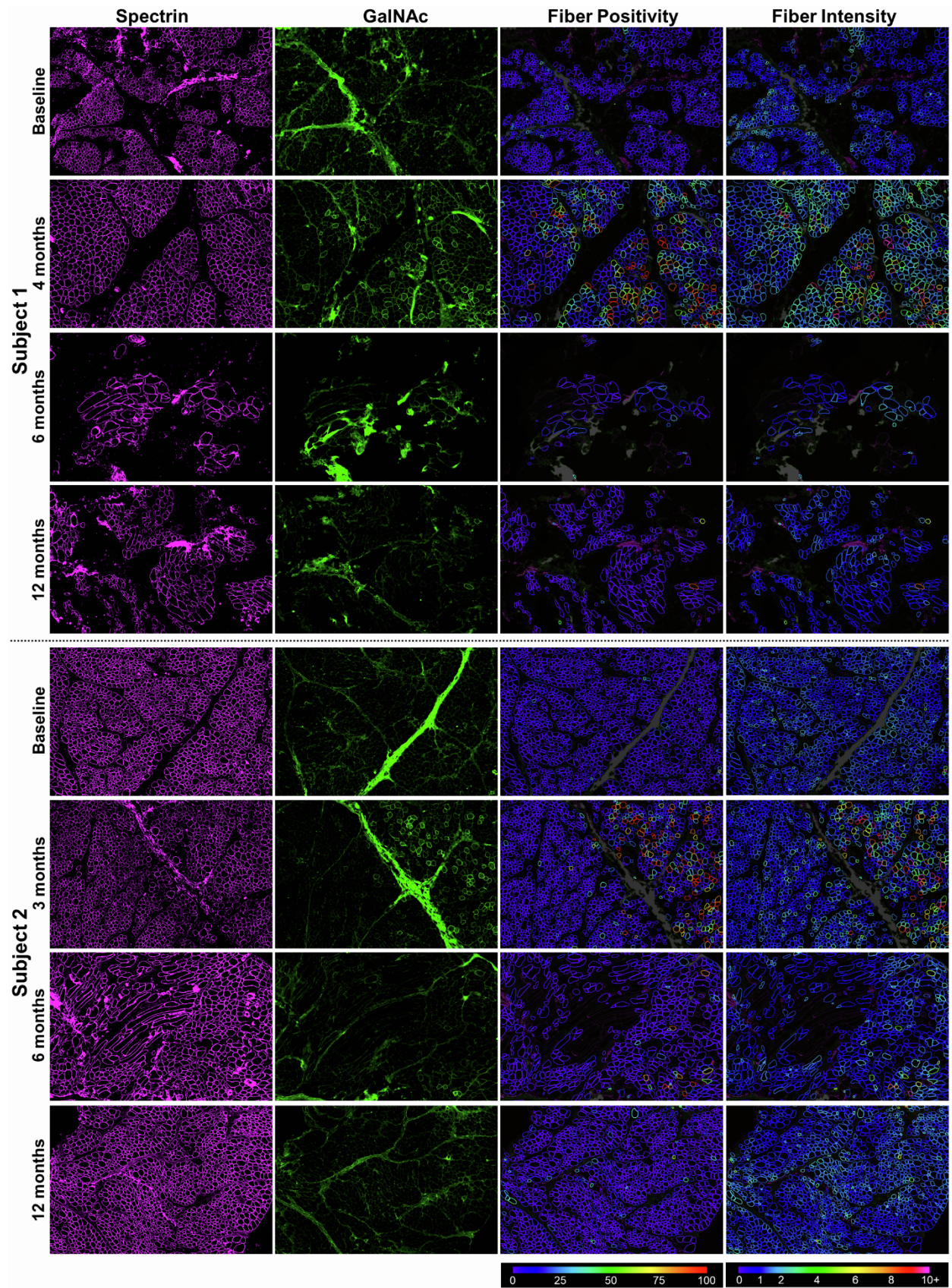


**Figure S1. Biopsy tissue morphology.** Representative images of hematoxylin and eosin (H&E) staining of muscle biopsy sections from Subjects 1 and 2 across all timepoints, and a representative healthy control sample. Scale bar = 0.5 mm.

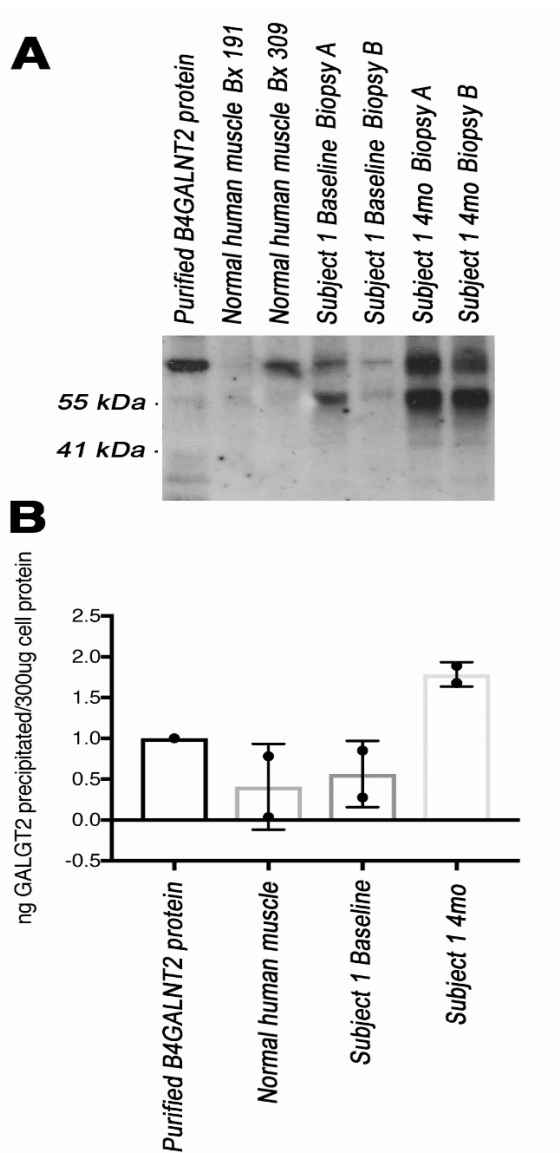


**Figure S2. qRT-PCR analysis of *GALGT2* gene expression.** *GALGT2* gene expression assessed using probes for exons 2-3 and 9-10 is shown for Subject 1, Subject 2, and 3 healthy controls (HC). The bars represent the mean  $\pm$  SD, and the individual points represent separate biopsy blocks from each timepoint. The horizontal dotted line at Y=1 reflects the mean HC value.



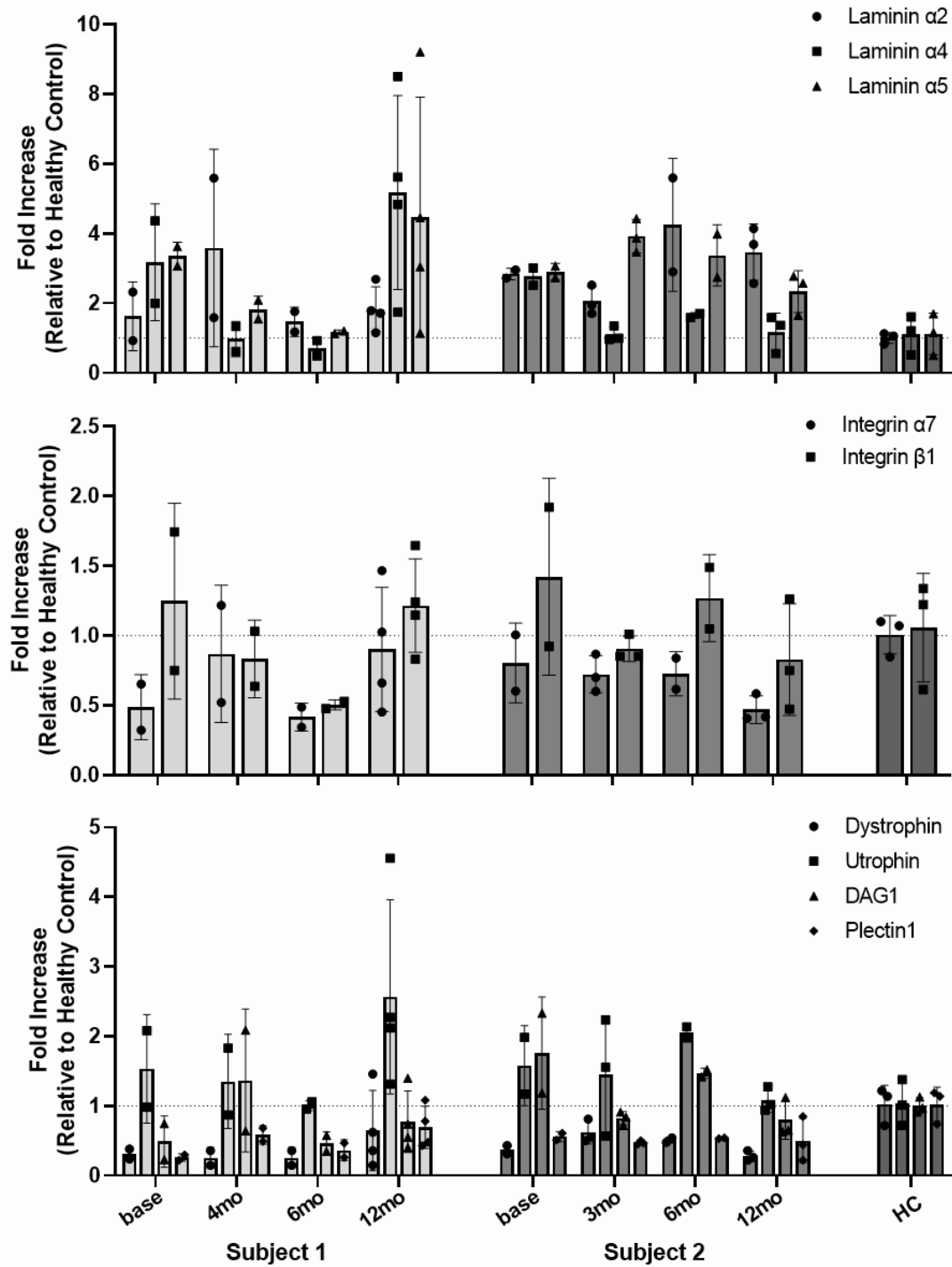


**Figure S3. Representative GalNAc expression from all study timepoints.** WFA-FITC fluorescence (green) labeling GalNAc in representative muscle regions counter-stained for spectrin (magenta) at baseline and all post-treatment timepoints. Fiber GalNAc positivity and normalized fiber GalNAc intensity analysis results for the same regions are shown in the third and fourth columns using color-coded heatmaps. Color keys indicating the conversion scale for % GalNAc-positive perimeter and fold GalNAc intensity (normalized to healthy control median) are shown below each column. Gray color indicates regions of high connective tissue accumulation that were masked to avoid interference with the automated analysis. All regions are 2 x 3 mm.

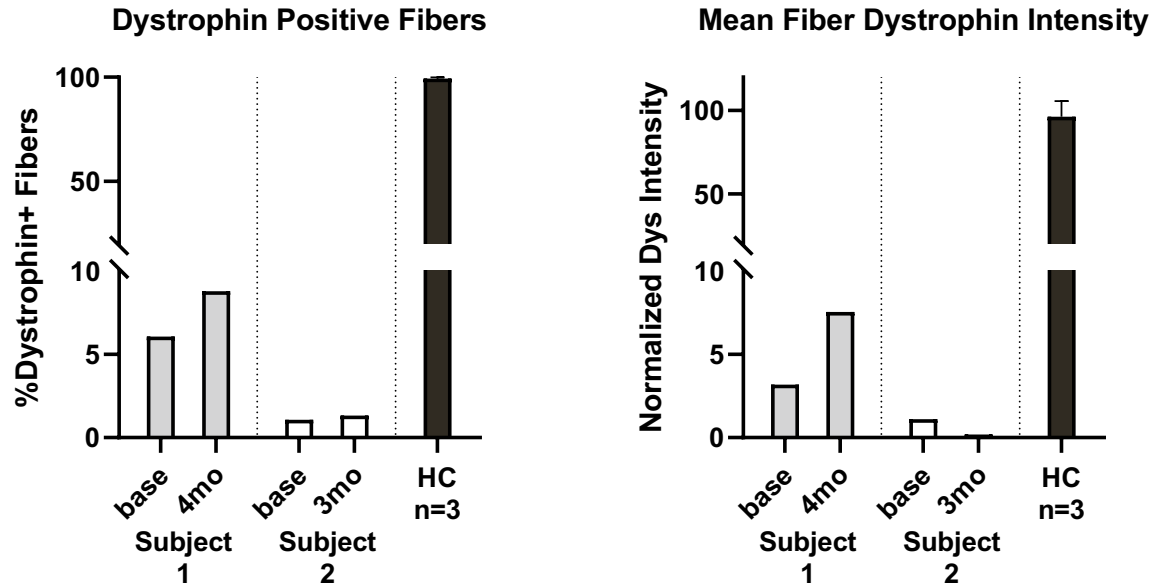


**Figure S4. GALGT2 protein expression.** (A) Representative western blot of GALGT2 protein precipitated using fetuin agarose, a GALGT2 substrate. Muscle biopsies from two normal subjects were compared to two pre-treatment biopsy samples from Subject 1 and two post-treatment biopsy samples from Subject 1 at 4 months. 1ng of purified GALGT2 (also B4GALNT2) protein was run as a control. Note that the standard used is the long-form of the GALGT2 protein, which is 6kDa larger than the short-form transgenic protein. (B) Western blot quantification results of baseline and the first post-treatment timepoints from of Subject 1 and from two different Normal human muscle biopsies. The bars represent the mean  $\pm$  SD, and the individual points represent separate blocks from the same biopsy for Subject 1.



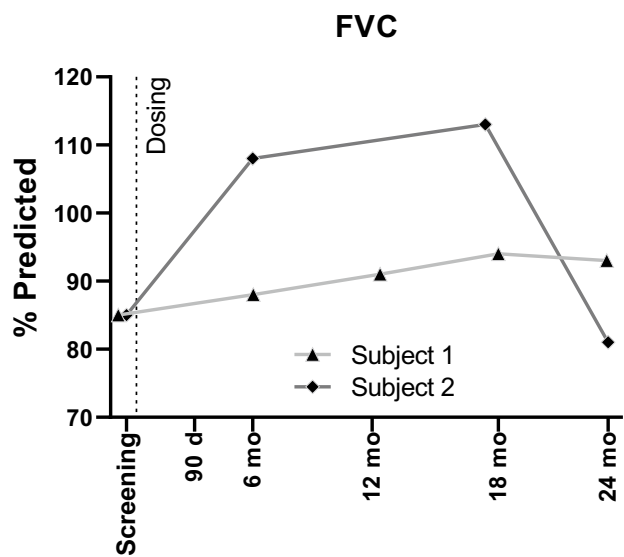


**Figure S5. qRT-PCR analysis of laminin, integrin, dystrophin, dystroglycan, and plectin gene expression.** Expression of multiple genes potentially affected by *GALGT2* overexpression was assessed for Subject 1, Subject 2, and for 3 healthy controls (HC). The bars represent the mean  $\pm$  SD, and the individual points represent separate biopsy blocks of an individual biopsy from each timepoint, except for HC, where each block is from a different individual. The horizontal dotted lines at Y=1 reflect the mean normalized HC expression values.



**Figure S6. Dystrophin expression in DMD Subject muscles.** Dystrophin immunofluorescence staining was quantified in muscle biopsies from Subject 1 (pre-treatment baseline (base) and 4 months post-treatment), Subject 2 (pre-treatment baseline (base) and 3 months post-treatment), and from 3 healthy controls (HC). Errors for HC are SD. The percentage of dystrophin-positive (+) fibers (left), and the mean fiber dystrophin (Dys) staining intensity (right), normalized to HC, were compared.





**Figure S7. Forced Vital Capacity.** Forced vital capacity (FVC) is expressed as the % predicted value for both subjects.