CERTIFICATE OF ANALYSIS

AICS-0113: MYH3-G769V mEGFP-ACTN2 (mono-allelic tag)

Cell Collection description	G769V mutation introduced into the MYH3 gene. Additionally, mEGFP added into C terminus of ACTN2.
Parental cell line	Human iPSC clonal line in which ACTN2 has been endogenously tagged with mEGFP using CRISPR/Cas9. Parental hiPSC line (WTC/AICS-0 passage 33 at acquisition) derived from dermal fibroblasts reprogrammed using episomal vectors (OCT3/4, shp53, SOX2, KLF4, LMYC, and LIN28).
Relevant publications	Kreitzer et al (2013) Am. J. Stem Cells, 30; 2(2): 119-31 . PMID: 23862100; PMCID: PMC3708511 Roberts et al (2019) Stem Cell Reports, 12(5): 1145-1158. doi: 10.1016/j.stemcr.2019.03.001
Number of passages at Coriell	0
Media	mTeSR1
Feeder or matrix substrate	Matrigel
Passage method	Accutase, single cell
Thaw	500K cells (per vial) in 10 cm plate - ready for passaging in 4-5 days

Test Description	Method	Specification	Results					
Clone Number	N/A	N/A	2	30	82	23	44	57
Transfection Replicate (A or B)	N/A	Clones were derived from separate replicated transfections. Comparisons between clones of different genotypes recommended from same replicate.	A	A	B	A	A	B
Clone PCR & Sanger	PCR and Sanger sequencing of MYH3 recombinant and wildtype alleles	Determine if predicted mutation occurred with no additional mutations present.	G769V / WT			WT / WT		
Passage of gene edited iPSC reported at submission	N/A	N/A	p48[a]	p48[a]	p48[a]	p48[a]	p48[a]	p48[a]
Seeding density	N/A	Recommended seeding densities in 10 cm plate every 4 days or every 3 days, consecutively (see culture protocol)	400K / 800K	400K / 800K	400K / 800K	400K / 800k	400k / 800K	400K / 800K
Post-Thaw Viable Cell Recovery	hiPSC culture on Matrigel	>50% confluency 4-5 days post-thaw (10cm plate)	Pass	Pass	Pass	Pass	Pass	Pass
Mono-Clonality Confirmation	ddPCR assay	Verification of genomic copy number of WT and mutant alleles	Pass	Pass	Pass	Pass	Pass	Pass

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Trisomy 12 Test	ddPCR assay (Chr12:RPP30)	pass = trisomy 12 not detected in quantitative ddPCR assay.	Pass	Pass	Pass	Pass	Pass	Pass
Karyotype	G-banding (30 cell analysis)	Normal karyotype, 46 XY	Pass	Pass	Pass	Pass	Pass	Pass
Skeletal Differentiation[d]	Differentiation protocol[e]	Desmin positivity of myoblasts ≥ 60% (by immunofluorescence) & Myoblast fusion and formation of sarcomeres when cultured in secondary differentiation media	N/A	N/A	Pass	N/A	Pass	N/A
Mycoplasma	qPCR (IDEXX)	Negative	Pass	Pass	Pass	Pass	Pass	Pass
Sterility (bacterial, yeast and fungal testing)	Direct inoculation and incubation for 10 days	No growth after 10 days	Pass	Pass	Pass	Pass	Pass	Pass
Viral Panel Testing[b]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass					
Identity of Unedited WTC-11 parental line[c]	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] 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
[c] 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.
[d] Skeletal Differentation was performed courtesy of collaborators Alina Greimal, BS, Christian Mandrycky, PhD and David Mack, PhD, *Institute for Stem Cell & Regenerative Medicine* (ISCRM) at the *University of Washington*
[e] Skeletal Differentiation Protocol: Smith et al (2022) J Tissue Eng. doi:10.1177/20417314221122127

BLUE = MUTANT CLONES; GREEN = WILDTYPE CLONES

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Tagging strategy: CRISPR-Cas9 methodology was used to introduce a single base pair mutation to MYH3, and mEGFP at C-terminus of ACTN2 as shown below.

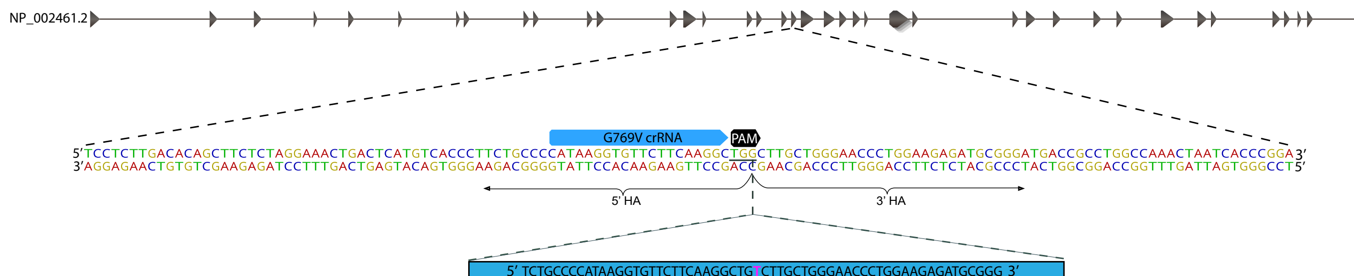


Figure 1: Top: MYH3 locus showing 1 MYH3 isoform; Bottom: Zoom in on mutation site at isoform NM_0027470.4(MYH3):c.2306G>T(p.Gly769Val)

HDR Editing Design for MYH3	
crRNA Target Site	5' CATAAGGTGTTCTTCAAGGCTGG 3'
DNA Donor Sequence	5' TCTGCCCCATAAGGTGTTCTTCAAGGCTG**T**CTTGCTGGG AACCCTGGAAGAGATGCGGG 3'
F primer for PCR/sequencing	5' TGACTCCGAGCTAGTTCCCT 3'
R primer for PCR/sequencing	5' CTCCGACTTGGCGAGTTCAT 3'

Red = PAM Site; Blue = Mutation

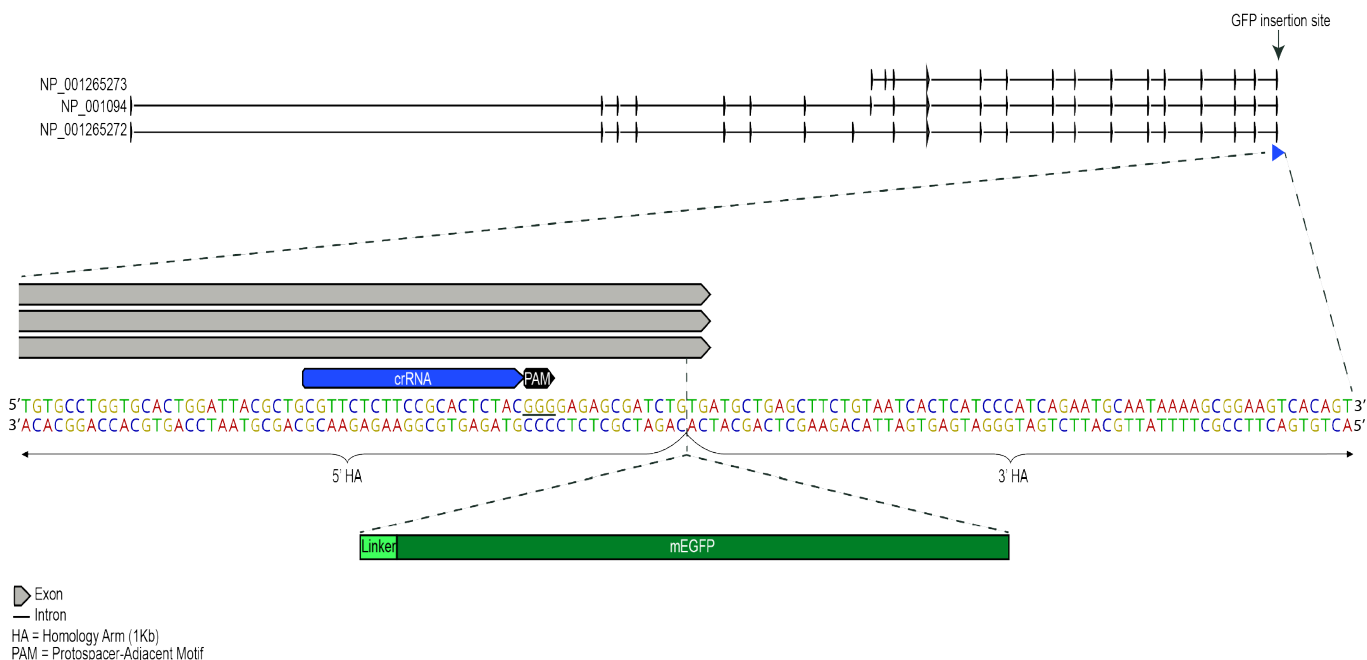


Figure 2: Top: ACTN2 locus showing 3 ACTN2 isoforms; Bottom: Zoom in on mEGFP insertion site at ACTN2 C-terminus.

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 four days post-thaw[1,2] using a Leica microscope at 4x and 10x magnification. 1. clone 44 (wt/wt), 2. clone 23 (wt/wt), and 3. clone 30 (G769V/wt) is shown here.

1 REPRESENTATIVE IMAGE FOR ALL CLONES (EXCEPT CLONE 23 AND CLONE 30, SEE BELOW)

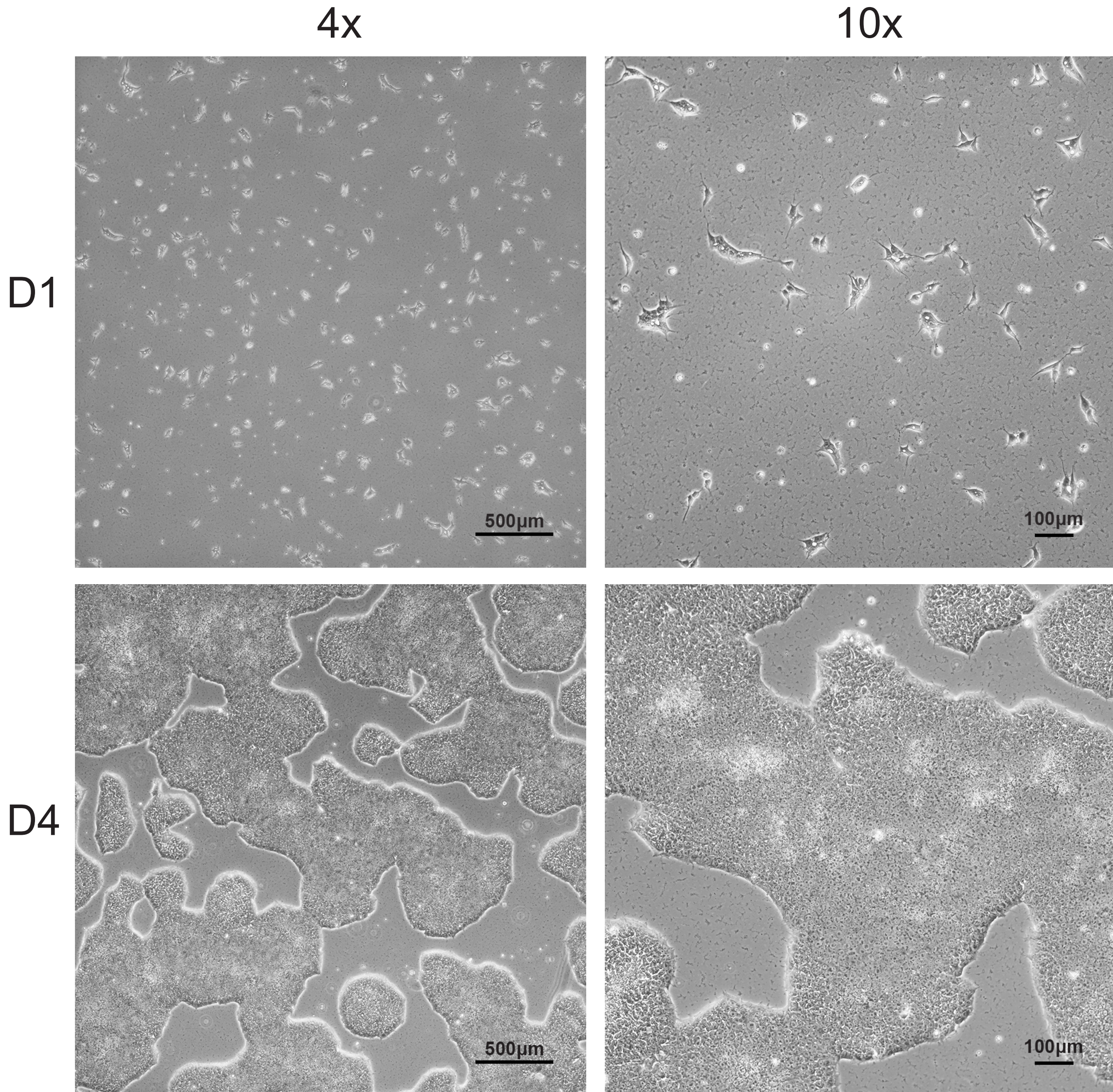


Figure 3: Four panel image of clone 44. Viability and colony formation one day and four days post-thaw. Scale bars are shown.

2 REPRESENTATIVE IMAGE FOR CLONE 23 (wt/wt)

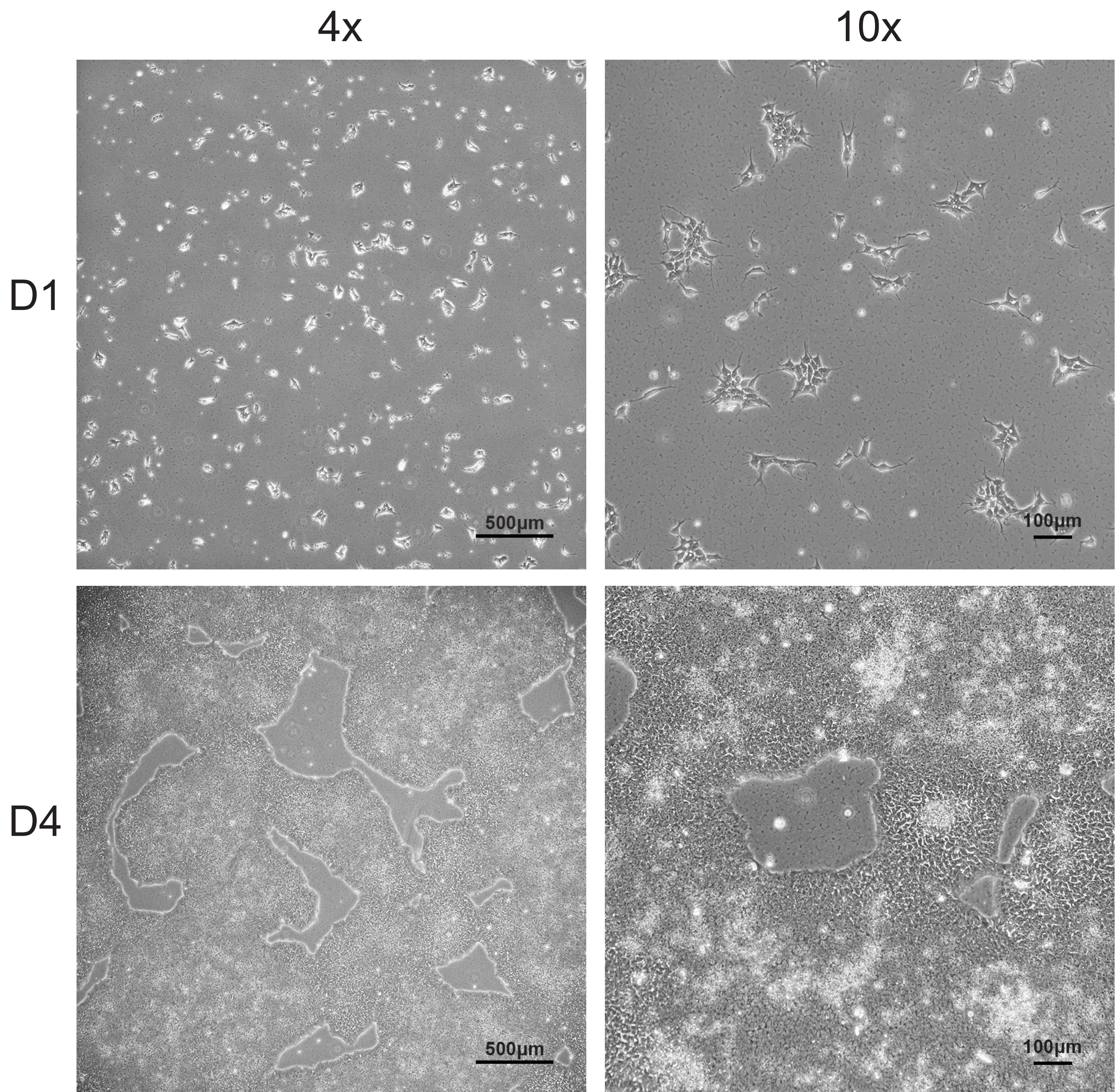


Figure 4: Four panel image of clone 23. Viability and colony formation one day and four days post-thaw. Scale bars are shown.

3 REPRESENTATIVE IMAGE FOR CLONE 30 (G769V/wt)

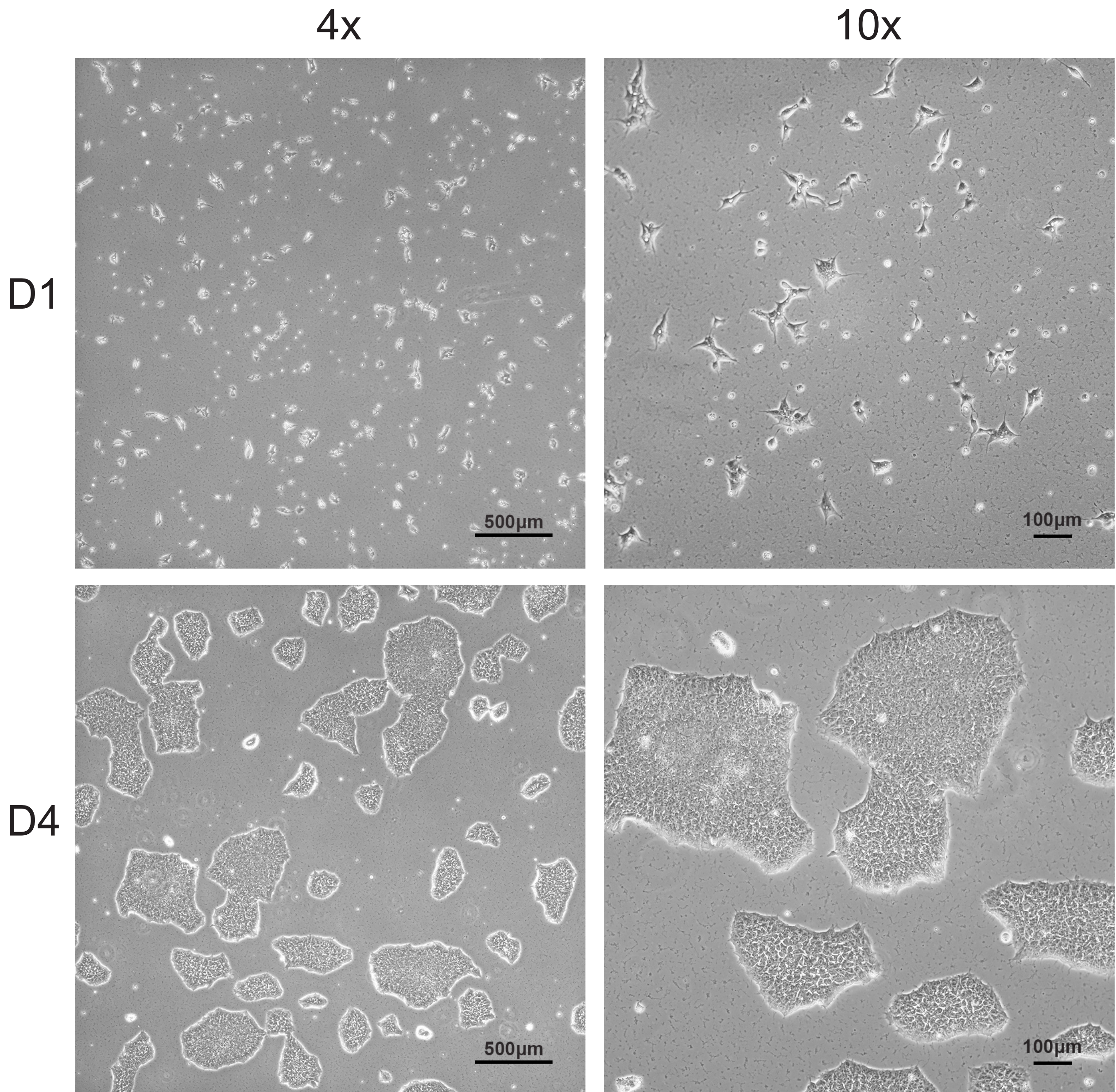
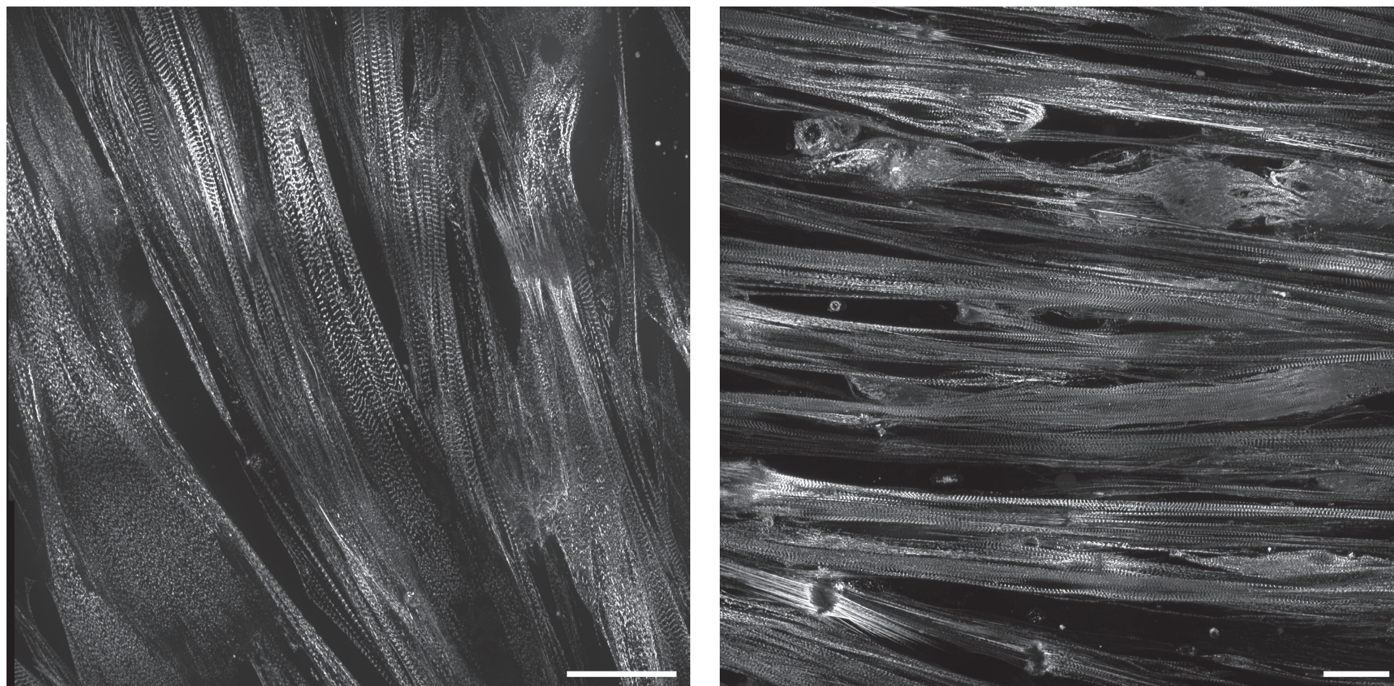


Figure 5: Four panel image of clone 30. Viability and colony formation one day and four days post-thaw. May take up to five days to recover post-thaw (Day 5 image not shown). Scale bars are shown.

[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

LIVE IMAGING OF CLONE 82 (G769V/wt)



Firgure 6: Live-cell imaging of skeletal muscle from the MYH3 G769V collection (Clone 82). Cells express mEGFP-tagged alpha-actinin-2. After 35 days of primary differentiation from hiPSC to myogenic progenitors, cells were replated onto Matrigel coated cover glass and induced to differentiate into skeletal muscle. Cells were imaged 11 days after this replating on a spinning disk confocal microscope. Images were acquired in a 3 x 3 tiled Z stack and are presented as maximum intensity projections of 10 slices. Scale bars are 50μm.

Image system details: Nikon Eclipse Ti microscope with a Yokogawa CSU-W1 spinning disk head imaging onto an Andor iXon 888 EMCCD. Objectives were either Nikon Plan Apo VC 60x/1.4 NA or Nikon Plan Apo 100x/1.4 NA.

Skeletal muscle sample and images was courtesy of Alina Greimal, BS, Christian Mandrycky, PhD and David Mack, PhD *Institute for Stem Cell & Regenerative Medicine (ISCRM)* at the *University of Washington.*