CERTIFICATE OF ANALYSIS
AICS-0063-096:WTC-DMD-mEGFP-cl96 (mono-allelic tag)

Product description	Human iPSC clonal line in which DMD has been endogenously tagged with mEGFP using CRISPR/Cas9 technology
Parental cell line	Parental hiPSC line (WTC/AICS-0 at passage 33) derived from fibroblasts reprogrammed using episomal vectors (OCT3/4, shp53, SOX2, KLF4, LMYC, and LIN28). Coriell catalog: GM25256
Publication(s) describing iPSC establishment	Kreitzer et al (2013) Am. J. Stem Cells, 30; 2(2): 119-31
Passage of gene edited iPSC reported at submission	p32[a]
Number of passages at Coriell	0
Media	mTeSR1
Feeder or matrix substrate	Matrigel
Passage method	Accutase
Thaw	1 million cells (ea vial) in 10-cm plate - ready for passaging in 3-4 days
Seeding density	400K cells/10-cm plate every 4 days or 800K cells/10-cm plate every 3 days (see culture protocol)

Test Description[b]	**Method**	**Specification**	**Result**
Post-Thaw Viable Cell Recovery	hiPSC culture on Matrigel	$>$ 50% confluency 3-4 days post-thaw (10-cm plate)	Pass
mEGFP insertion(s) at genomic locus - precise editing	PCR and Sanger sequencing of the recombinant allele	C-term insertion of mEGFP in frame with exact predicted recombinant allele junctions. No additional mutations.	Pass
Copy number	ddPCR[c] assay for FP(s) and RPP30 reference gene[d]	FP/RPP30: $\sim$ 0.5 = Mono-allelic $\sim$ 1.0 = Bi-allelic	Mono-allelic (0.42)
Plasmid integration	ddPCR assay to detect plasmid integration into the genome	AmpR/RPP30: $<$ 0.1 = no plasmid integration	Pass (0.00)
Mutational analysis	Whole exome sequencing[f]	Check for acquired mutations (not detected in p8[a] parental line) that: 1) Correspond to off-target sites predicted by Cas-OFFinder[e] 2) Affect genes in Cosmic Cancer Gene Census	Sequencing planned
mEGFP localization	Spinning Disk confocal live cell imaging	Localization to the costamere	mEGFP-tagged dystrophin is present at the cell periphery in hiPSC-derived cardiomyocytes and localizes to the cell membrane and in tubular structures in hiPSC-derived skeletal muscle myofibers. This is an expected pattern as dystrophin, a member of the dystrophin-glycoprotein complex, links the contractile apparatus of muscle cells to the sarcolemma.
Expression of tagged protein	Western blot	Expression of expected size product	Not performed

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Growth rate	ATP quantitation[g]	Comparable to parental line	Pass (measured at p31)[a]
Expression of stem cell markers	Flow cytometry	Transcription factors: OCT4, SOX2, NANOG $\geq$ 85% Surface markers: SSEA4, TRA-1-60 $\geq$ 85%; SSEA1 $\leq$ 15%	Pass
Germ layer differentiation	Trilineage differentiation[h] as assayed by ddPCR gene expression analysis	Expression of endoderm (SOX17), mesoderm (Brachyury), and ectoderm (PAX6) markers upon directed differentiation to all three germ layers	Pass
Cardiomyocyte differentiation	Modified small molecule differentiation, Lian et al (2012)[i]	Beating initiated (D7-D14) and Cardiac Troponin T expression (D11-D30) by flow cytometry	Pass
Skeletal muscle myofiber differentiation[j]	Modified from Chal et al (2016)[k]	Spontaneous contraction of nascent fibers (D29); morphological assessment	Pass
Karyotype	G-banding (30 cell analysis)	Normal karyotype, 46 XY	Pass
Mycoplasma	qPCR (IDEXX)	Negative	Pass
Sterility (bacterial, yeast and fungal testing)	Direct inoculation and incubation for 10 days	No growth after 10 days	Pass
Viral Panel Testing[l]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass
Identity of unedited parental line[m]	STR	29 allelic polymorphisms across 15 STR loci compared to donor fibroblasts	Identity matched

[a] This is the number of passages beyond the original parental line (WTC/AICS-0 at passage 33).
[b] All QC assays are performed on stem cells except when noted otherwise.
[c] Droplet digital PCR using Bio-Rad QX200.
[d] RPP30 is a reference 2 copy gene used for normalization.
[e] Bae et al (2014) Bioinformatics. 30(10): 1473-1475
[f] Nextera rapid capture exome
[g] Promega CellTiter-Glo Luminescent Cell Viability Assay (Catalog #G7571)
[h] STEMCELL Technologies STEMdiff Trilineage Differentiation Kit (Catalog #05230)
[i] Lian et al (2012) PNAS. 109(27):E1848-E1857
[j] We thank Shawn Luttrell and David Mack (University of Washington) for myofiber differentiation of AICS-0063.
[k] Chal et al (2016) Nature Protocols. 11(10):1833-1850
[l] Viral panel testing was conducted for the parental WTC line prior to editing. Sterility (bacterial, fungal) and mycoplasma testing were conducted in both the parental and edited lines.
[m] STR tests were conducted for the WTC parental line prior to editing. WTC is the only cell line used by AICS. Edited WTC cells were not re-tested because they did not come into contact with any other cell lines.

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Tagging strategy: CRISPR-Cas9 methodology was used to introduce mEGFP at C-terminus of DMD as shown below.

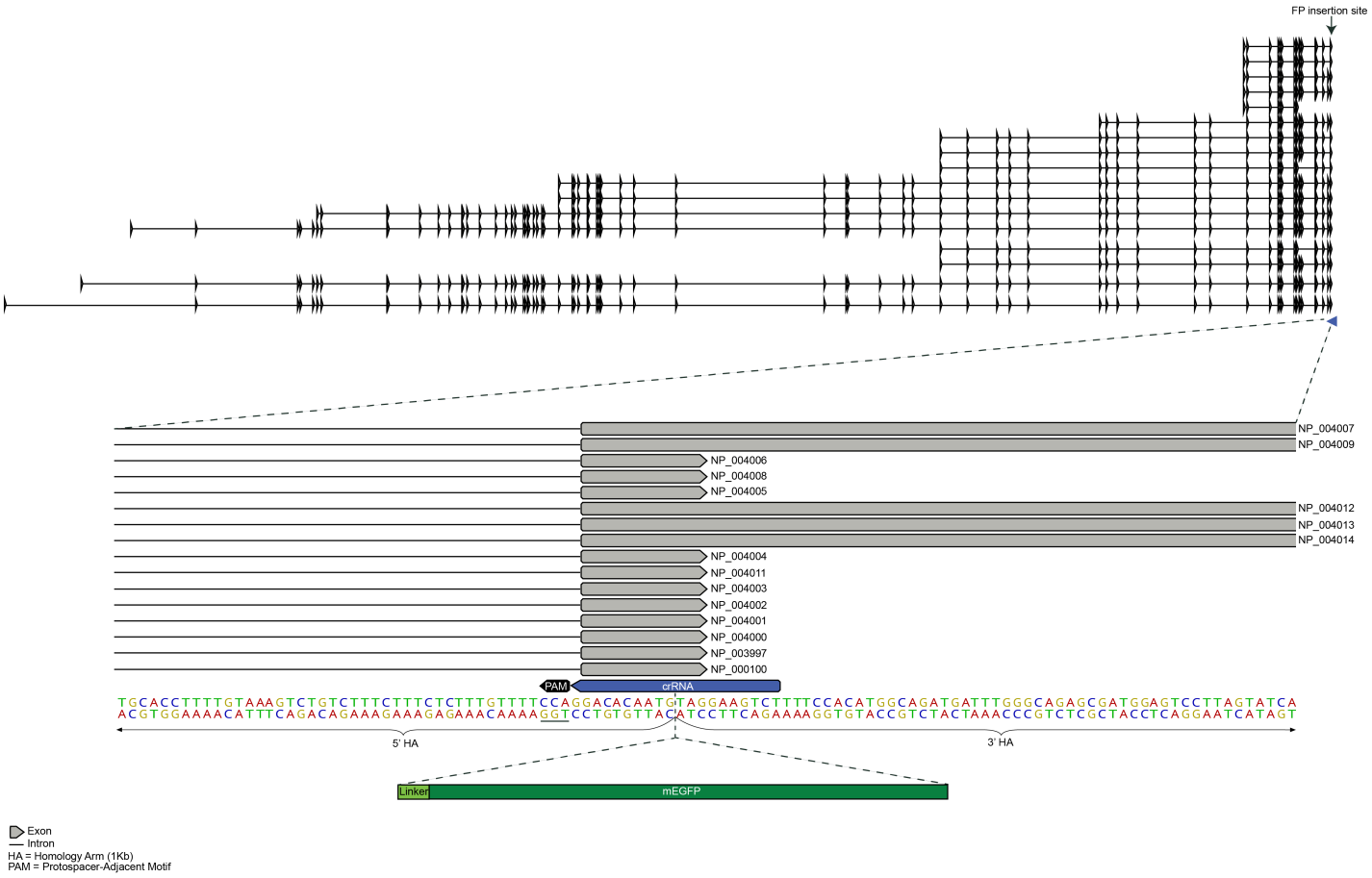

Figure 1: Top: DMD locus showing DMD isoforms; Bottom: Zoom in on mEGFP insertion site at DMD C-terminal exon

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Post-thaw imaging: One vial of distribution lot was thawed (cells were treated with ROCK inhibitor for 24hrs post-thaw - refer to culture protocol). Cultures were observed daily. Colonies were imaged one and three days post-thaw[1,2] using a Leica microscope.

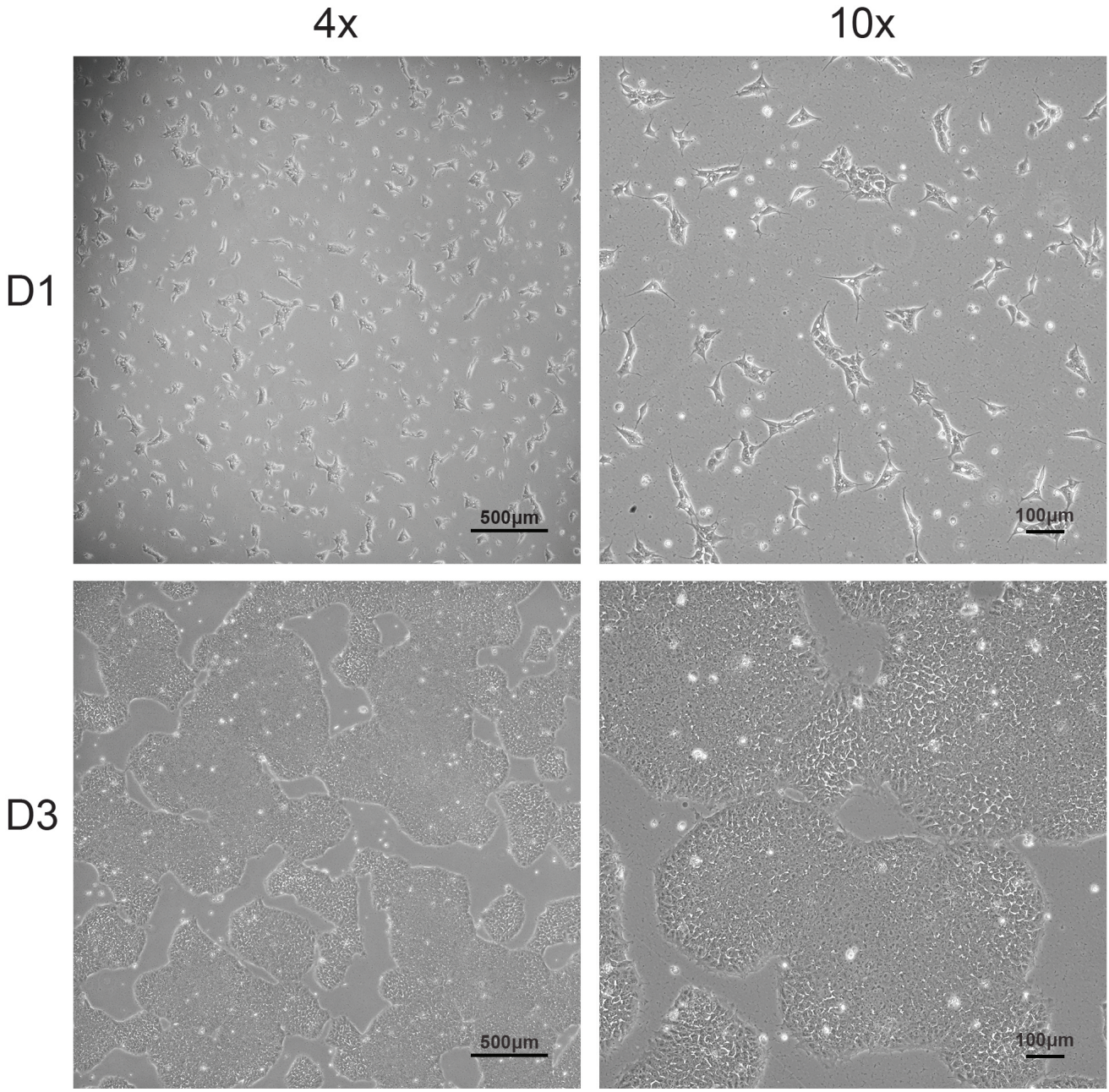

Figure 2: Viability and colony formation one day and three days post-thaw

[1]Cells may take up to 3 passages to recover after thaw
[2]Morphologies observed post-thaw are representative of cell morphologies observed post-passage

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Imaging labeled structures in endogenously tagged hiPSC derived cells: The tagged proteins are expressed endogenously and therefore may not appear as bright as they would in an overexpressed system. For imaging cardiomyocytes, we plate cells onto high-quality glass bottom 24-well plates (Cellvis) coated with 0.1% w/vol polyethylenimine (PEI) and 25μg/ml laminin. Cells are imaged in phenol red-free RPMI 1640 media (Gibco) supplemented with B-27 containing insulin (Gibco). For imaging skeletal muscle myofibers, cells were plated onto high-quality glass bottom 24-well plates (Cellvis) coated with Matrigel diluted 1:60. Cells were imaged in DMEM with high glucose (Gibco) supplemented with KnockOut SR (Gibco), small molecules, and growth factors (unpublished data, Mack lab). Cells were imaged on a 3i spinning disk fluorescence microscope with a Yokogawa CSU-W1 head, Hamamatsu CMOS camera, a Zeiss 63x 1.2 NA water immersion objective, and 488 laser at approximately 24 mW. Cells were maintained at 37°C and 5% CO_2 in a temperature-controlled chamber during imaging.

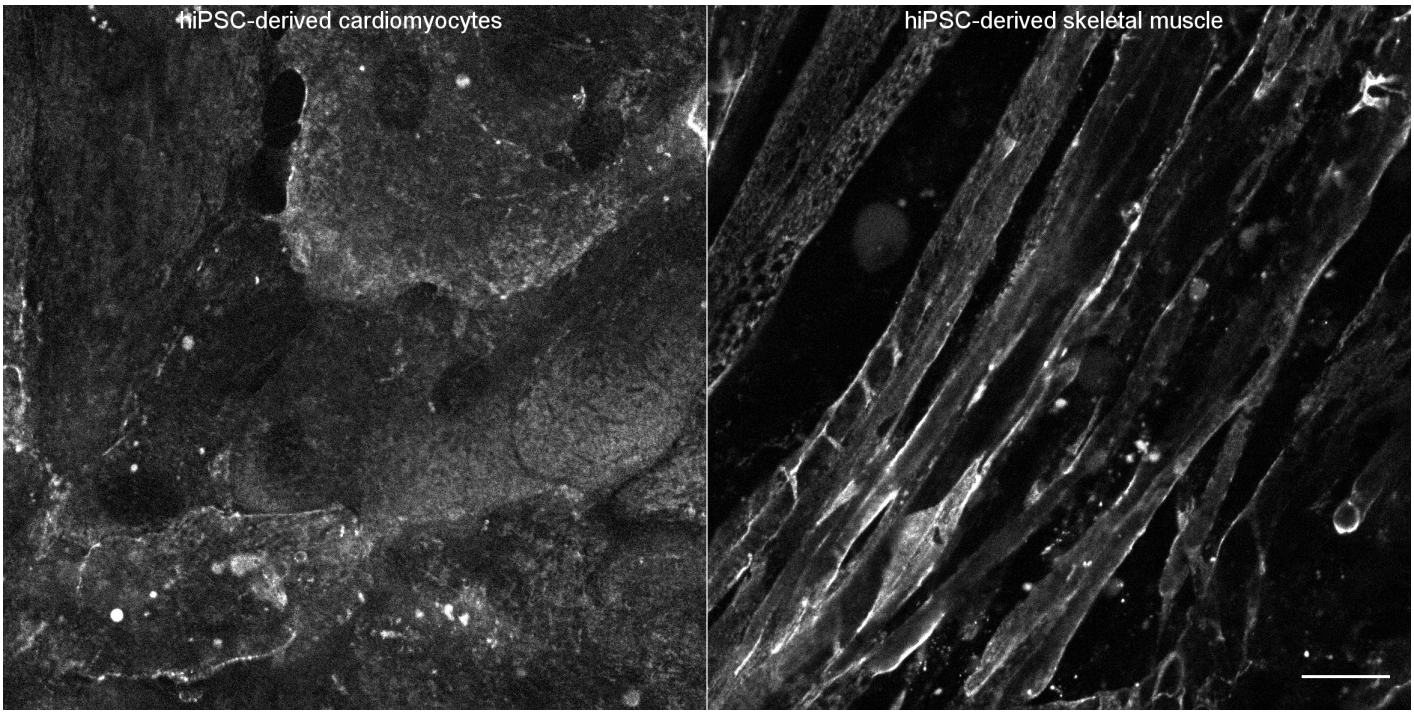

Figure 3: Single, mid-level planes of hiPSC-derived cardiomyocytes (left) and hiPSC-derived skeletal muscle myofibers (right) expressing mEGFP-tagged dystrophin imaged live in 3D on a spinning disk microscope. Left: Twelve days after the onset of differentiation, cells were plated on PEI and laminin coated glass and imaged 18 days later (30 days total after the onset of differentiation). Right: 25 days after the onset of differentiation, cells were plated on Matrigel (diluted 1:60) coated glass. Cell fusion was induced one day later, and muscle fibers were imaged 7 days after fusion induction (imaging was performed 33 days total after the onset of differentiation). Skeletal muscle sample was courtesy of Shawn Luttrell and David Mack (University of Washington). Brightness and contrast display settings are optimized for each panel; display settings are not the same between cell types. Scale bar, 20 μm.