CERTIFICATE OF ANALYSIS

AICS-0086-147:WTC FBL-mEGFP / NPM1-mTagRFP-T / UBTF-HaloTag-cl147 (mono-allelic tags)

Product description	Human iPSC clonal line in which FBL, NPM1, and UBTF have been endogenously tagged with mEGFP, mTagRFP-T, and HaloTag, respectively, 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	p65[a]
Number of passages at Coriell	0
Media	mTeSR1
Feeder or matrix substrate	Matrigel
Passage method	Accutase
Thaw	500K cells (ea vial) in 10 cm plate - ready for passaging in 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; Flattened, loosely packing cells at colony edges observed at d4 post-thaw. Morphology improves after 4 passages with small clump passaging using Versene (see Fig. 4)
mEGFP / mTagRFP-T / HaloTag 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. C-term insertion of mTagRFP-T in frame with exact predicted recombinant allele junctions. N-term insertion of HaloTag in frame with exact predicted recombinant allele junctions. No additional mutations.	FBL-mEGFP: Pass; Acquired K157M mutation in mEGFP that does not affect fluorescence NPM1-mTagRFP-T: Pass UBTF-HaloTag: Pass
Copy number	ddPCR[c] assay for FP(s) and RPP30 reference gene[d]	FP/RPP30: ~ 0.5 = Mono-allelic ~ 1.0 = Bi-allelic	FBL-mEGFP: Mono-allelic (0.521) NPM1-mTagRFP-T: Mono-allelic (0.492) UBTF-HaloTag: Mono-allelic (0.486)
Plasmid integration	ddPCR assay to detect plasmid integration into the genome	AmpR/RPP30: < 0.1 = no plasmid integration	Pass (0.000)

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Mutational analysis	Whole exome sequencing[f]	Check for acquired mutations (not detected in p8[a] parental line) that: 1) Correspond to off-target sites predicted by Cas-OFFinder[e] 2) Affect genes in Cosmic Cancer Gene Census	Sequencing planned
mEGFP, mTagRFP-T, and HaloTag localization	Spinning Disk confocal live cell imaging	Localization of mEGFP to the dense fibrillar component of the nucleolus, localization of mTagRFP-T to the granular component of the nucleolus, and localization of HaloTag to the fibrillar center of the nucleolus.	mTagRFP-T-tagged nucleophosmin is visible as textured shells surrounding spheres of mEGFP-tagged fibrillarin. HaloTag-tagged nucleolar transcription factor UBF is found in smaller spheres within mEGFP-tagged fibrillarin. This pattern is consistent with the localization of the tags to the nucleolar granular component, nucleolar dense fibrillar component, and fibrillar center respectively for nucleophosmin, fibrillarin, and nucleolar transcription factor UBF. Localization of all three changes throughout the course of cell division.
Expression of tagged protein	Western blot	Expression of expected size product	Expected size bands for untagged and mEGFP-tagged fibrillarin, untagged and mTagRFP-T-tagged nucleophosmin, and untagged and HaloTag-tagged nucleolar transcription factor UBF. Semi-quantitative results show that 28% of FBL-encoded protein product is mEGFP labeled, 29% of NPM1-encoded protein product is mTagRFP-T labeled, and 49% of nucleolar transcription factor UBF is HaloTag labeled.
Growth rate	ATP quantitation[g]	Comparable to parental line	Pass (measured at p60)[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[h] 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
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

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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 FBL. An FBL clone (AICS-0014 cl.6) was selected, and mTagRFP-T was introduced at C-terminus of NPM1 to make a dual tag line (AICS-0084 cl.18). This dual tagged line was then used as the starting point to introduce HaloTag at N-terminus of UBTF as shown below to make a triple tagged line.

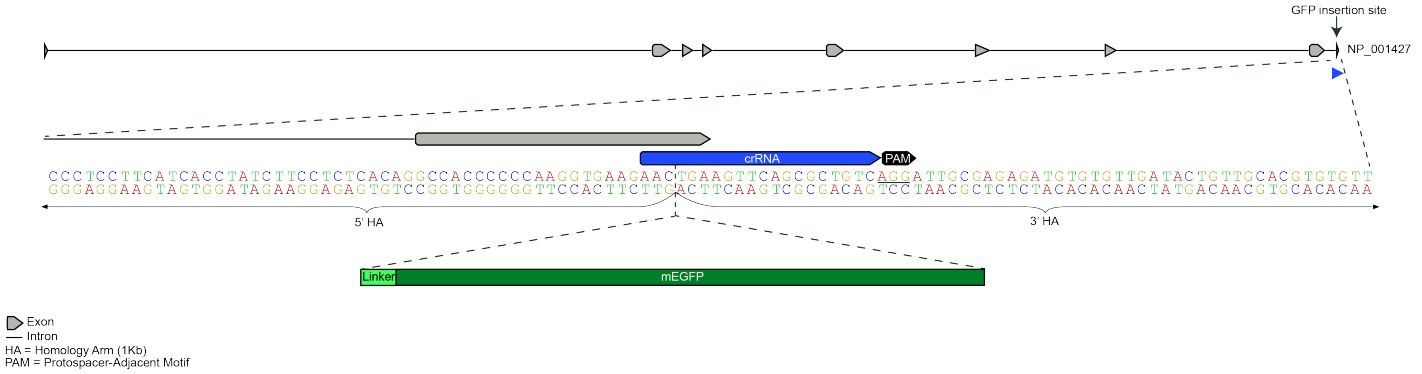

Figure 1: Top: FBL locus; Bottom: Zoom in on mEGFP insertion site at FBL C-terminal exon

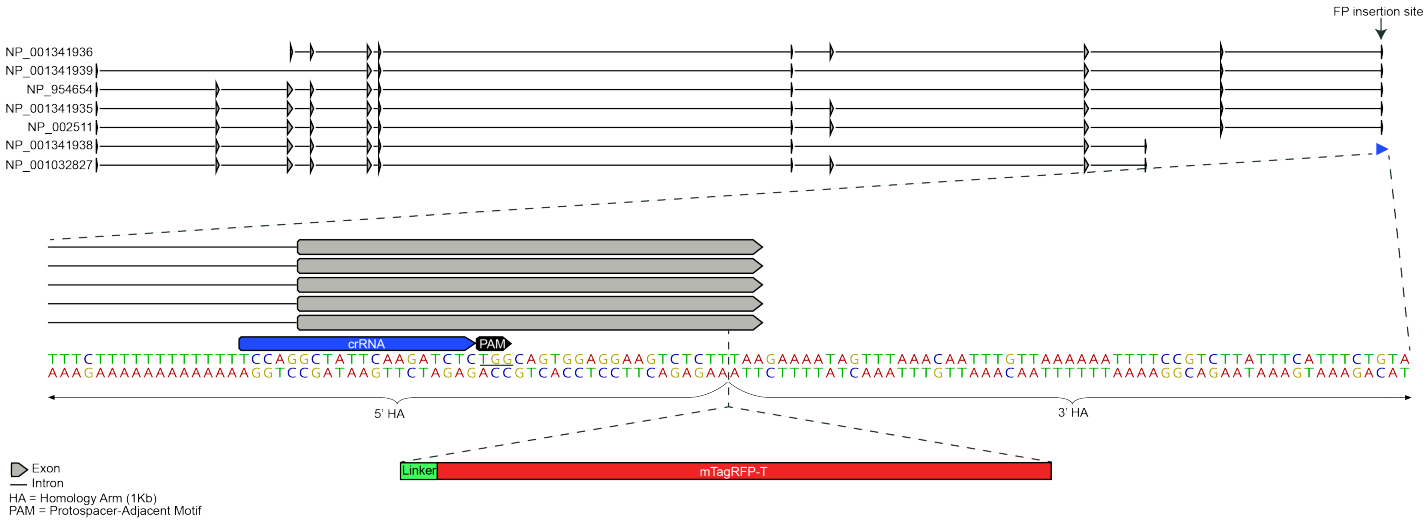

Figure 2: Top: NPM1 locus showing 7 NPM1 isoforms; Bottom: Zoom in on mTagRFP-T insertion site at NPM1 C-terminal exon

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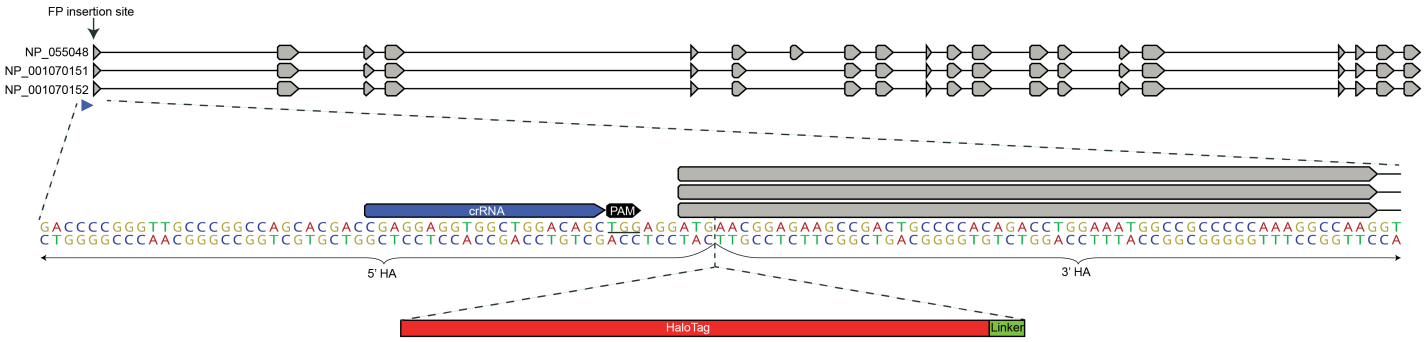

Figure 3: Top: UBTF locus showing 3 UBTF isoforms; Bottom: Zoom in on HaloTag insertion site at UBTF N-terminal exon

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Post-thaw imaging: One vial of the distribution lot was thawed (cells were treated with ROCK inhibitor for 24hrs post-thaw - refer to culture protocol). Cultures were observed daily and imaged at 4x and 10x magnifications as indicated below[1,2] using a Leica microscope.

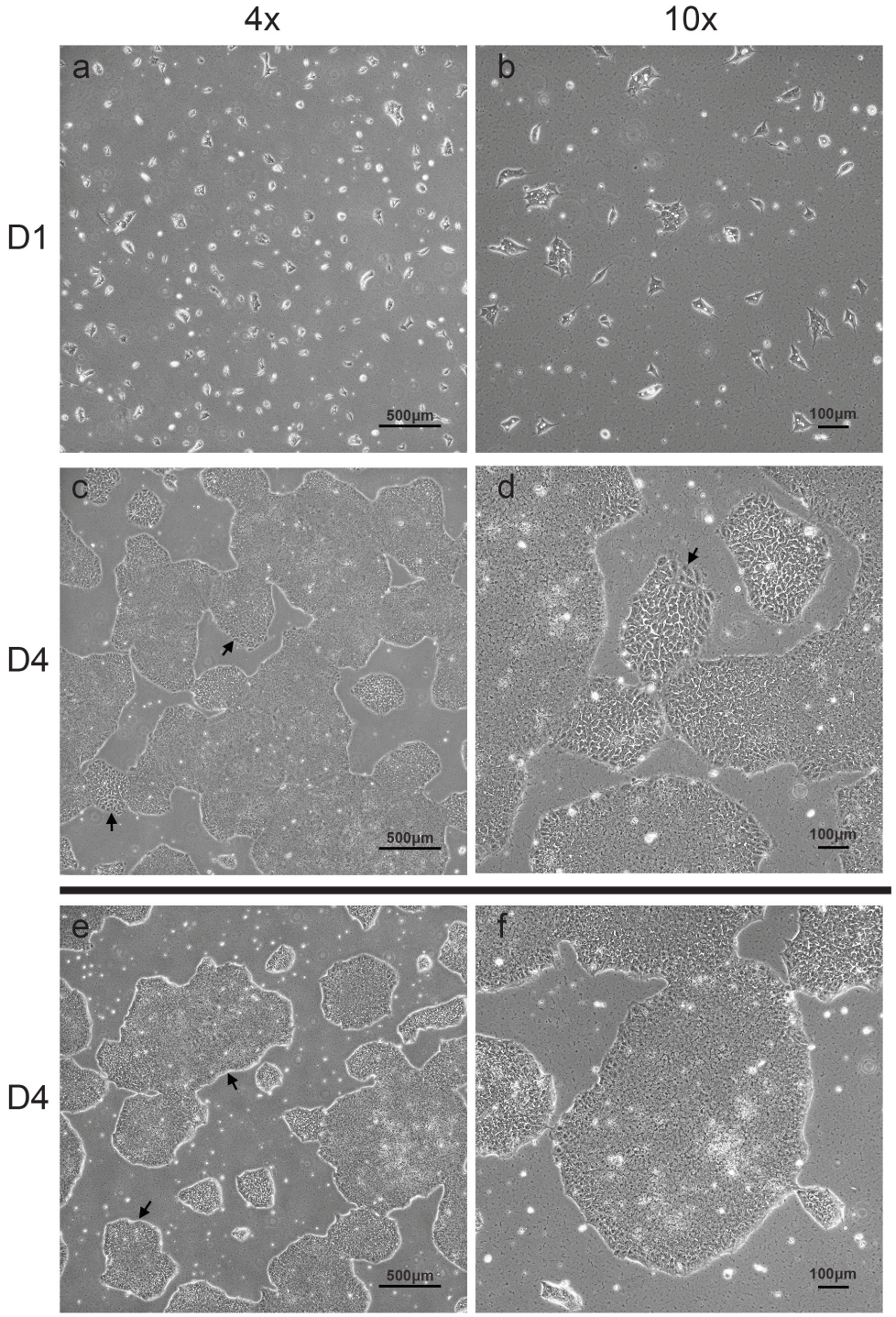

Figure 4: Viability and colony formation one day (a,b) and four days post-thaw (c,d). Flattened, loosely packed cells around colony edges were observed (Fig. 4c, d; see arrows) in many mature stem cell colonies on day 4 post thaw, which is suboptimal. Small clump passaging with Versene improved the colony morphology after 4 passages (Fig. 4e, f; see arrows).

[1] Cells may take up to 4 passages to recover after thaw using small clump passaging with Versene

[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), 561 laser (mTagRFP-T), and 638 laser (HaloTag). 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.

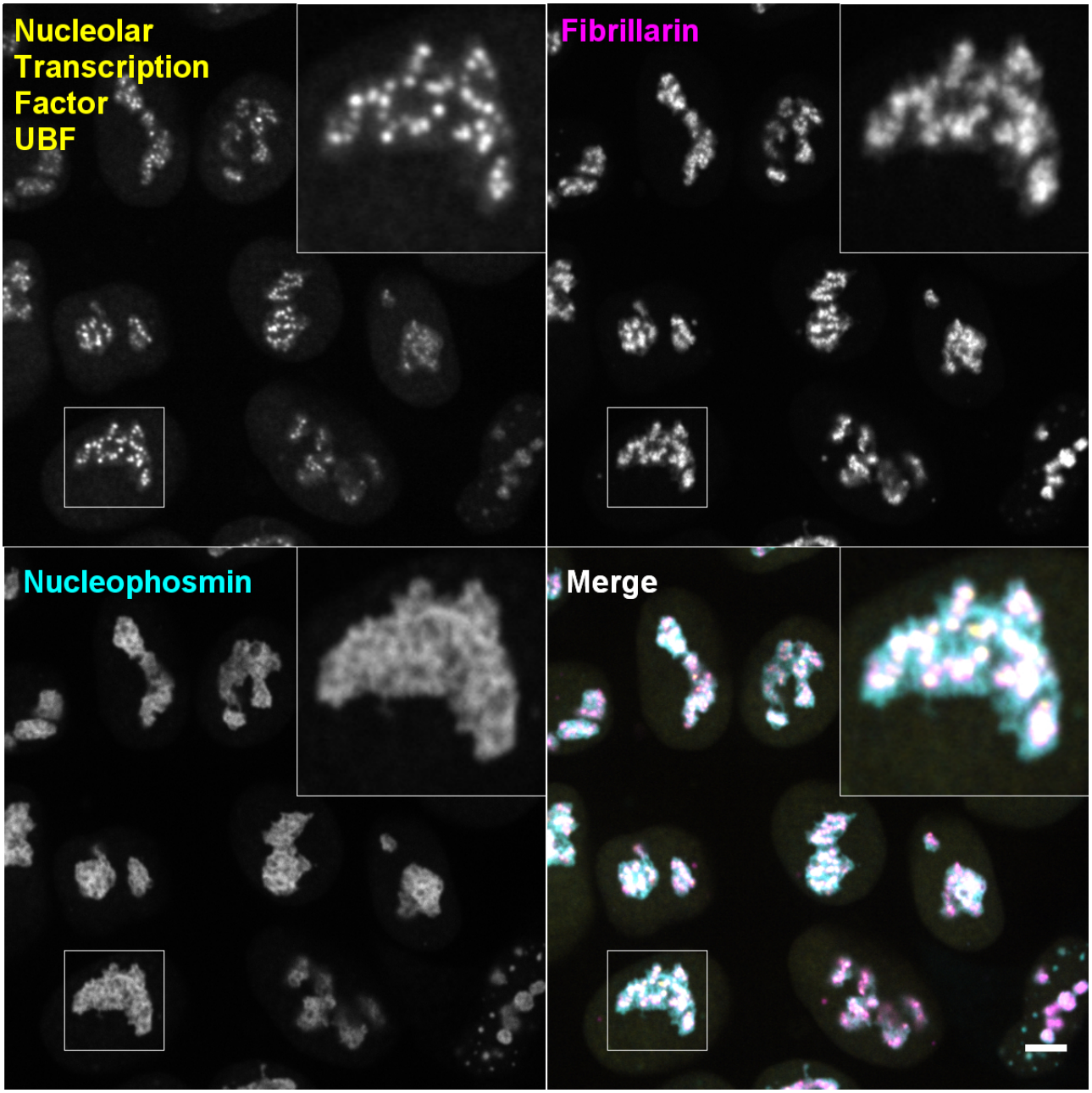

Figure 5: Single, mid-level plane of cells in a live hiPS cell colony expressing mEGFP-tagged fibrillarin, mTagRFP-T-tagged nucleophosmin, and HaloTag-tagged nucleolar transcription factor UBF visualized with ligand Janelia Fluor 646 (Promega). Panels show individual channels for fibrillarin, nucleolar transcription factor UBF, nucleophosmin, and an overlay of the three (counterclockwise from top right). Cells were imaged in 3D on a spinning-disk confocal microscope. Scale bar, 5 μm.