CERTIFICATE OF ANALYSIS
AICS-0126-068:WTC-mEGFP-CDH5-cl68 (mono-allelic tag)

Product description	Human iPSC clonal line in which CDH5 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	p28[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-terminus 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 (0.577)
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 to adherens junctions	In endothelial cells, vascular-endothelial (VE)-cadherin (encoded by CDH5) appears as puncta at cell-cell contact sites, consistent with localization to endothelial adherens junctions formed between membranes of adjacent cells.
Expression of tagged protein	Western blot	Expression of expected size product	Expected size band for mEGFP-tagged VE-cadherin. Semi-quantitative results demonstrate that $\sim$23% of VE-cadherin encoded protein product is mEGFP labeled.

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Test Description[b]	Method	Specification	Result
Growth rate	ATP quantitation[g]	Comparable to parental line	Pass (measured at p28)[a]
Expression of stem cell markers	Flow cytometry	Transcription factors: OCT4/SOX2/NANOG $\geq$ 90% Surface markers: SSEA4 $\geq$ 90%; SSEA1$\leq$ 10%	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
Endothelial differentiation	Directed differentiation (Patsch et al. 2015)[i]	Platelet endothelial cell adhesion molecule (PECAM1) and VE-cadherin (CDH5) expression (D6) 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] Patsch et al (2015) Nature Cell Bio. 10.1038/protex.2015.055
[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 CDH5 as shown below.

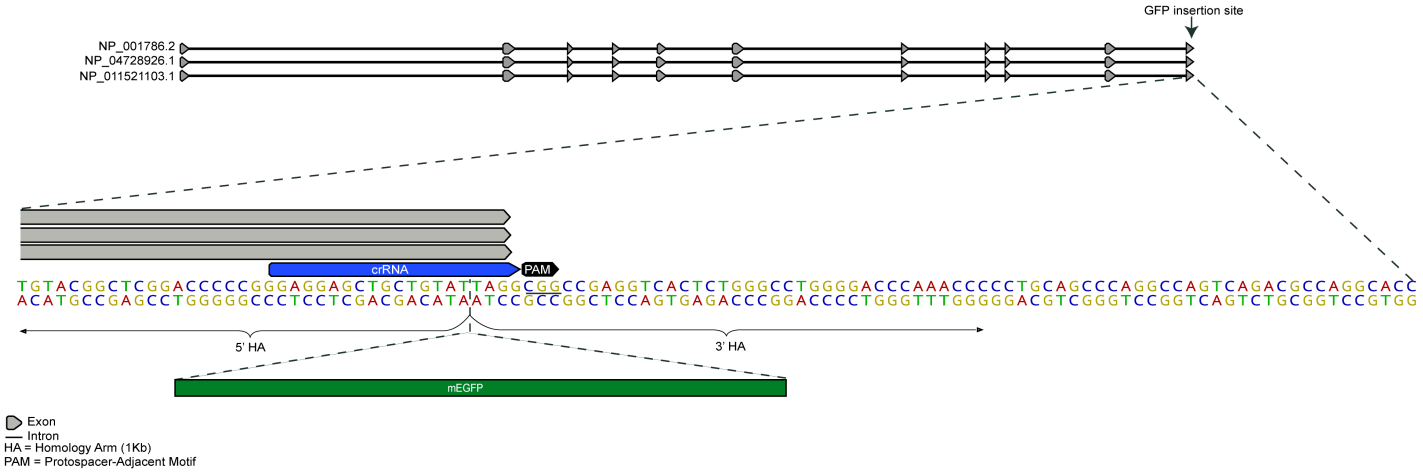

Figure 1: Top: CDH5 locus showing 3 CDH5 isoforms; Bottom: Zoom in on mEGFP insertion site at CDH5 C-terminal exon

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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.

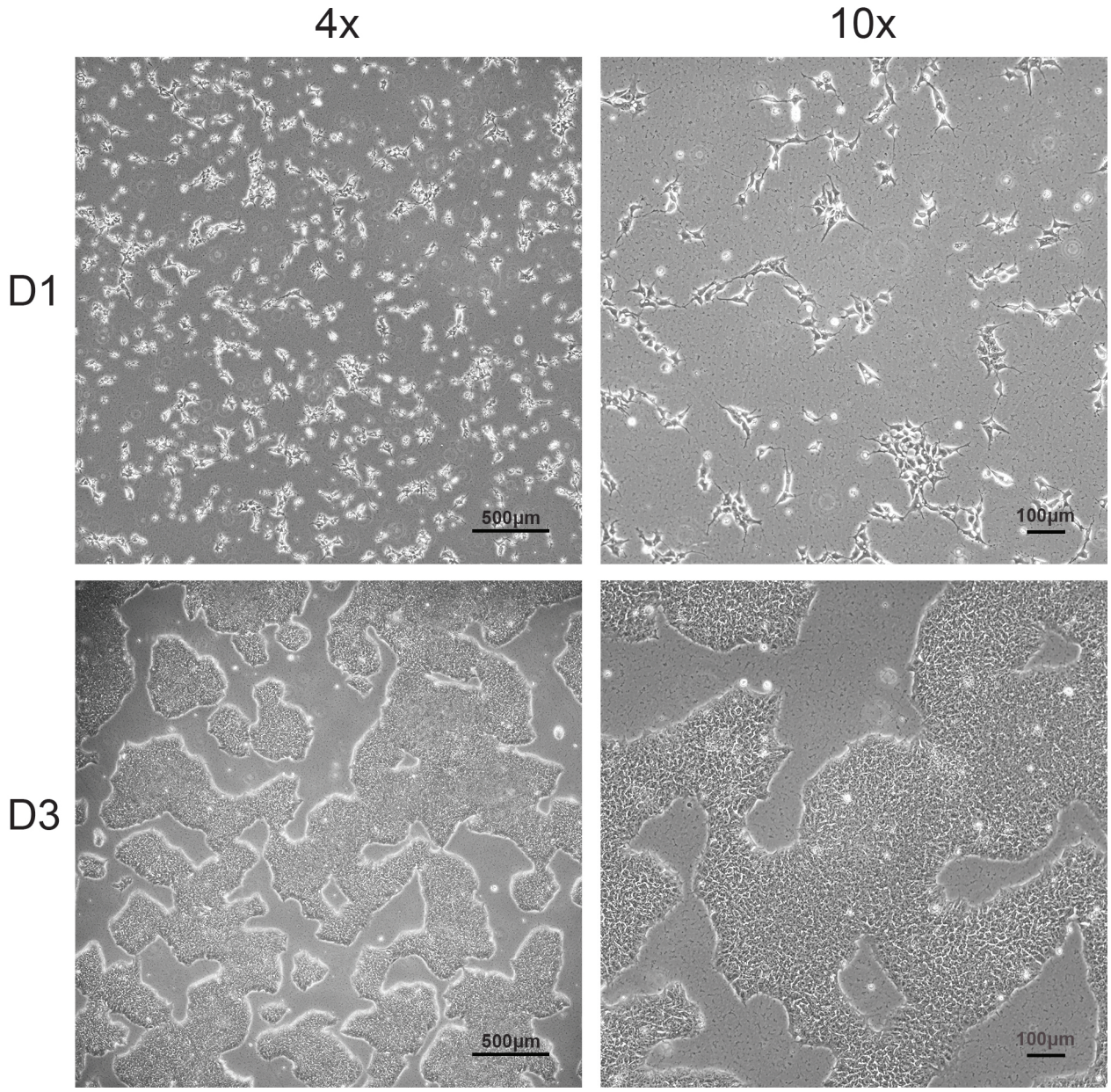

Figure 2: Viability and colony formation post-thaw. Scale bars are as shown

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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, cells were plated onto high-quality glass bottom 24-well plates (Cellvis) coated with 5 µg/ml fibronectin (Gibco). Cells are imaged in StemPro-34 media (Gibco) supplemented with 50 ng/mL VEGF (PeproTech). Our microscope configuration for imaging endothelial cells is a 3i spinning disk fluorescence microscope system that includes a Zeiss AxioObserver inverted microscope, a Yokogawa CSU-W1 scanhead, two Photometrics Prime BSI sCMOS cameras, and a laserstack with 405/488/561/640 laser lines (488 is used for GFP). Cells are imaged with a 63x 1.2 NA water immersion objective at 37°C and 5% CO_2 in a temperature-controlled chamber. The approximate laser power measured at the sample for our standard 63x images is 14 mW and the camera exposure time is typically 100 ms.

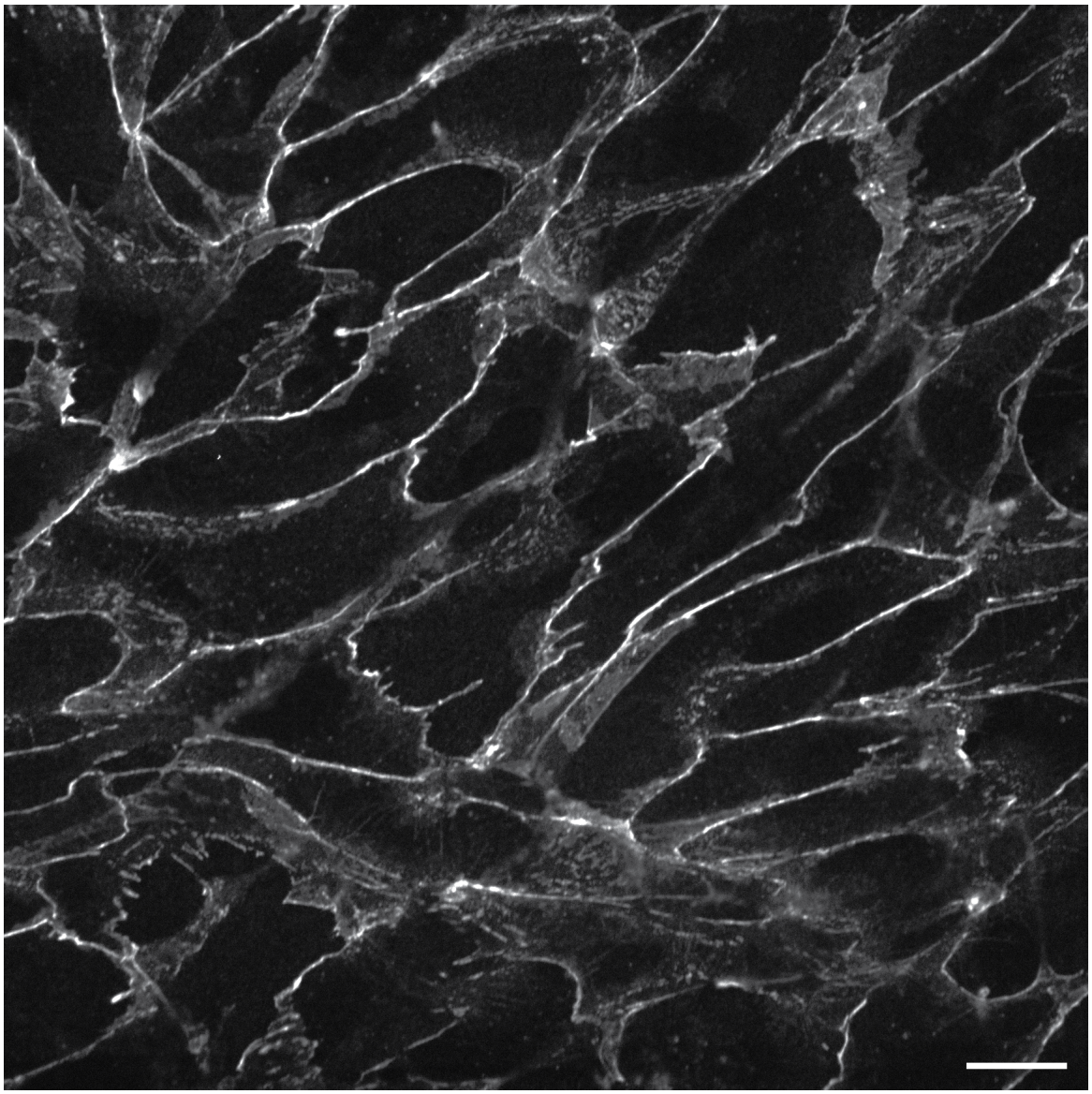

Figure 3: Single, mid-level plane of hiPSC-derived endothelial cells expressing mEGFP-tagged VE-cadherin (scalebar, 20 µm). Five days after the onset of differentiation, cells were magnetically sorted using CD144 antibodies and subsequently cryopreserved. Upon thawing, the cells were grown on fibronectin-coated tissue culture plates until they reached confluence. They were then re-plated onto fibronectin-coated glass plates and imaged four days later. Cells were imaged live in 3D on a spinning-disk confocal microscope.