CERTIFICATE OF ANALYSIS

AICS-0122-077:WTC-EOMES-mEGFP-cl77 (bi-allelic tag)

Product description	Human induced pluripotent stem cell (hiPSC) clonal line in which EOMES 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 33) derived from fibroblasts reprogrammed using episomal vectors (OCT3/4, shp53, SOX2, KLF4, LMYC, and LIN28). Coriell catalog: GM25256
Publication(s) describing hiPSC establishment	Kreitzer et al (2013) Am. J. Stem Cells, 30; 2(2): 119-31
Passage of gene edited hiPSC line reported at submission	p30[a]
Number of passages at Coriell	0
Media	mTeSR1
Feeder or matrix substrate	Matrigel
Passage method	Accutase, single cell
Thaw	1 million cells (ea vial) in 10 cm plate - ready for passaging in 3-4 days
Seeding density	450K cells/10-cm plate every 4 days or 900K cells/10-cm plate every 3 days (see culture protocol)

Test Description	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 at genomic locus - precise editing	PCR and Sanger sequencing of recombinant alleles	Insertion of mEGFP at the EOMES locus with exact predicted recombinant allele junctions.	Pass
Copy number	ddPCR[b] assay for FP(s) and RPP30 reference gene[c]	FP/RPP30: ~ 0.5 = Mono-allelic ~ 1.0 = Bi-allelic	mEGFP: Bi-allelic (1.19)
To determine the presence of the PPM1D mutation[d] in clonal line	ddPCR assay (PPM1D:PPM1D REF)	PPM1D mutation present in parental line	positive (+)
mEGFP tag localization	Spinning-disk confocal live cell imaging	Localization of mEGFP to nuclei	mEGFP-tagged Eomesodermin (Eomes) rises and falls during the epithelial to mesenchymal transition (EMT) induced in hiPSCs. Using the imaging parameters described below, mEGFP-tagged Eomes appeared in cells as diffuse nuclear signal approximately five hours after EMT induction and peaked around 15 hours later.
Epithelial to mesenchymal transition	GSK3-β inhibition with 5 µM CHIR99021[h]	Presence of endomesodermal marker Eomesodermin in live imaging and immunofluorescence after induction of EMT by GSK3-β inhibition	Pass

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Growth rate	ddPCR assay for RPP30 reference gene	Comparable to parental line	Pass (measured at p32)[a]
Expression of stem cell markers	Flow cytometry	Transcription factors: OCT4/SOX2/NANOG $\geq$ 85% Surface markers: SSEA4, TRA-1-60 $\geq$ 85%; SSEA1 $\leq$ 15%	Pass
Germ layer differentiation	Trilineage differentiation[e] 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
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[f]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass
Identity of unedited parental line[g]	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] Droplet digital PCR using Bio-Rad QX200
[c] RPP30 is a reference 2 copy gene used for normalization.
[d] Identifier NM_003620.4(PPM1D):c.1426G>T(p.Glu159X)
[e] STEMCELL Technologies STEMdiff Trilineage Differentiation Kit (Catalog #05230)
[f] 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.
[g] 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.
[h] GSK3-β inhibition with 5 µM CHIR99021: Hookway, Borensztejn, Harris et. al (2024) bioRxiv, doi.org/10.1101/2024.08.16.608353.

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Tagging strategy:

CRISPR-Cas9 methodology was used to introduce mEGFP at the C-terminus of EOMES as shown below.

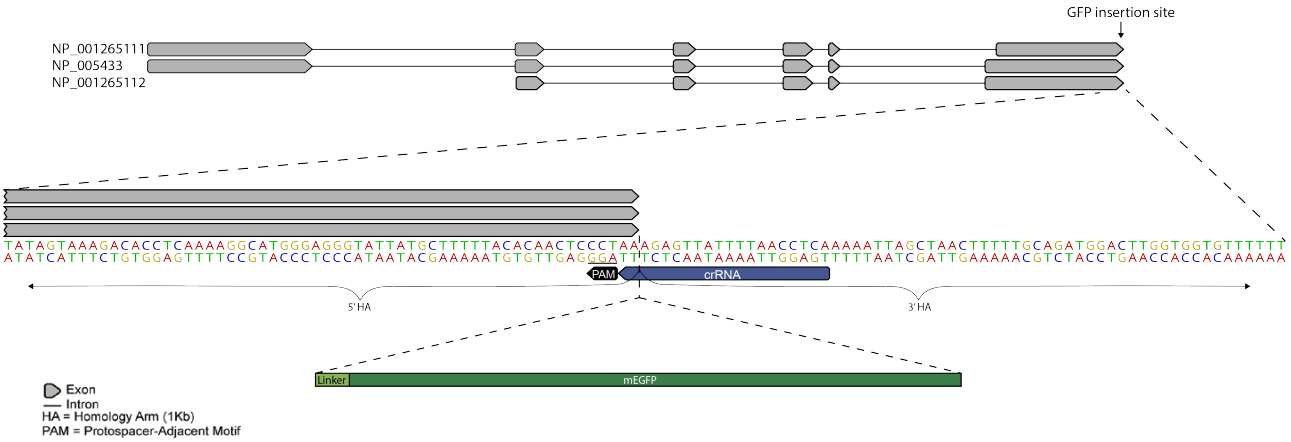

Figure 1: Top: EOMES locus showing 3 EOMES isoforms; Bottom: Zoom in on mEGFP insertion site at EOMES C-terminal exon.

Post-thaw imaging:

One vial of distribution lot was thawed (cells were treated with ROCK inhibitor for 24hrs post-thaw). Cultures were observed daily. Colonies were imaged one (a,b), and three (c,d) days post-thaw using a Leica microscope. Morphologies observed post-thaw are representative of cell morphologies observed post-passage.

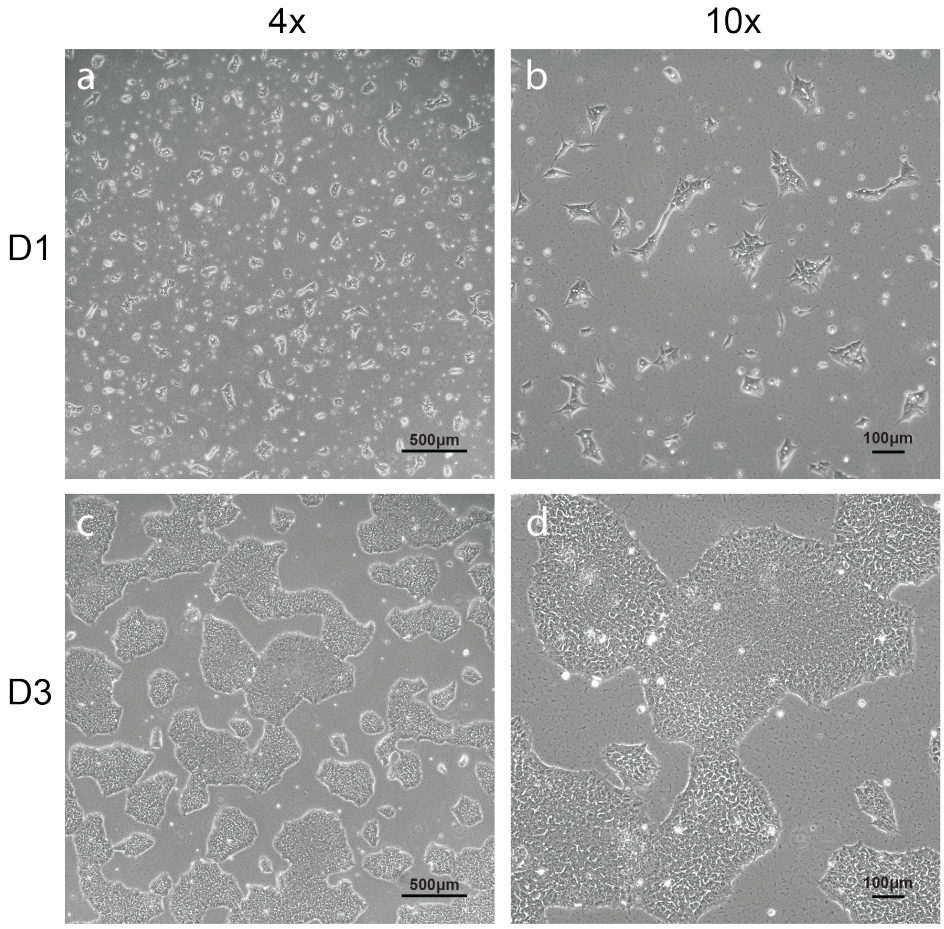

Figure 2: Viability and colony formation one day and three days post-thaw.

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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, high-quality glass bottom 96-well plates (Cellvis) were coated with 1:52 dilution of Matrigel (Corning, Cat. # 356231) in DMEM/F12 (Gibco, Cat. #21041025). Cells were seeded and grown for four days in mTeSR media (STEMCELL Technologies, Cat. # 85850). Immediately prior to imaging, media was changed to RPMI-1640 media (Gibco, Cat. # 11835-030) with 5 µM CHIR99021 (Cayman Chemical, Cat. # 13122), B27 supplement devoid of insulin (Gibco, Cat. # A1895601) and a 1:60 Matrigel dilution. Our microscope configuration for the images shown is a Zeiss spinning disk fluorescence microscope system that includes a Yokogawa CSUX1 head, Hamamatsu CMOS camera, and a 488nm laser (mEGFP). Cells were imaged with a 20x 0.8NA objective at 37°C and 5% CO_2 in a temperature-controlled chamber. The approximate laser power measured at the sample for shown 20x images is 0.77 mW and the camera exposure time was 50 ms.

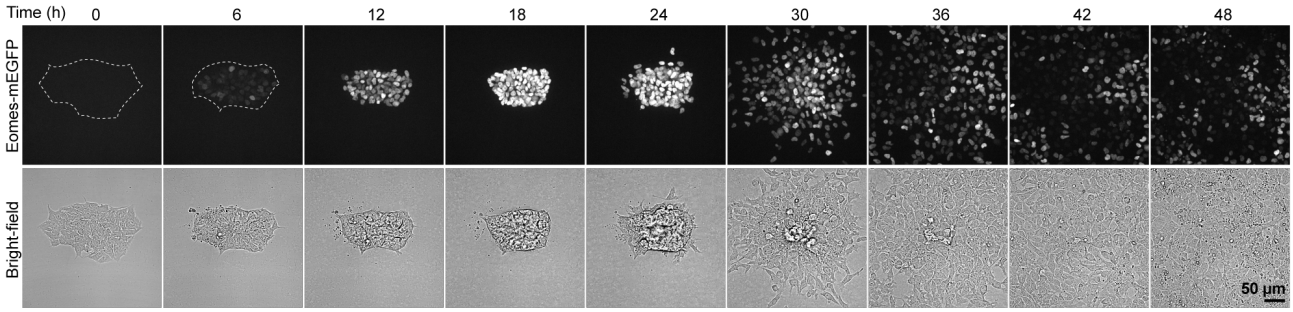

Figure 3: Cells expressing mEGFP-tagged Eomesodermin (Eomes) after induction of the epithelial to mesenchymal transition in hiPSCs by activation of the WNT pathway using GSK3-β inhibition (5 µM CHIR99021). Top row shows a maximum intensity projection of cells expressing mEGFP-tagged Eomes. Prior to expression, the colony perimeter is indicated by the dashed white line (0-hour and 6-hour timepoints). Bottom row shows a bright-field single, mid-level plane for each time point. Cells were imaged live in 3D on a spinning-disk confocal microscope every 30 minutes for 48 hours (six hour increments shown). Scale bar, 50 µm.