CERTIFICATE OF ANALYSIS
AICS-0120-204:WTC Dual tagged TOMM20-mEGFP/mTagRFP-T-COXVIII (mitoTag)-Safe harbor locus (AAVS1)-cl204 (mono-allelic tags)

Product description	Human iPSC clonal line in which TOMM20 has been endogenously tagged with mEGFP and mTagRFP-T has been localized to the mitochondrial matrix via COXVIII (mitoTag) under the control of a CAG promoter at a safe harbor locus (AAVS1) edited 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	p48[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	400K cells/10-cm plate every 4 days or 800K 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 / mTagRFP-T 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. Insertion of mTagRFPT at COXVIII (mitoTag) at the AAVS1 locus with exact predicted recombinant allele junctions.	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	TOMM20-mEGFP: Mono-allelic (0.545) mTagRFP-T-COXVIII (mitoTag): Mono-allelic (0.560)
Plasmid integration	ddPCR assay to detect plasmid integration into the genome	AmpR/RPP30 $<$ 0.1: no plasmid integration	Pass (0.00)
To determine the prescence of the PPM1D mutation[e] in clonal line	ddPCR assay (PPM1D:PPM1D REF)	PPM1D mutation present in parental line	positive (+)

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mEGFP localization and mTagRFP-T localization	Spinning Disk confocal live cell imaging	Localization of mEGFP to the outer mitochondria and mTagRFP-T to the mitochondrial matrix.	mEGFP-tagged Tom20 surrounds mTagRFP-T-tagged mitochondrial targeting sequence of COXVIII (mitoTag), consistent with the labeling of the outer membrane and inner matrix compartments of mitochondria, respectively.
Expression of tagged protein	Western blot	Expression of expected size product	Expected size band for untagged and mEGFP-tagged outer mitochondrial membrane receptor Tom20 (TOMM20). Semi-quantitative results show that 34% of TOMM20-encoded protein product is mEGFP labeled. Expected size band for mTagRFP-T-tagged mitochondrial targeting sequence of COXVIII (mitoTag).
Growth rate	ATP quantitation[f]	Comparable to parental line	Pass (measured at p46)[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[g] 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[h]	PCR	Negative when assayed for CMV, EBV, HepB, HepC, HIV1, and HPV	Pass
Identity of unedited parental line[i]	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] Identifier NM_003620.4(PPM1D):c.1426G>T(p.Glu159X)
[f] Promega CellTiter-Glo Luminescent Cell Viability Assay (Catalog #G7571)
[g] STEMCELL Technologies STEMdiff Trilineage Differentiation Kit (Catalog #05230)
[h] 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.
[i] 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 mTagRFP-T localized to the mitochondrial matrix via COXVIII (mitoTag) at the safe harbor locus (AAVS1) as shown below. A population was selected, and mEGFP was introduced at the C-terminus of TOMM20 as shown below to make a dual tag line.

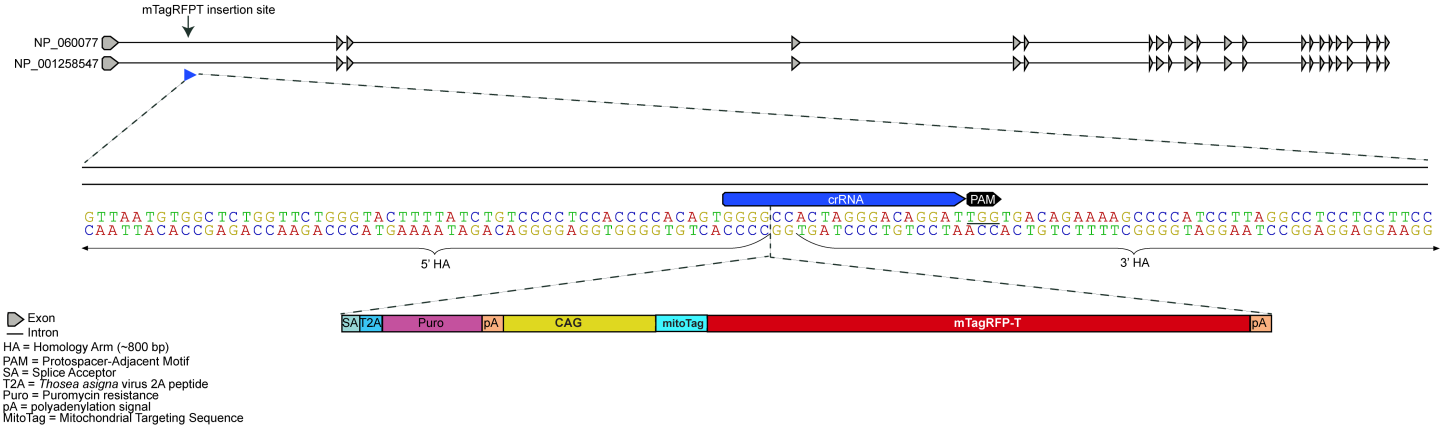

Figure 1: Top: mTagRFPT insertion site at safe harbor locus (AAVS1) in PPP1R12C intron; Bottom: Zoom in on mTagRFPT insertion site at safe harbor locus (AAVS1); insertion into AAVS1 is based on Hockmeyer et al (2011) Nat. Biotechnology, 29(8): 731-734

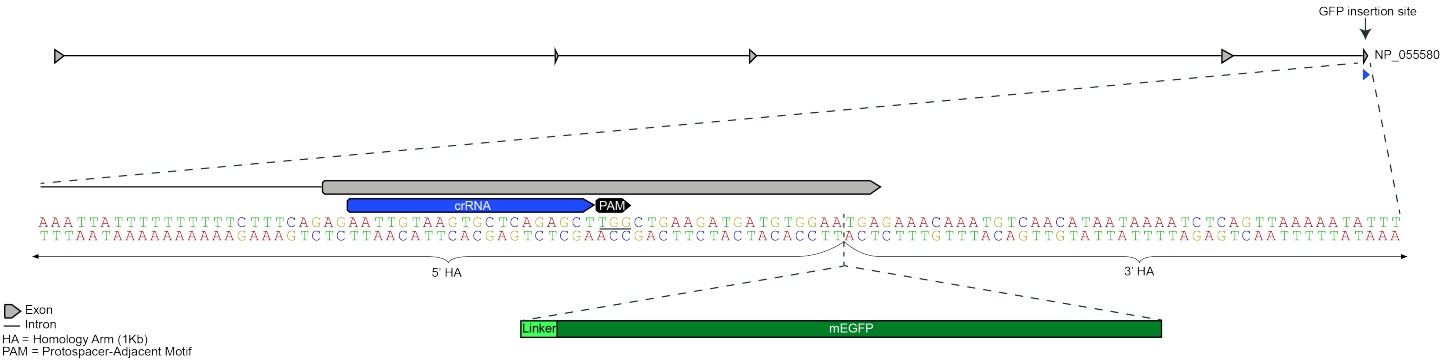

Figure 2: Top: TOMM20 locus; Bottom: Zoom in on mEGFP insertion site at TOMM20 C-terminal exon.

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Post-thaw imaging: One vial of distribution lot was thawed (cells were treated with ROCK inhibitor for 24hrs post-thaw). Cultures were observed daily. Colonies were imaged one and three days post-thaw[1,2] using a Leica microscope.

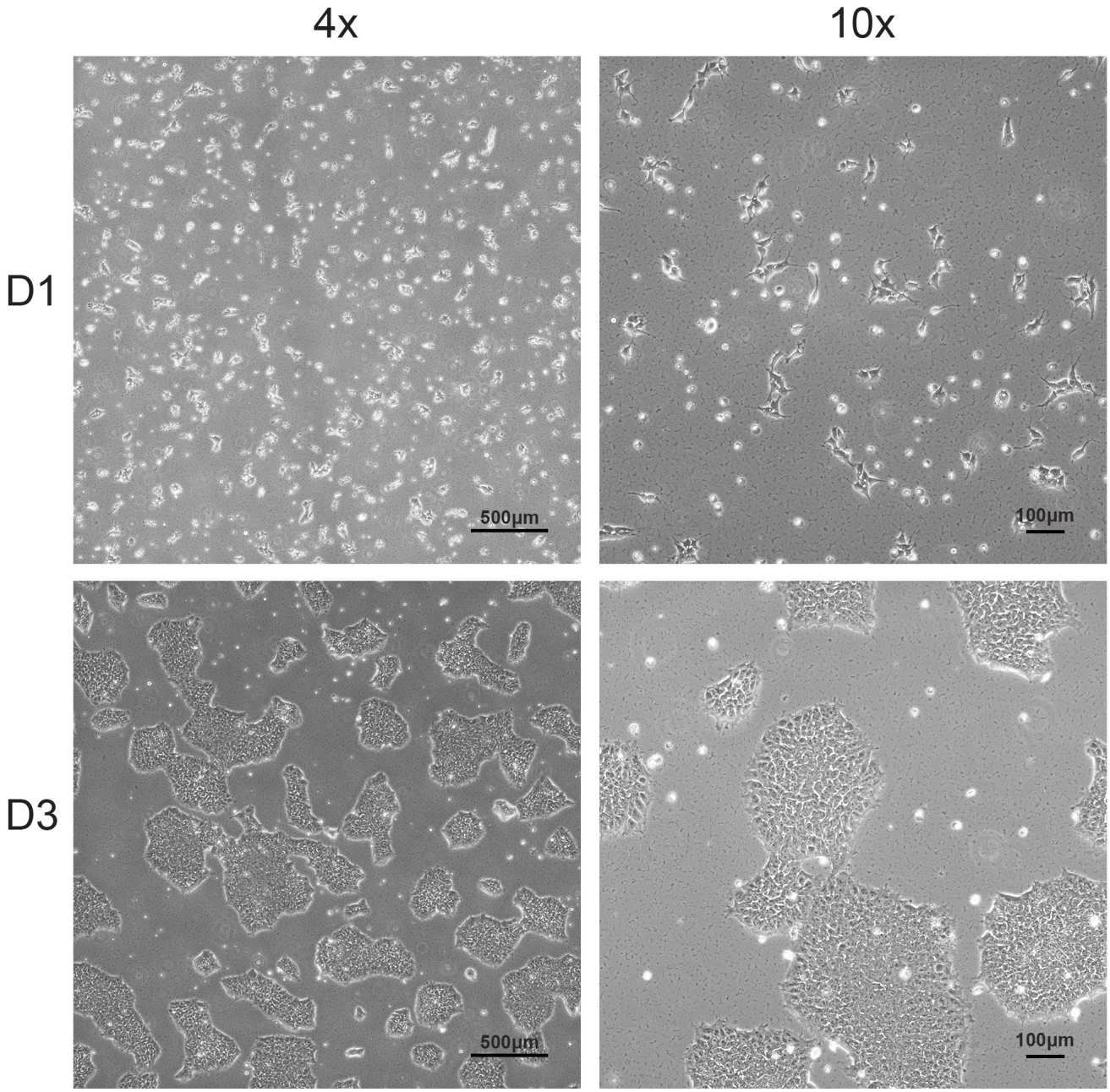

Figure 3: Viability and colony formation one day and three days post-thaw.

[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

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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 mTeSR 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) and 561 laser (mTagRFP-T). 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.

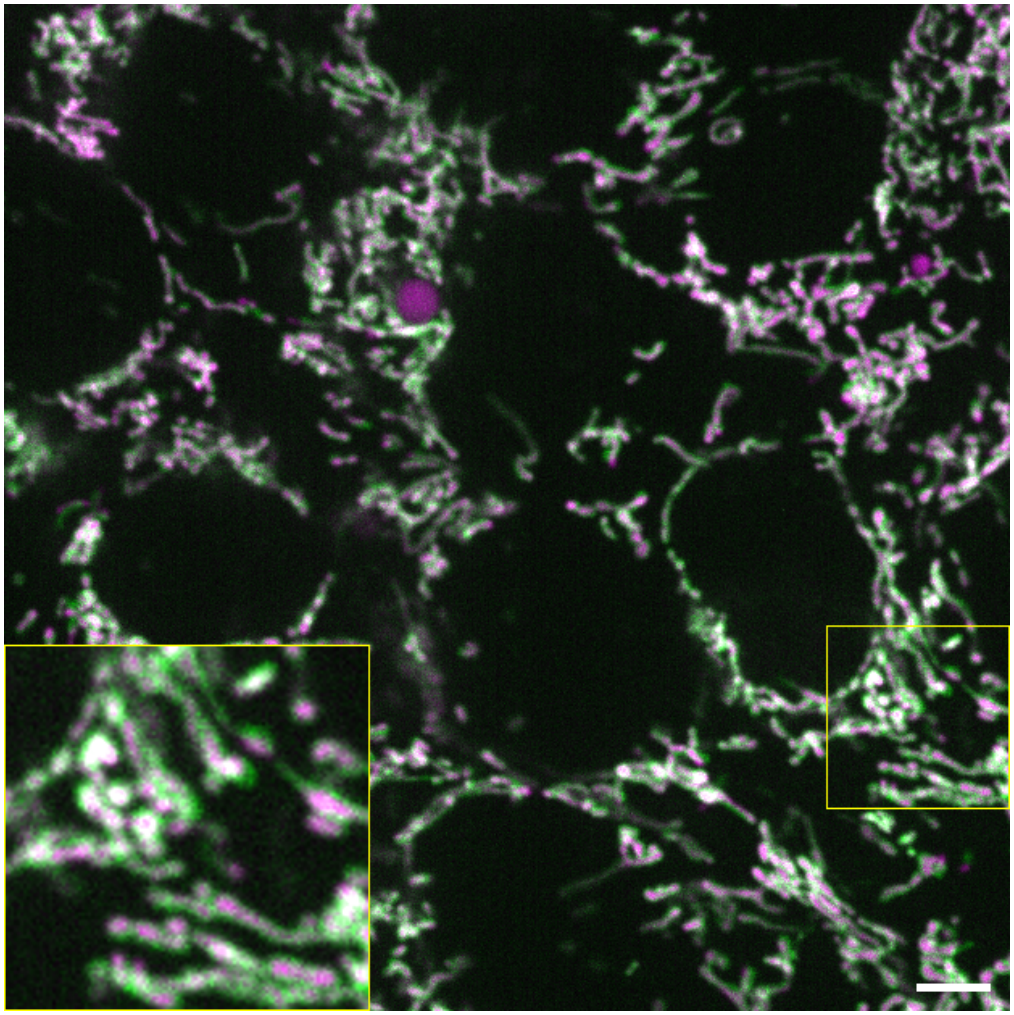

Figure 4: hiPS cells expressing mEGFP-tagged outer mitochondrial membrane receptor Tom20 (TOMM20) and mTagRFP-T-tagged mitochondrial targeting sequence of COXVIII (mitoTag). Image is a single slice near the bottom of the cell (scalebar, 5 μm). Inset image is 2x enlargement of boxed area to show detailed relationship between tagged proteins. Cells were imaged live in 3D on a spinning-disk confocal microscope.