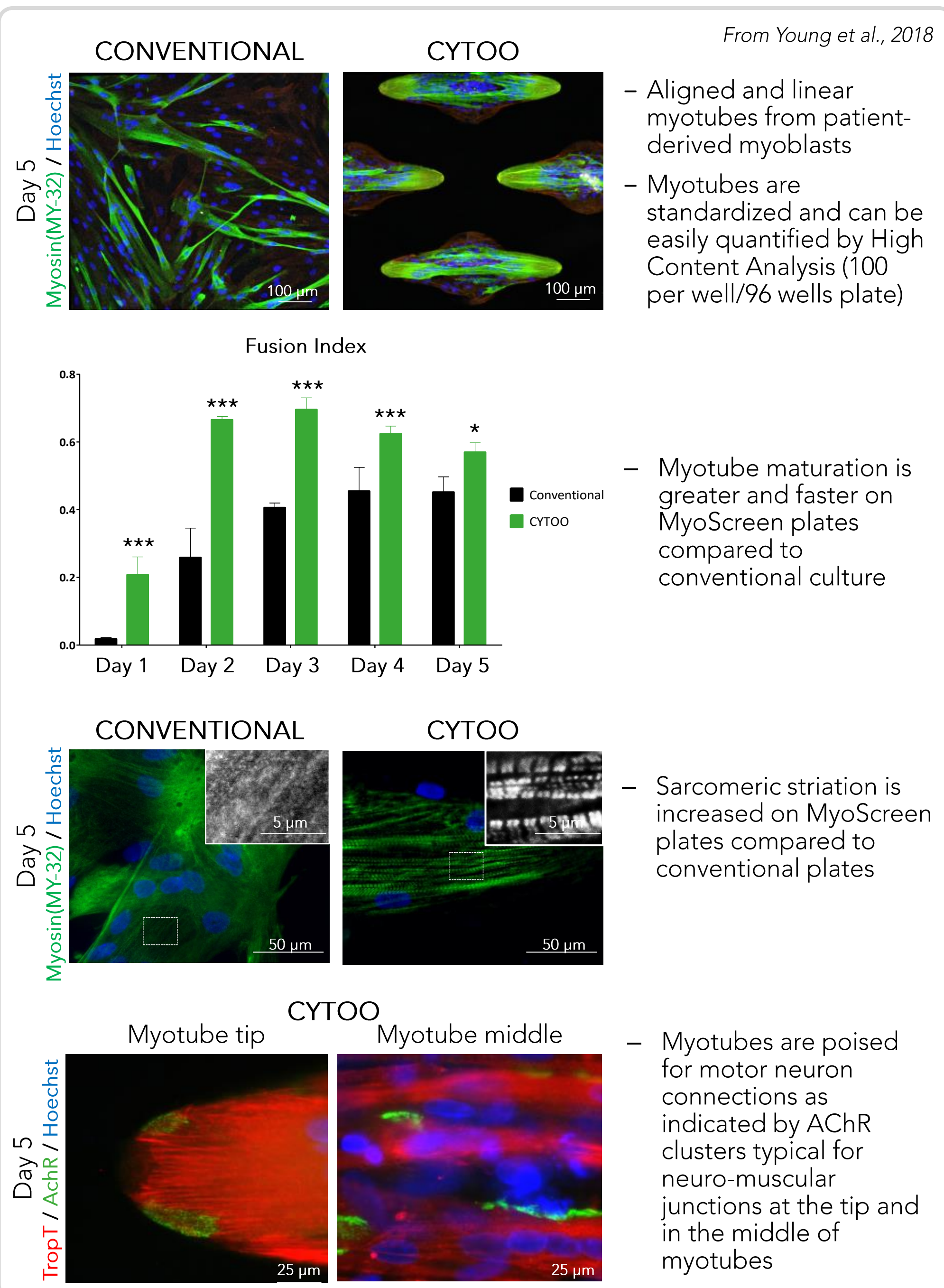


CYTOO

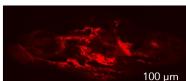
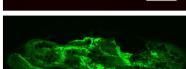
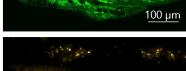
MyoScreen™ Discovery Platform: Patient-derived primary myoblasts



The diagram illustrates the integration of three components:

- Myotubes on MyoScreen**: Represented by two circular images showing myotubes with red and green fluorescence.
- State of the Art Image Analysis**: Represented by a computer monitor and keyboard.
- Quantitative functional assays**: Represented by a line graph showing Readout (Y-axis, 0 to 100) versus Treatment (X-axis). The graph shows a sigmoidal curve starting at 0 and reaching 100.

The components are connected by a plus sign (+) and an equals sign (=), indicating a workflow or integration process.

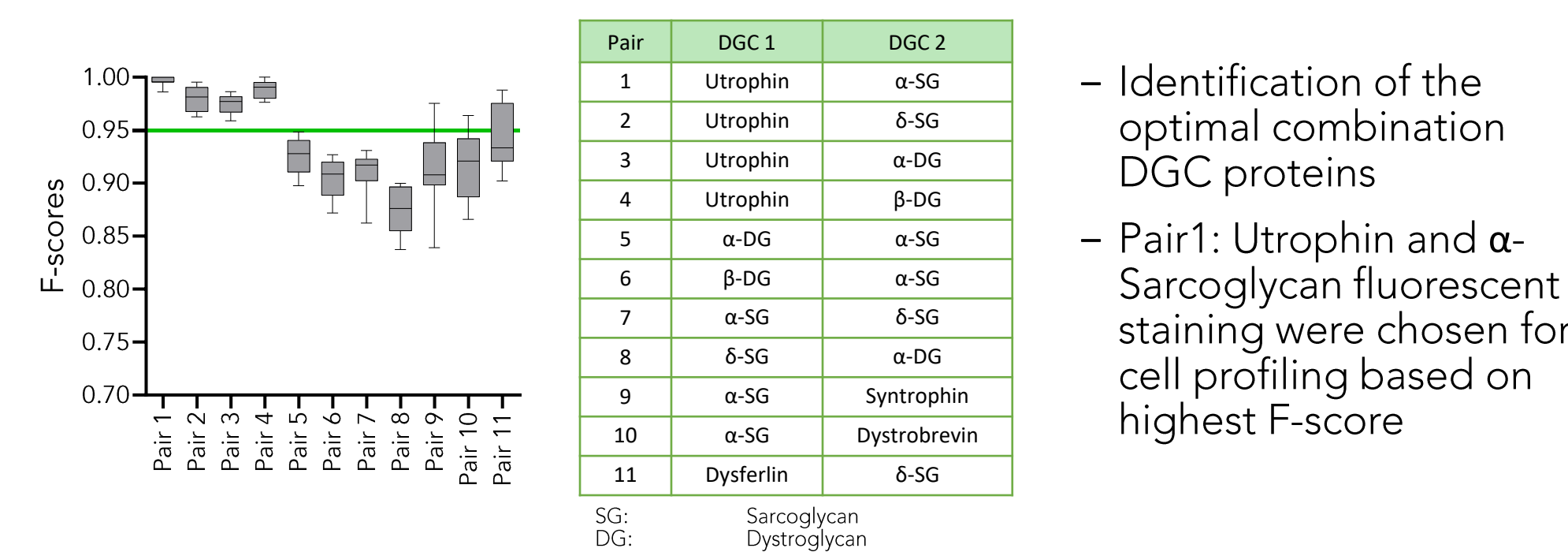
Disease drivers		Dystrophin → DMD
Proteins involved in disease-altered muscle functions		Dystrophin-Glycoprotein Complex → DMD, DM1, LGMD and others
Disease-associated morphological features		Nuclear RNA foci → DM1, DM2

- Segmentation of myotubes using CellProfiler™
- Phenotypic image features extracted for each MyoScreen pattern
- 594 features extracted per pattern

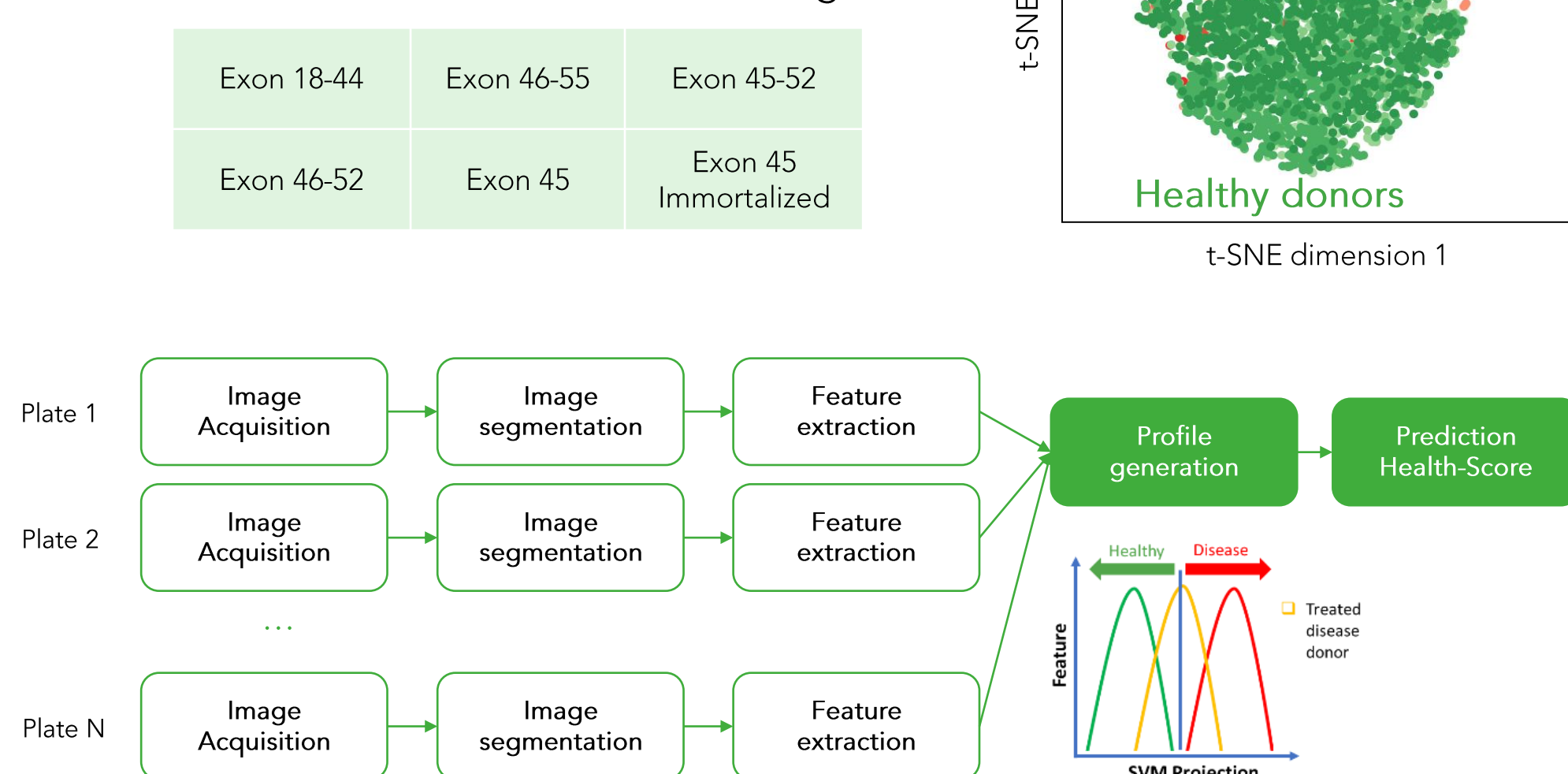


The figure displays four panels illustrating the segmentation and feature extraction process for myotubes. Each panel shows a myotube image with a vertical line indicating the segmentation axis. The panels are labeled: Granularity, Intensity, Texture, and Intensity distribution. The Intensity distribution panel shows a segmented myotube with a green outline and internal green regions, representing the extracted features.

- Dystrophin expression and DGC assembly is impaired in DMD
- DGC assembly is critical for DMD therapy development



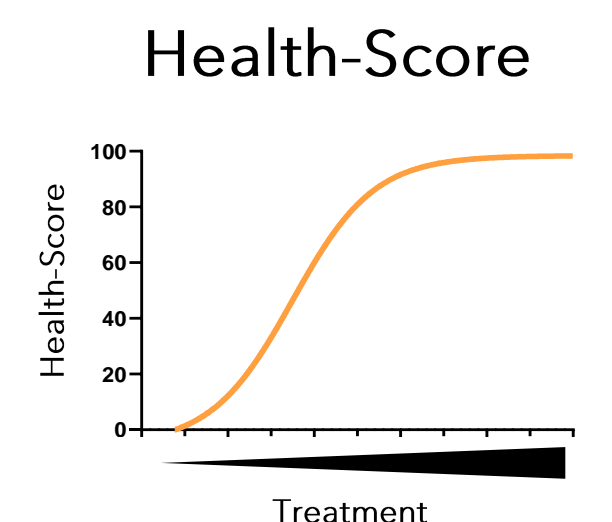
- Generalization to a variety of phenotypes and morphologies
- 15,000 replicates per phenotype
- 5 healthy donors
- 7 DMD donors with deletions in following exons:



The diagram illustrates an iterative process for model training and validation. It starts with an 'Initial plate' leading to 'Model training', which produces an 'F1-score'. This leads to 'New plate', which leads to 'Model validation', also producing an 'F1-score'. A curved arrow labeled 'Add plates 2...n' loops back from the validation stage to the training stage. A large curved arrow on the right indicates the entire process is repeated '200 times'.

- With the introduction of a new plate, there is a notable decrease in the F1-score attributed to the emergence of new donors
- Convergence of F1-score after 8 plates

- Analysis of treated disease donors
- Quantify the number of myotubes that have recovered healthy-like phenotype through treatment
- Health-Score = % phenotypically healthy myotubes out of total



- AI-powered cell profiling assays are cell-based functional assays that quantify the ability of therapies to reverse disease phenotypes in patient-derived cells using a HealthScore
- Profiling of phenotypic features does not require knowledge of disease mechanism of action
- AI-powered cell profiling assays serve all stages of drug discovery/development

Target ID & Validation	Lead ID/OP	Candidate Selection	Pre-clinical Development	Clinical Development	Market
<ul style="list-style-type: none"> - Disease reversal 	<ul style="list-style-type: none"> - Potency - Efficacy - Specificity 		<ul style="list-style-type: none"> - MOA - Biomarker ID 	<ul style="list-style-type: none"> - GMP-compliant potency assay - Patient stratification 	

DMD

HealthScore (phenotypically healthy cells)

Legend:

- Healthy Donor 1 Exp 1
- Healthy Donor 1 Exp 2
- Healthy Donor 2 Exp 1
- Healthy Donor 2 Exp 2
- untreated donors

– RNAi-induced downregulation of DMD in healthy donors

– Cells with dystrophin levels <20% display a disease phenotype

– Cells with dystrophin levels >60% display a healthy phenotype

– Aligns with results from animal studies

Healthy

ASD1 ASD2 ASD3 ASD4 ASD5 ASD6 ASD7 ASD8 ASD9 ASD10 ASD11 ASD12 ASD13 ASD14 ASD15 ASD16 ASD17 ASD18 ASD19 ASD20 ASD21 ASD22 ASD23 ASD24 ASD25 ASD26 ASD27 ASD28 ASD29 ASD30 ASD31 ASD32 ASD33 ASD34 ASD35 ASD36 ASD37 ASD38 ASD39 ASD40 ASD41 ASD42 ASD43 ASD44 ASD45 ASD46 ASD47 ASD48 ASD49 ASD50 ASD51 ASD52 ASD53 ASD54 ASD55 ASD56 ASD57 ASD58 ASD59 ASD60 ASD61 ASD62 ASD63 ASD64 ASD65 ASD66 ASD67 ASD68 ASD69 ASD70 ASD71 ASD72 ASD73 ASD74 ASD75 ASD76 ASD77 ASD78 ASD79 ASD80 ASD81 ASD82 ASD83 ASD84 ASD85 ASD86 ASD87 ASD88 ASD89 ASD90 ASD91 ASD92 ASD93 ASD94 ASD95 ASD96 ASD97 ASD98 ASD99 ASD100 ASD101 ASD102 ASD103 ASD104 ASD105 ASD106 ASD107 ASD108 ASD109 ASD110 ASD111 ASD112 ASD113 ASD114 ASD115 ASD116 ASD117 ASD118 ASD119 ASD120 ASD121 ASD122 ASD123 ASD124 ASD125 ASD126 ASD127 ASD128 ASD129 ASD130 ASD131 ASD132 ASD133 ASD134 ASD135 ASD136 ASD137 ASD138 ASD139 ASD140 ASD141 ASD142 ASD143 ASD144 ASD145 ASD146 ASD147 ASD148 ASD149 ASD150 ASD151 ASD152 ASD153 ASD154 ASD155 ASD156 ASD157 ASD158 ASD159 ASD160 ASD161 ASD162 ASD163 ASD164 ASD165 ASD166 ASD167 ASD168 ASD169 ASD170 ASD171 ASD172 ASD173 ASD174 ASD175 ASD176 ASD177 ASD178 ASD179 ASD180 ASD181 ASD182 ASD183 ASD184 ASD185 ASD186 ASD187 ASD188 ASD189 ASD190 ASD191 ASD192 ASD193 ASD194 ASD195 ASD196 ASD197 ASD198 ASD199 ASD200 ASD201 ASD202 ASD203 ASD204 ASD205 ASD206 ASD207 ASD208 ASD209 ASD210 ASD211 ASD212 ASD213 ASD214 ASD215 ASD216 ASD217 ASD218 ASD219 ASD220 ASD221 ASD222 ASD223 ASD224 ASD225 ASD226 ASD227 ASD228 ASD229 ASD230 ASD231 ASD232 ASD233 ASD234 ASD235 ASD236 ASD237 ASD238 ASD239 ASD240 ASD241 ASD242 ASD243 ASD244 ASD245 ASD246 ASD247 ASD248 ASD249 ASD250 ASD251 ASD252 ASD253 ASD254 ASD255 ASD256 ASD257 ASD258 ASD259 ASD260 ASD261 ASD262 ASD263 ASD264 ASD265 ASD266 ASD267 ASD268 ASD269 ASD270 ASD271 ASD272 ASD273 ASD274 ASD275 ASD276 ASD277 ASD278 ASD279 ASD280 ASD281 ASD282 ASD283 ASD284 ASD285 ASD286 ASD287 ASD288 ASD289 ASD290 ASD291 ASD292 ASD293 ASD294 ASD295 ASD296 ASD297 ASD298 ASD299 ASD300 ASD301 ASD302 ASD303 ASD304 ASD305 ASD306 ASD307 ASD308 ASD309 ASD310 ASD311 ASD312 ASD313 ASD314 ASD315 ASD316 ASD317 ASD318 ASD319 ASD320 ASD321 ASD322 ASD323 ASD324 ASD325 ASD326 ASD327 ASD328 ASD329 ASD330 ASD331 ASD332 ASD333 ASD334 ASD335 ASD336 ASD337 ASD338 ASD339 ASD340 ASD341 ASD342 ASD343 ASD344 ASD345 ASD346 ASD347 ASD348 ASD349 ASD350 ASD351 ASD352 ASD353 ASD354 ASD355 ASD356 ASD357 ASD358 ASD359 ASD360 ASD361 ASD362 ASD363 ASD364 ASD365 ASD366 ASD367 ASD368 ASD369 ASD370 ASD371 ASD372 ASD373 ASD374 ASD375 ASD376 ASD377 ASD378 ASD379 ASD380 ASD381 ASD382 ASD383 ASD384 ASD385 ASD386 ASD387 ASD388 ASD389 ASD390 ASD391 ASD392 ASD393 ASD394 ASD395 ASD396 ASD397 ASD398 ASD399 ASD400 ASD401 ASD402 ASD403 ASD404 ASD405 ASD406 ASD407 ASD408 ASD409 ASD410 ASD411 ASD412 ASD413 ASD414 ASD415 ASD416 ASD417 ASD418 ASD419 ASD420 ASD421 ASD422 ASD423 ASD424 ASD425 ASD426 ASD427 ASD428 ASD429 ASD430 ASD431 ASD432 ASD433 ASD434 ASD435 ASD436 ASD437 ASD438 ASD439 ASD440 ASD441 ASD442 ASD443 ASD444 ASD445 ASD446 ASD447 ASD448 ASD449 ASD450 ASD451 ASD452 ASD453 ASD454 ASD455 ASD456 ASD457 ASD458 ASD459 ASD460 ASD461 ASD462 ASD463 ASD464 ASD465 ASD466 ASD467 ASD468 ASD469 ASD470 ASD471 ASD472 ASD473 ASD474 ASD475 ASD476 ASD477 ASD478 ASD479 ASD480 ASD481 ASD482 ASD483 ASD484 ASD485 ASD486 ASD487 ASD488 ASD489 ASD490 ASD491 ASD492 ASD493 ASD494 ASD495 ASD496 ASD497 ASD498 ASD499 ASD500 ASD501 ASD502 ASD503 ASD504 ASD505 ASD506 ASD507 ASD508 ASD509 ASD510 ASD511 ASD512 ASD513 ASD514 ASD515 ASD516 ASD517 ASD518 ASD519 ASD520 ASD521 ASD522 ASD523 ASD524 ASD525 ASD526 ASD527 ASD528 ASD529 ASD530 ASD531 ASD532 ASD533 ASD534 ASD535 ASD536 ASD537 ASD538 ASD539 ASD540 ASD541 ASD542 ASD543 ASD544 ASD545 ASD546 ASD547 ASD548 ASD549 ASD550 ASD551 ASD552 ASD553 ASD554 ASD555 ASD556 ASD557 ASD558 ASD559 ASD560 ASD561 ASD562 ASD563 ASD564 ASD565 ASD566 ASD567 ASD568 ASD569 ASD570 ASD571 ASD572 ASD573 ASD574 ASD575 ASD576 ASD577 ASD578 ASD579 ASD580 ASD581 ASD582 ASD583 ASD584 ASD585 ASD586 ASD587 ASD588 ASD589 ASD590 ASD591 ASD592 ASD593 ASD594 ASD595 ASD596 ASD597 ASD598 ASD599 ASD600 ASD601 ASD602 ASD603 ASD604 ASD605 ASD606 ASD607 ASD608 ASD609 ASD610 ASD611 ASD612 ASD613 ASD614 ASD615 ASD616 ASD617 ASD618 ASD619 ASD620 ASD621 ASD622 ASD623 ASD624 ASD625 ASD626 ASD627 ASD628 ASD629 ASD630 ASD631 ASD632 ASD633 ASD634 ASD635 ASD636 ASD637 ASD638 ASD639 ASD640 ASD641 ASD642 ASD643 ASD644 ASD645 ASD646 ASD647 ASD648 ASD649 ASD650 ASD651 ASD652 ASD653 ASD654 ASD655 ASD656 ASD657 ASD658 ASD659 ASD660 ASD661 ASD662 ASD663 ASD664 ASD665 ASD666 ASD667 ASD668 ASD669 ASD670 ASD671 ASD672 ASD673 ASD674 ASD675 ASD676 ASD677 ASD678 ASD679 ASD680 ASD681 ASD682 ASD683 ASD684 ASD685 ASD686 ASD687 ASD688 ASD689 ASD690 ASD691 ASD692 ASD693 ASD694 ASD695 ASD696 ASD697 ASD698 ASD699 ASD700 ASD701 ASD702 ASD703 ASD704 ASD705 ASD706 ASD707 ASD708 ASD709 ASD710 ASD711 ASD712 ASD713 ASD714 ASD715 ASD716 ASD717 ASD718 ASD719 ASD720 ASD721 ASD722 ASD723 ASD724 ASD725 ASD726 ASD727 ASD728 ASD729 ASD730 ASD731 ASD732 ASD733 ASD734 ASD735 ASD736 ASD737 ASD738 ASD739 ASD740 ASD741 ASD742 ASD743 ASD744 ASD745 ASD746 ASD747 ASD748 ASD749 ASD750 ASD751 ASD752 ASD753 ASD754 ASD755 ASD756 ASD757 ASD758 ASD759 ASD760 ASD761 ASD762 ASD763 ASD764 ASD765 ASD766 ASD767 ASD768 ASD769 ASD770 ASD771 ASD772 ASD773 ASD774 ASD775 ASD776 ASD777 ASD778 ASD779 ASD780 ASD781 ASD782 ASD783 ASD784 ASD785 ASD786 ASD787 ASD788 ASD789 ASD790 ASD791 ASD792 ASD793 ASD794 ASD795 ASD796 ASD797 ASD798 ASD799 ASD800 ASD801 ASD802 ASD803 ASD804 ASD805 ASD806 ASD807 ASD808 ASD809 ASD810 ASD811 ASD812 ASD813 ASD814 ASD815 ASD816 ASD817 ASD818 ASD819 ASD820 ASD821 ASD822 ASD823 ASD824 ASD825 ASD826 ASD827 ASD828 ASD829 ASD830 ASD831 ASD832 ASD833 ASD834 ASD835 ASD836 ASD837 ASD838 ASD839 ASD840 ASD841 ASD842 ASD843 ASD844 ASD845 ASD846 ASD847 ASD848 ASD849 ASD850 ASD851 ASD852 ASD853 ASD854 ASD855 ASD856 ASD857 ASD858 ASD859 ASD860 ASD861 ASD862 ASD863 ASD864 ASD865 ASD866 ASD867 ASD868 ASD869 ASD870 ASD871 ASD872 ASD873 ASD874 ASD875 ASD876 ASD877 ASD878 ASD879 ASD880 ASD881 ASD882 ASD883 ASD884 ASD885 ASD886 ASD887 ASD888 ASD889 ASD890 ASD891 ASD892 ASD893 ASD894 ASD895 ASD896 ASD897 ASD898 ASD899 ASD900 ASD901 ASD902 ASD903 ASD904 ASD905 ASD906 ASD907 ASD908 ASD909 ASD910 ASD911 ASD912 ASD913 ASD914 ASD915 ASD916 ASD917 ASD918 ASD919 ASD920 ASD921 ASD922 ASD923 ASD924 ASD925 ASD926 ASD927 ASD928 ASD929 ASD930 ASD931 ASD932 ASD933 ASD934 ASD935 ASD936 ASD937 ASD938 ASD939 ASD940 ASD941 ASD942 ASD943 ASD944 ASD945 ASD946 ASD947 ASD948 ASD949 ASD950 ASD951 ASD952 ASD953 ASD954 ASD955 ASD956 ASD957 ASD958 ASD959 ASD960 ASD961 ASD962 ASD963 ASD964 ASD965 ASD966 ASD967 ASD968 ASD969 ASD970 ASD971 ASD972 ASD973 ASD974 ASD975 ASD976 ASD977 ASD978 ASD979 ASD980 ASD981 ASD982 ASD983 ASD984 ASD985 ASD986 ASD987 ASD988 ASD989 ASD990 ASD991 ASD992 ASD993 ASD994 ASD995 ASD996 ASD997 ASD998 ASD999 ASD1000 ASD1001 ASD1002 ASD1003 ASD1004 ASD1005 ASD1006 ASD1007 ASD1008 ASD1009 ASD1010 ASD1011 ASD1012 ASD

Disease	Donor	Sex	Age	Form	CTG repeat size (blood)	Muscle weakness	Myotonia	
Healthy		F	20	-	5-37	-	-	
DM1	DM1-1	M	27	Infantile	1300	+	++	+ low
	DM1-2	M	38	Juvenile	350	++	+++	++ moderate
	DM1-3	M	34	Late onset	300	None	None	+++ severe

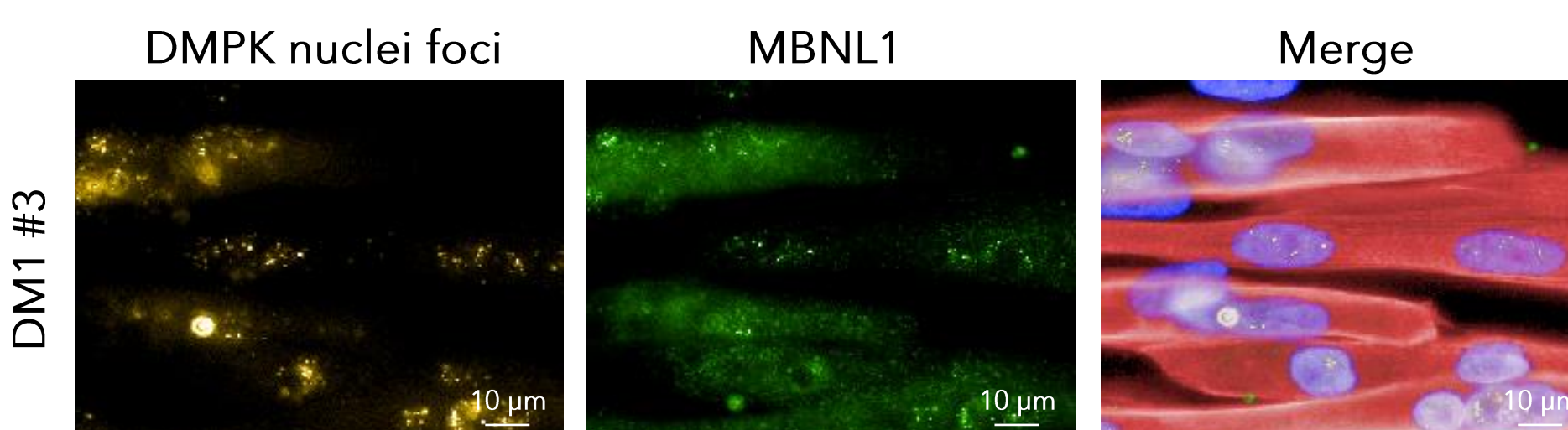


Figure 3: F1-score and ASO dose response.

Left Panel: F1-score by Category

Category	F1-score (approx. median)
MBNL1	0.99
FOCI	0.98
All	0.99

Right Panel: ASO Dose Response (Health-Score vs ASO Dose)

ASO Dose (nM)	DM1 #1 (Health-Score)	DM1 #2 (Health-Score)	DM1 #3 (Health-Score)
0	0	0	0
2	0	0	0
4	0	0	0
6	~10	~15	~20
8	~15	~25	~30
10	~20	~30	~40

- MyoScreen enables the development of novel quantitative and functional cell-based assays

- are robust, versatile assays that can be applied to human primary cells or immortalized cells lines with disease-associated phenotypes
- serve various stages from drug discovery and development, to potency assays for commercial release of gene therapy products
- can be applied to High Content Screening and batch release testing

- evaluating the efficacy of therapies aiming to restore the activity of a disease driver
- analyzing the responses of different patients to a therapy

- AI-powered cell profiling will enable drug discovery for disorders with complex genetic backgrounds, multifactorial mechanisms, or unidentified mode of action, such as metabolic myopathies

Acknowledgment

We thank Dr Jack Puymirat and Dominic Jauvin from the CHU Québec for DM1 donor cells and CBC Biotech, Lyon, France, for the DMD and healthy donor cells