CERTIFICATE OF ANALYSIS
AICS-0114-035:WTC-mEGFP-CDH1-cl35 (mono-allelic tag)

Product description	Human iPSC clonal line in which CDH1 has been endogenously tagged with mEGFP using CRISPR/Cas9 technology and an AAV6 donor template
Parental cell line	Parental hiPSC line (WTC/AICS-0 at passage 30) 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	p30[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	500K cells/10-cm plate every 4 days or 1M 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 recombinant and wildtype alleles	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 (0.55)
Off-target mutations	1) PCR and Sanger sequencing of 5-10 sites predicted by Cas-OFFinder[e] 2) Whole exome sequencing[f]	No mutations at off-target sites assayed	Pass
Other mutations	Whole exome sequencing[f]	Check for acquired mutations (not detected in p8[a] parental line) that affect genes in Cosmic Cancer Gene Census	PPM1D G1426T/E476X
mEGFP localization	Spinning Disk confocal live cell imaging	Localization adherens junctions	E-cadherin (encoded by CDH1) appears as puncta at cell-cell contact sites, consistent with localization to adherens junctions formed between membranes of adjacent cells.
Expression of tagged protein	Western blot	Expression of expected size product	Expected size band for untagged and mEGFP-tagged E-cadherin. Semi-quantitative results demonstrate that $\sim$34% of E-cadherin encoded protein product is mEGFP labeled.
Growth rate	ATP quantitation[g]	Comparable to parental line	Pass (measured at p28)[a]

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Test Description[b]	Method	Specification	Result
Expression of stem cell markers	Flow cytometry	Transcription factors: OCT4/SOX2/NANOG $\geq$ 85% Surface markers: SSEA4 $\geq$ 85%; SSEA1$\leq$ 15%	Pass
Germ layer differentiation	Trilineage differentiation[h]	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
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[j]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass
Identity of unedited parental line[k]	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] 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.
[k] 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 CDH1 as shown below.

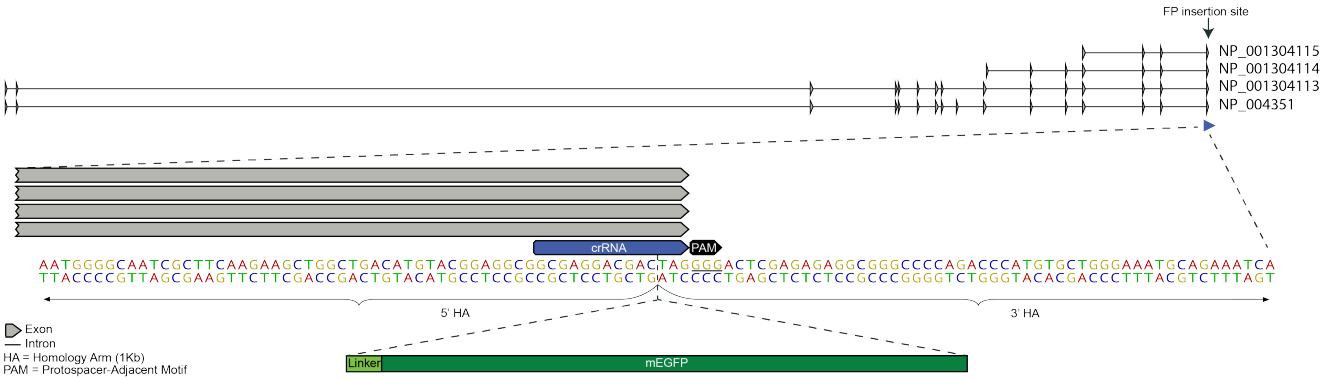

Figure 1: Top: CDH1 locus showing 4 CDH1 isoforms; Bottom: Zoom in on mEGFP insertion site at CDH1 C-terminal exon

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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 using a Leica microscope at 4x and 10x magnification (a-d). Cells were then passaged and seeded into plates for three day and four day growth and imaged at 4x magnification (e, f). This cell line may exhibit transient poor morphology in the first few passages post-thaw. However, this sub-optimal phenotype resolves with continued passage.

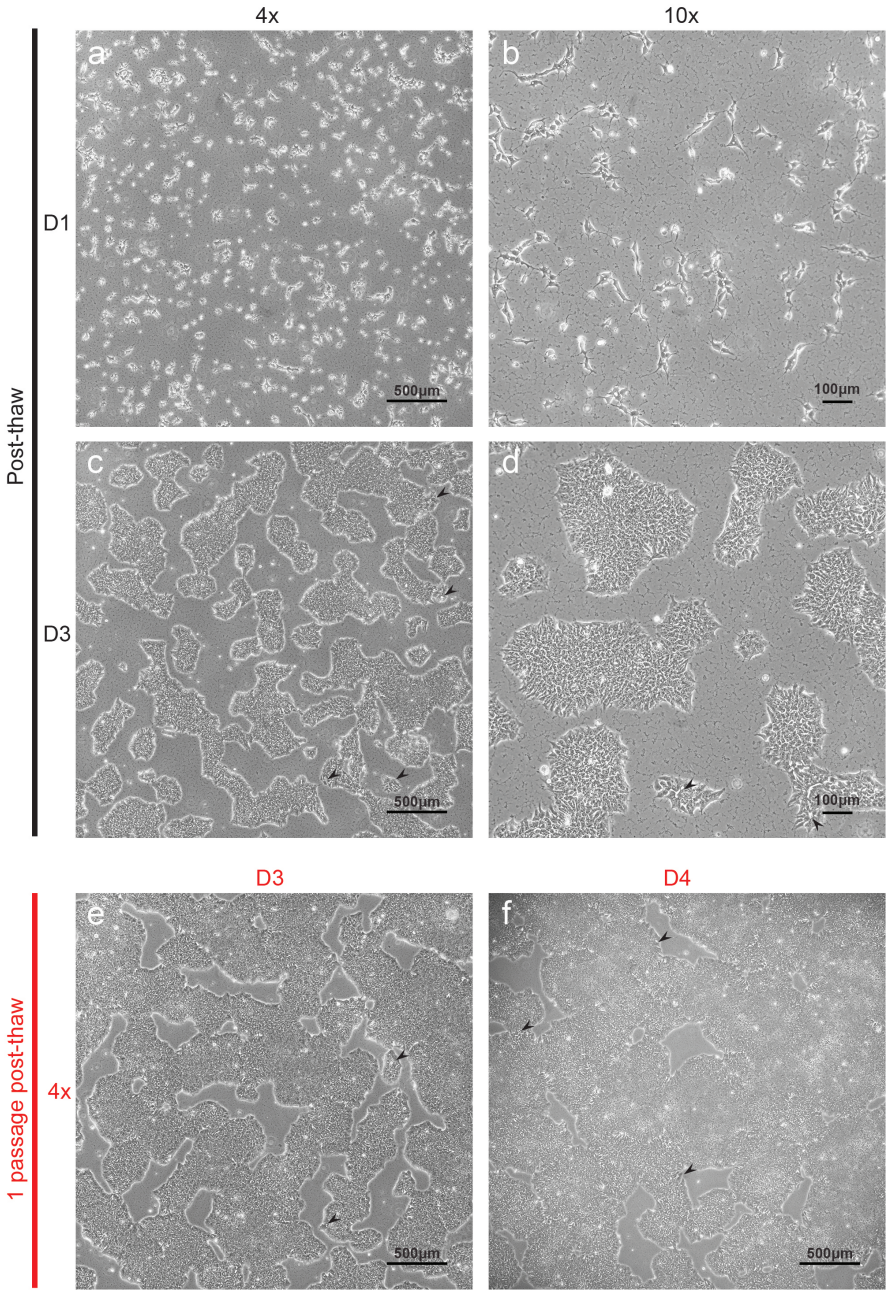

Figure 2: Viability and colony formation post-thaw. a-d: Morphological abnormalities (indicated by black arrows) may be visible post-thaw (∼5% of population). e-f: Morphology improves the first few passages post-thaw when seeding at 1M/10cm dish for three days and 500K cells/10cm dish for four days. Morphological abnormalities can be seen in ∼1-5% of colonies in three and four days of growth. Colony morphology will resolve with continued passage. Scale bars are as shown.

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Imaging labeled structures in endogenously tagged cells: The tagged proteins are expressed endogenously and therefore may not appear as bright as they would in an overexpressed system. For imaging we plate cells onto matrigel-coated high-quality glass bottom coverslips (Cellvis) and image cells in phenol red-free mTeSR1 media (STEMCELL Technologies). Our most common microscope configuration is a Zeiss spinning disk fluorescence microscope with a Yokogawa CSUX1 head, Hamamatsu CMOS camera, and a 488 laser (mEGFP). Cells are imaged either with a 20x 0.8NA objective for lower magnification or 100x 1.25NA water immersion objective for higher magnification, at 37°C and 5% CO_2 in a temperature-controlled chamber. The approximate laser power measured at the sample for our standard 100x images is ∼2.5 mW.

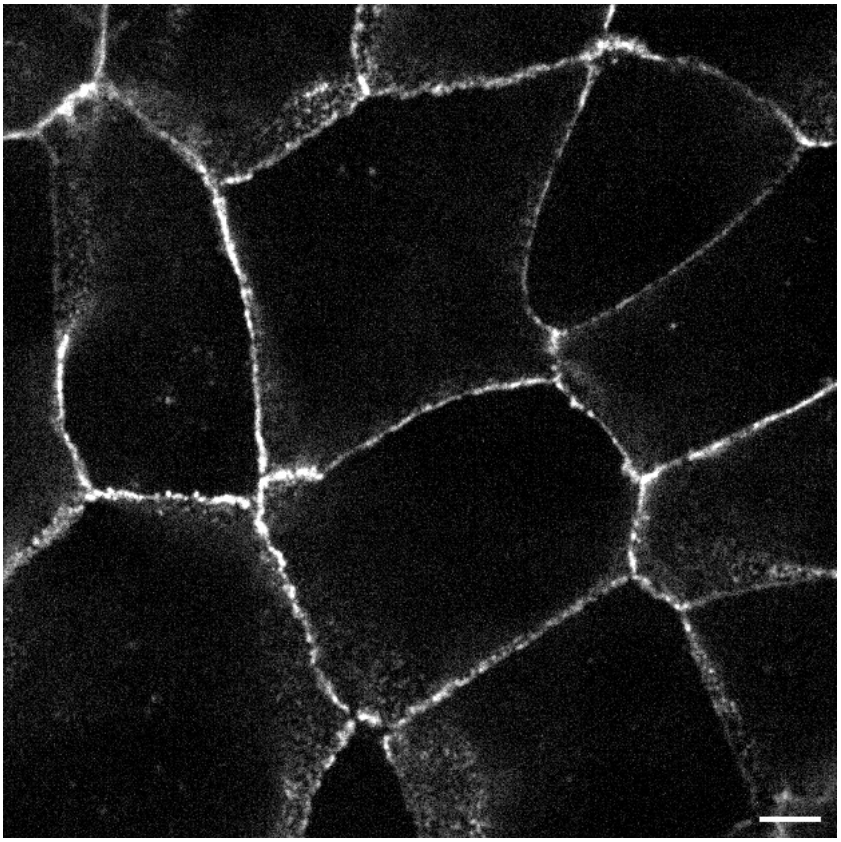

Figure 3: Single plane image near the top of hiPS cells expressing mEGFP-tagged E-cadherin. Cells were imaged live in 3D on a spinning-disk confocal microscope. Scale bar, 5 µm.