CERTIFICATE OF ANALYSIS
AICS-0105: MYH7-R369Q mEGFP-ACTN2 (mono-allelic tag)

Cell Collection description	R369Q mutation introduced into the MYH7 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 Lee et al (2023) bioRxiv (preprint). doi: 10.1101/2023.06.08.54476
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	31	30	32	57	22	89
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	A	B	A	B
Clone PCR & Sanger	PCR and Sanger sequencing of MYH7 recombinant and wildtype alleles	Determine if predicted mutation occurred with no additional mutations present.	R369Q / R369Q	R369Q / WT			WT / WT	
Passage of gene edited iPSC reported at submission	N/A	N/A	p49[a]	p50[a]	p50[a]	p50[a]	p49[a]	p49[a]
Seeding density	N/A	Recommended seeding densities in 10 cm plate every 4 days or every 3 days, consecutively (see culture protocol)	500K / 1M	500K / 1M	500K / 1M	500K / 1M	500K / 1M	500K / 1M
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
Cardiac Differentation	Modified small molecule differentiation **(see cardiac differentiation protocol)**	Beating initiated (D7-D14) and Cardiac Troponin T expression (D11- D30) by flow cytometry	Pass	Pass	Pass	Pass	Pass	Pass
Avg % cTnT+	Flow Cytometry	% cTnT+ cells compared to isotype control	48%	52%	54%	36%	50%	57 %
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.

RED = BI-ALLELIC MUTANT CLONE; 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 MYH7, and mEGFP at C-terminus of ACTN2 as shown below.

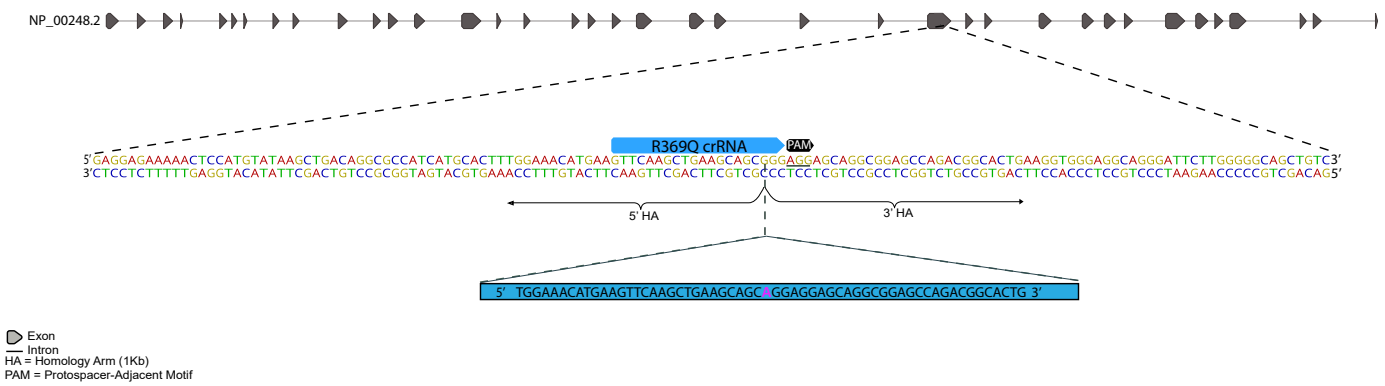

Figure 1: Top: MYH7 locus showing 1 MYH7 isoform; Bottom: Zoom in on mutation site at isoform NM_000257.4(MYH7):c.1106G>A(p.Arg369Gln)

HDR Editing Design for MYH7	
crRNA Target Site	5' GTTCAAGCTGAAGCAGCGGGAGG 3'
DNA Donor Sequence	5' TGGAAACATGAAGTTCAAGCTGAAGCAGCAGGAGGAGC AGGCGGAGCCAGACGGCACTG 3'
F primer for PCR/sequencing	5' GCCAGGAAGCATAAGTGGGT 3'
R primer for PCR/sequencing	5' GGTGACGTACTCATTGCCCA 3'

Red = PAM Site; Blue = Mutation

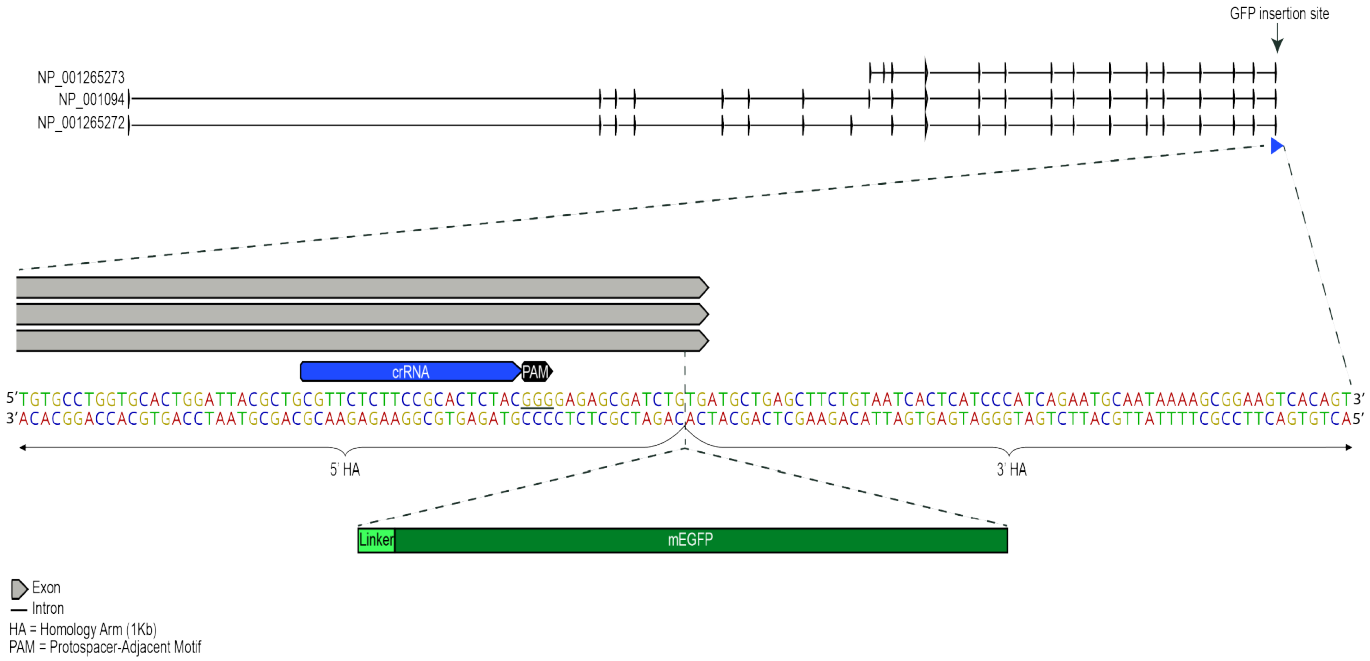

Figure 2: Top: ACTN2 locus showing 3 ACTN2 isoforms; Bottom: Zoom in on mEGFP insertion site at ACTN2 C-terminus

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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 four days post-thaw[1,2] using a Leica microscope at 4x and 10x magnification. 1. clone 57 (R369Q/wt) and 2. clone 89 (wt/wt) is shown here.

1 REPRESENTATIVE IMAGE FOR ALL CLONES (EXCEPT CLONE 89, SEE BELOW)

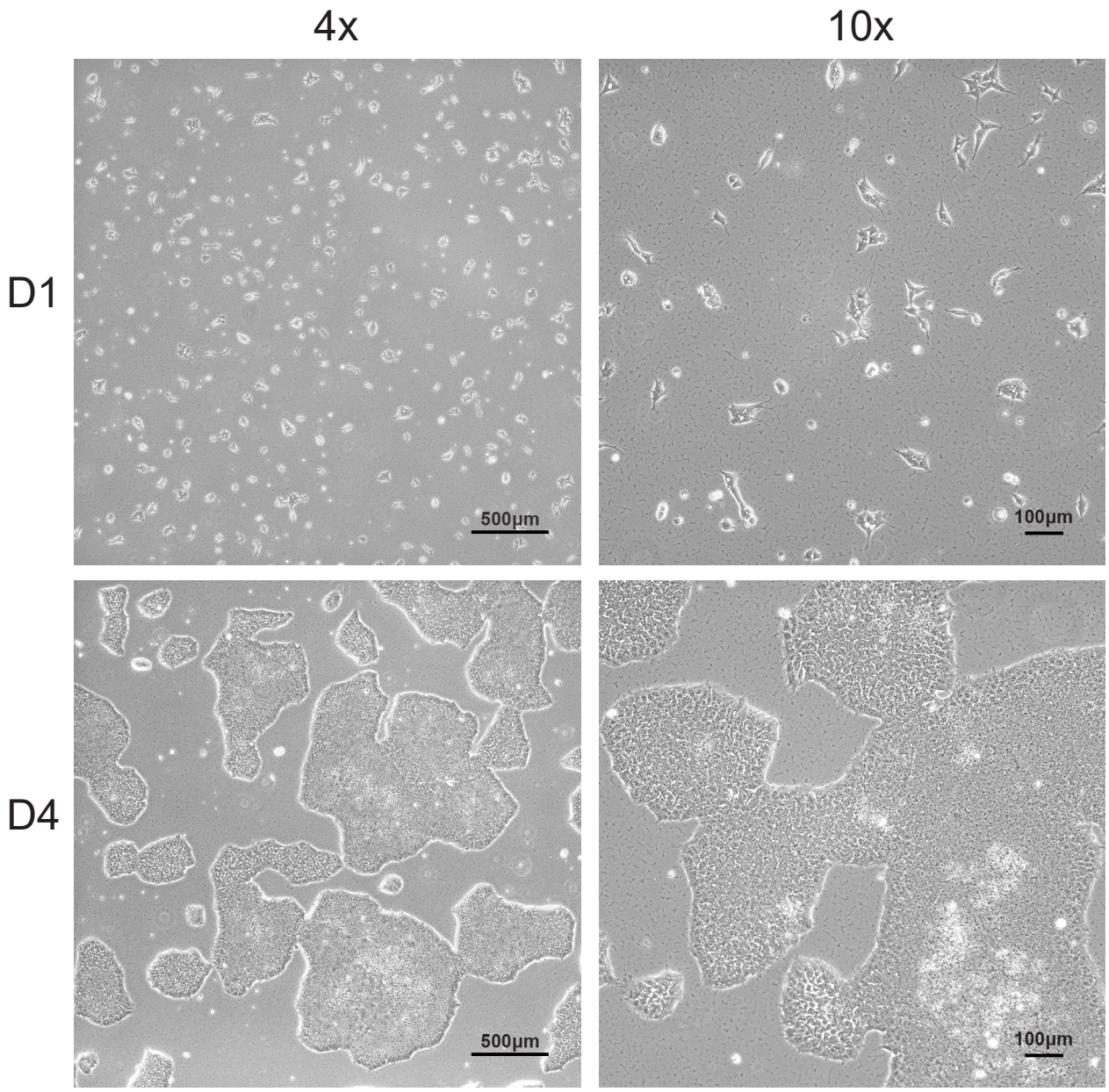

Figure 3: Four panel image of clone 57. Viability and colony formation one day and four days post-thaw. Scale bars are shown.

2 REPRESENTATIVE IMAGE FOR CLONE 89 (wt/wt)

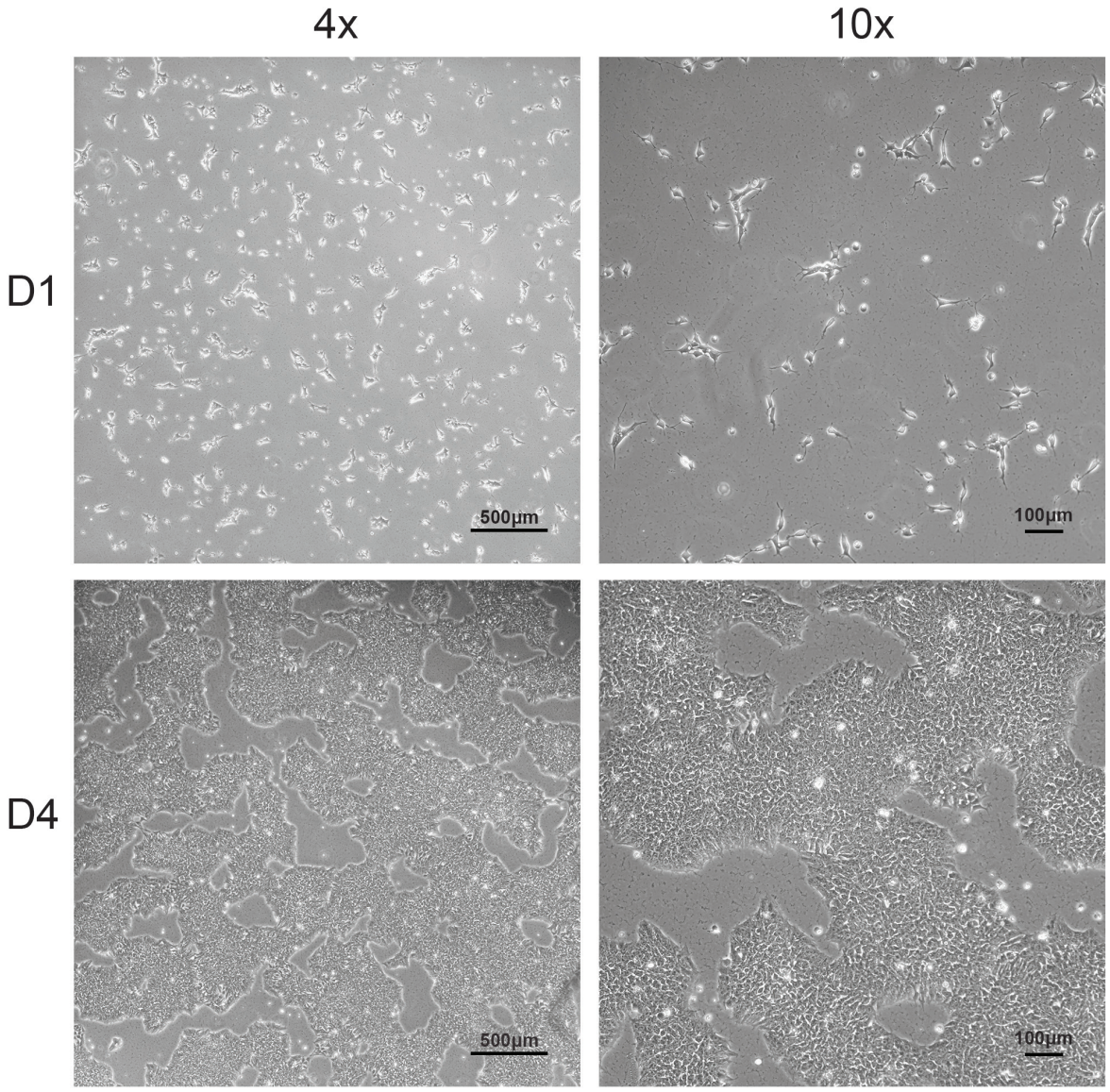

Figure 4: Four panel image of clone 89. Viability and colony formation one day and four days post-thaw. This cell line may exhibit transient poor morphology in the first three passages post-thaw. However, this sub-optimal phenotype resolves with continued passage. Scale bars are shown.

[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