CERTIFICATE OF ANALYSIS
AICS-0119: LMNA-G608G mEGFP-LMNB1 (mono-allelic tag)

Cell Collection description	G608G mutation introduced into the LMNA gene. Additionally, mEGFP added into N terminus of LMNB1.
Parental cell line	Human iPSC clonal line in which LMNB1 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
Number of passages at Coriell	0
Media	mTeSR1
Feeder or matrix substrate	Matrigel
Passage method	Accutase, single cell
Thaw	500K thousand 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	10	39	23	45
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	A
Clone PCR & Sanger	PCR and Sanger sequencing of LMNA recombinant and wildtype alleles	Determine if predicted mutation occurred with no additional mutations present.	G608G / WT	G608G / WT	WT / WT	WT / WT
Passage of gene edited iPSC reported at submission	N/A	N/A	p49[a]	p49[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)	450K / 900K	450K / 900K	450K / 900K	450K / 900K
Post-Thaw Viable Cell Recovery	hiPSC culture on Matrigel	>50% confluency 4-5 days post-thaw (10cm plate)	Pass	Pass	Pass	Pass
Mono-Clonality Confirmation	ddPCR assay	Verification of genomic copy number of WT and mutant alleles	Pass	Pass	Pass	Pass
Trisomy 12 Test	ddPCR assay (Chr12:RPP30)	pass = trisomy 12 not detected in quantitative ddPCR assay.	Pass	Pass	Pass	Pass
PPM1D	ddPCR assay (PPM1D:PPM1D REF)	PPM1D present in parental line	+	+	+	+
Off-Target assessment	PCR and Sanger sequencing of top 3 intronic and exonic sites	No mutations at off-target sites assayed	Pass	Pass	Pass	Pass

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Growth rate	ATP quantitation[b]	Mutant clones comparable to control clones	Pass	Pass	Pass	Pass
Expression of stem cell markers	Flow Cytometry	Transcription factors: OCT4/SOX2/NANOG $\geq$ 90% Surface markers: SSEA4, SSEA1 $\leq$ 10%	Pass	Pass	Pass	Pass
Germ layer differentiation	Trilineage Differentiation[c] 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	Pass	Pass	Pass
Karyotype	G-banding (30 cell analysis)	Normal karyotype, 46 XY	Pass	Pass	Pass	Pass
Mycoplasma	qPCR (IDEXX)	Negative	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
Viral Panel Testing[d]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass			
Identity of Unedited WTC-11 parental line[e]	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] Promega CellTiter-Glo Luminescent Cell Viability Assay (Catalog #G7571)
[c] STEMCELL Technologies STEMdiff Trilineage Differentiation Kit (Catalog #05230)
[d] 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
[e] 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.

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 LMNA, and mEGFP at N-terminus of LMNB1 as shown below.

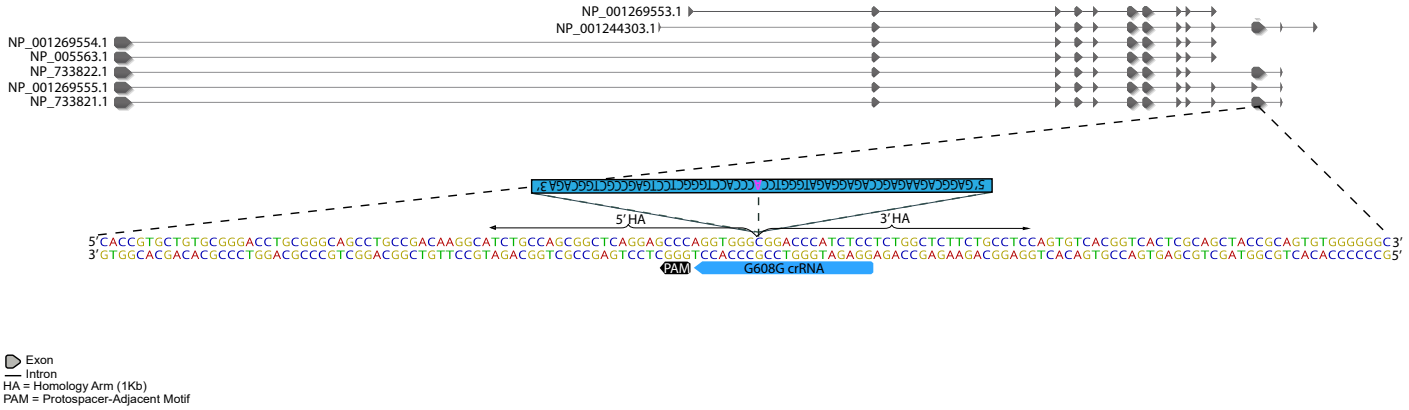

Figure 1: Top: LMNA locus showing 7 LMNA isoforms; Bottom: Zoom in on mutation site at isoform NM_170707.4(LMNA):c.1824C>T(p.Gly608=)

HDR Editing Design for LMNA	
crRNA Target Site	5' GGAGATGGGTCCGCCCACCTGGG 3'
DNA Donor Sequence	5' GAGGCAGAAGAGCCAGAGGAGATGGGTCCACCCACCTGGGCTC CTGAGCCGCTGGCAGA 3'
F primer for PCR/sequencing	5' GCCTCTCTCCCCCATTCTTG 3'
R primer for PCR/sequencing	5' ATGATGCTGCAGTTCTGGGG 3'

Red = PAM Site; Blue = Mutation

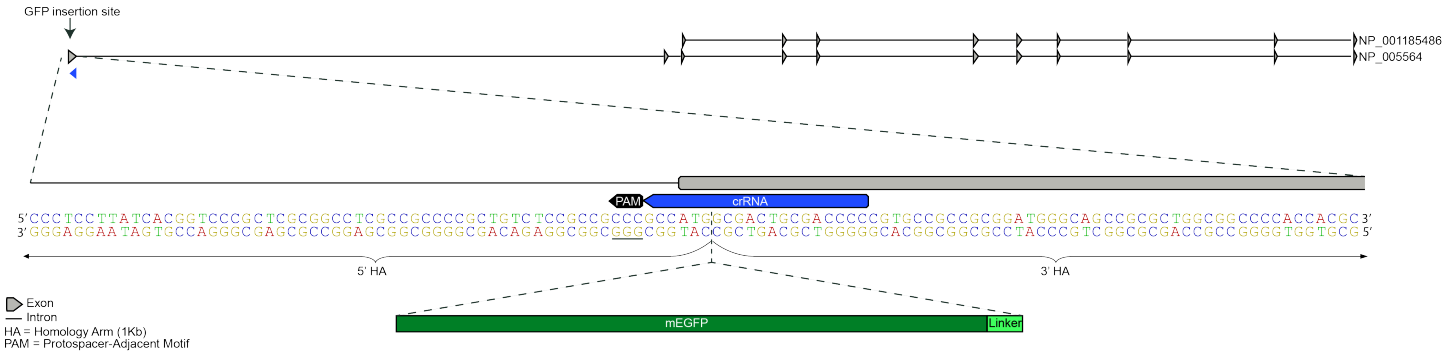

Figure 2: Top: LMNB1 locus showing 2 LMNB1 isoforms; Bottom: Zoom in on mEGFP insertion mutation site at N-terminal exon

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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 four days post-thaw[1,2] using a Leica microscope at 4x and 10x magnification. 1. clone 10 (G608G/wt), 2. clone 23 (wt/wt), and 3. clone 45 (wt/wt) is shown here.

1 REPRESENTATIVE IMAGE FOR ALL CLONES (EXCEPT CLONE 23 AND CLONE 45, SEE BELOW)

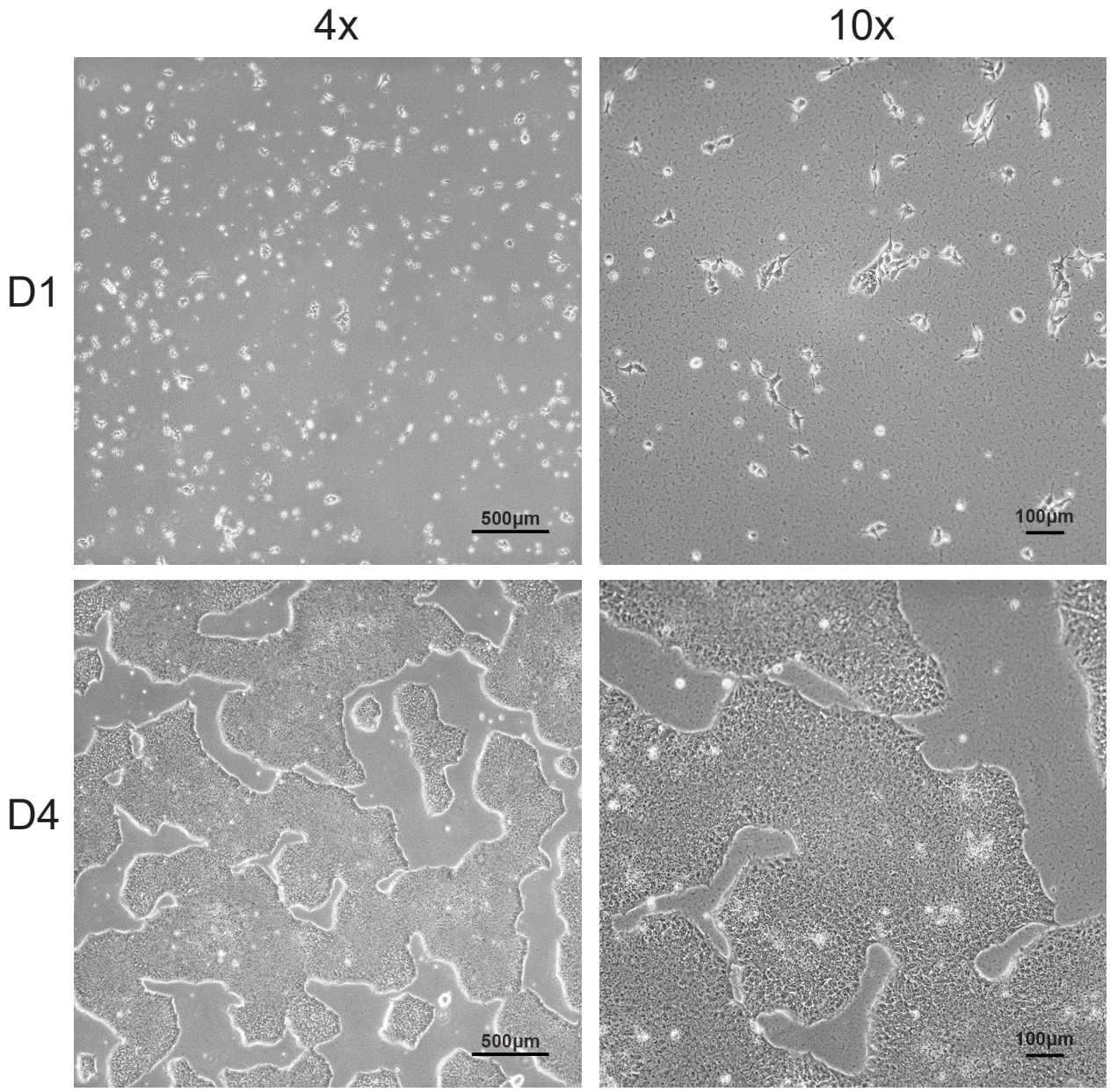

Figure 3: Four panel image of clone 10. Viability and colony formation one day and four days post-thaw. Scale bars are shown.

2 REPRESENTATIVE IMAGE FOR CLONE 23 (wt/wt)

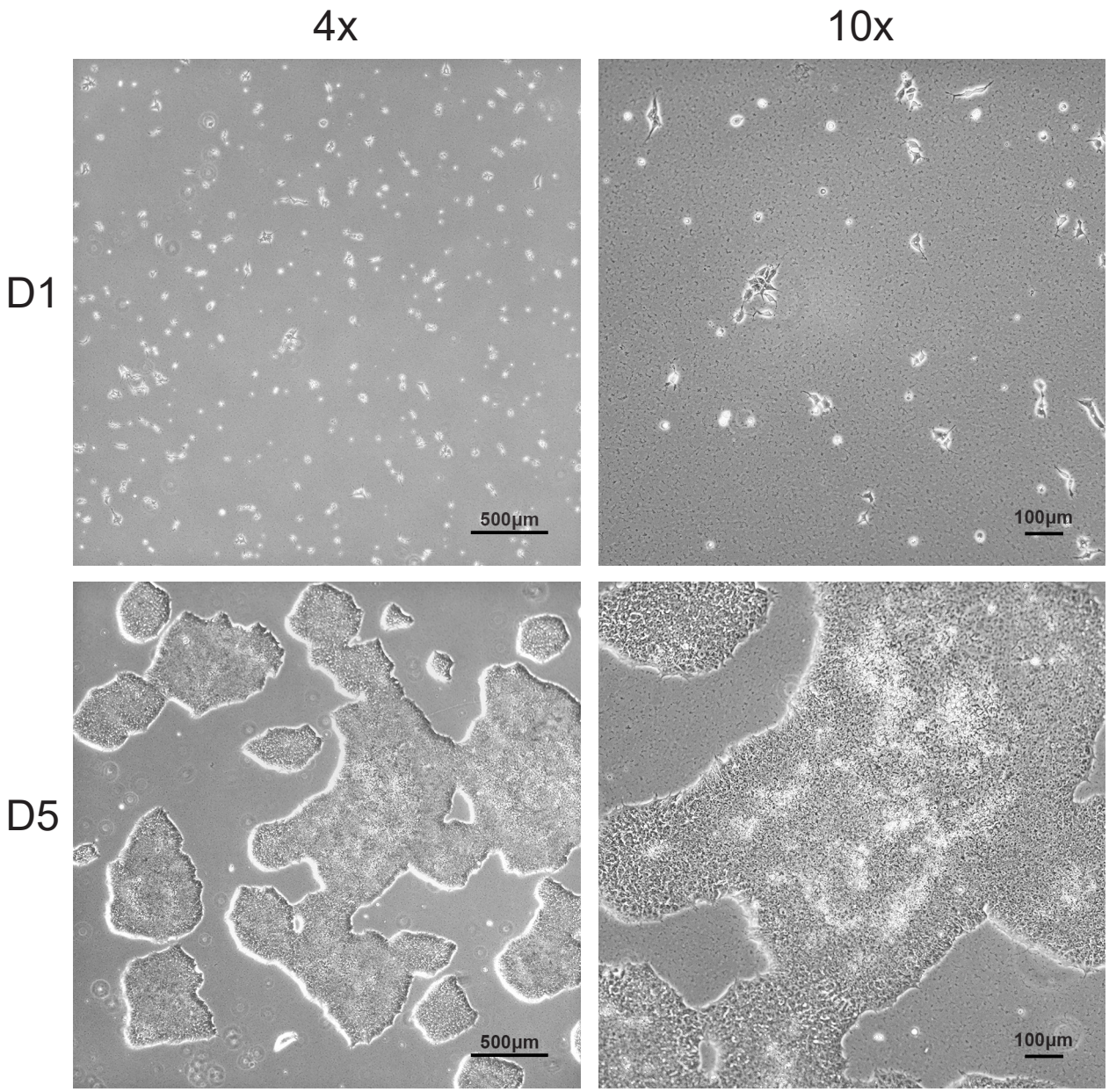

Figure 4: Four panel image of clone 23. Viability and colony formation one day and five days post-thaw. Scale bars are shown.

3 REPRESENTATIVE IMAGE FOR CLONE 45 (wt/wt)

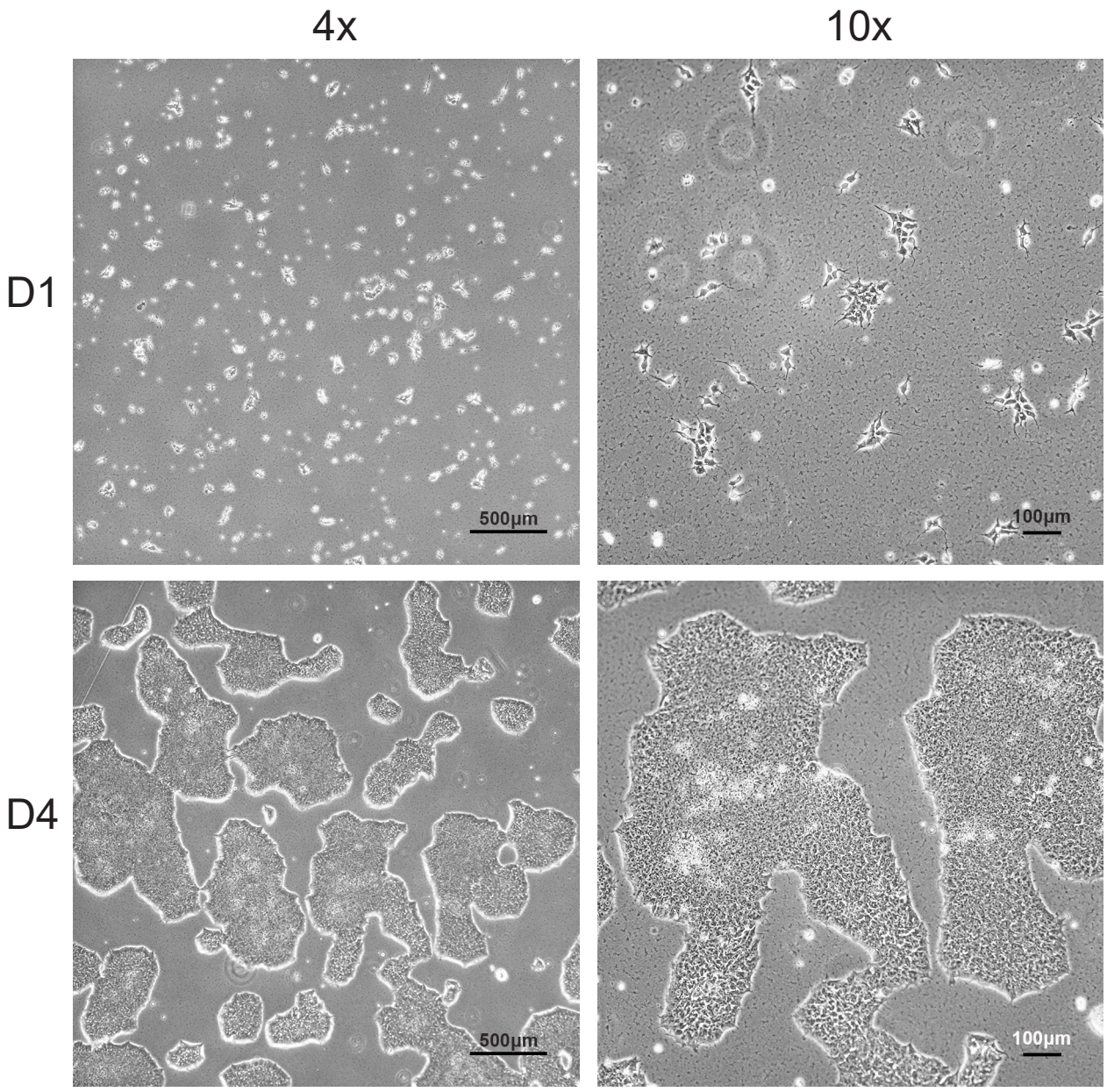

Figure 5: Four panel image of clone 45. Viability and colony formation one day and four days post-thaw. Scale bars are shown.

[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

IMMUNOFLOURESCENT LABELING OF CLONES 23 (wt/wt) and 39 (G608G/wt)

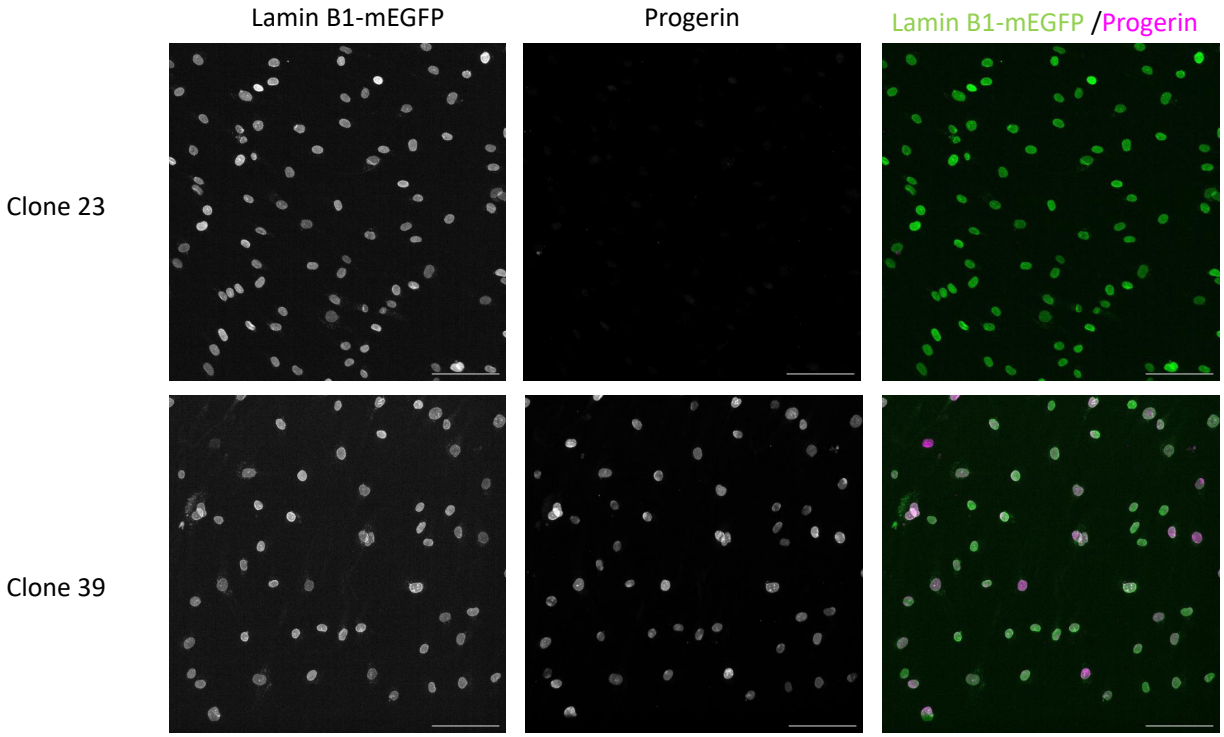

Figure 6: Immunofluorescent labeling of clones 23 and 39 to demonstrate expression of progerin proteins. hiPSCs were differentiated using the StemDIFF Endothelial Differentiation Kit (StemCell Technologies cat. No. 08005). Cells were cryopreserved in CryoStor CS10 (StemCell Technologies cat. No. 100-1061), and subsequently thawed into STEMdiff™ Endothelial Expansion Medium Kit (Catalog #08007) onto plastic tissue culture plates coated with Animal Component-Free Cell Attachment Substrate. Cells were passaged according to the provided protocol onto plastic tissue culture plates until Passage 7, at which point they were plated onto glass coated with Animal Component-Free Cell Attachment Substrate. After 4 days of culture the cells were fixed with a 4% paraformaldehyde in DPBS solution. Cells were then blocked and permeabilized in a solution of 1.5% normal goat serum + 0.4% Triton-100x in PBS for 1 hour at RT. Cells were labeled with an anti-progerin antibody (Santa Cruz cat. No. sc-81611, diluted 1:60) overnight at 4C. The following day cells were labeled with anti-mouse AlexaFluor594 (ThermoFisher cat. No. A-11005, diluted 1:500) for 1 hour at RT. Cells were imaged on a widefield microscope. All scale bars are 100um.