Creating a Cardiomyocyte Pipeline for Gene Edited Human iPSCs

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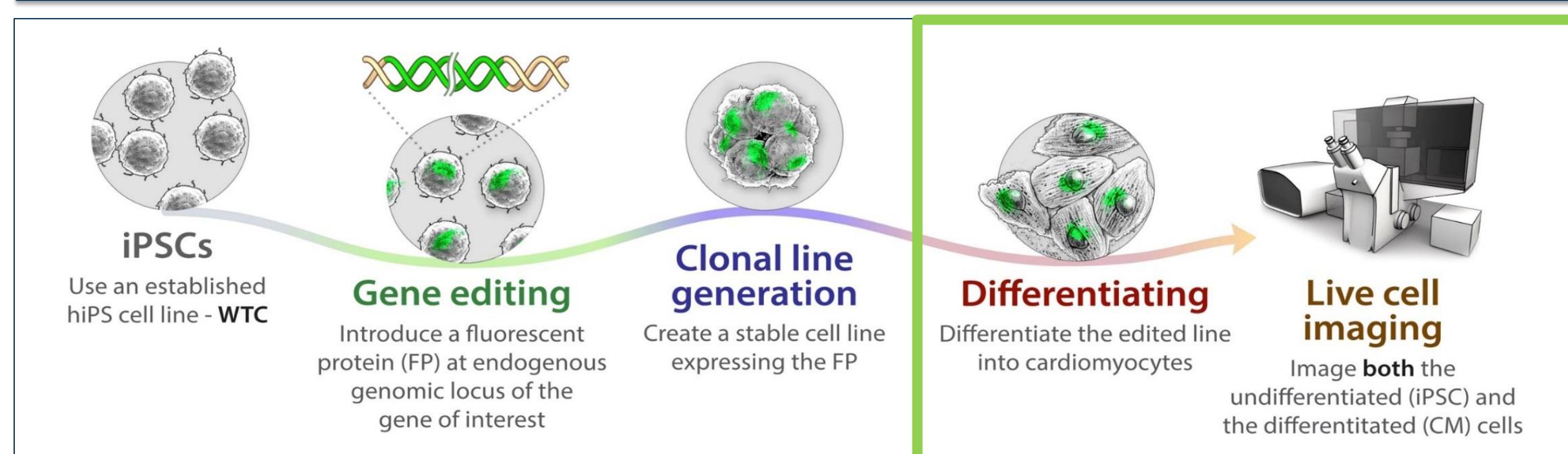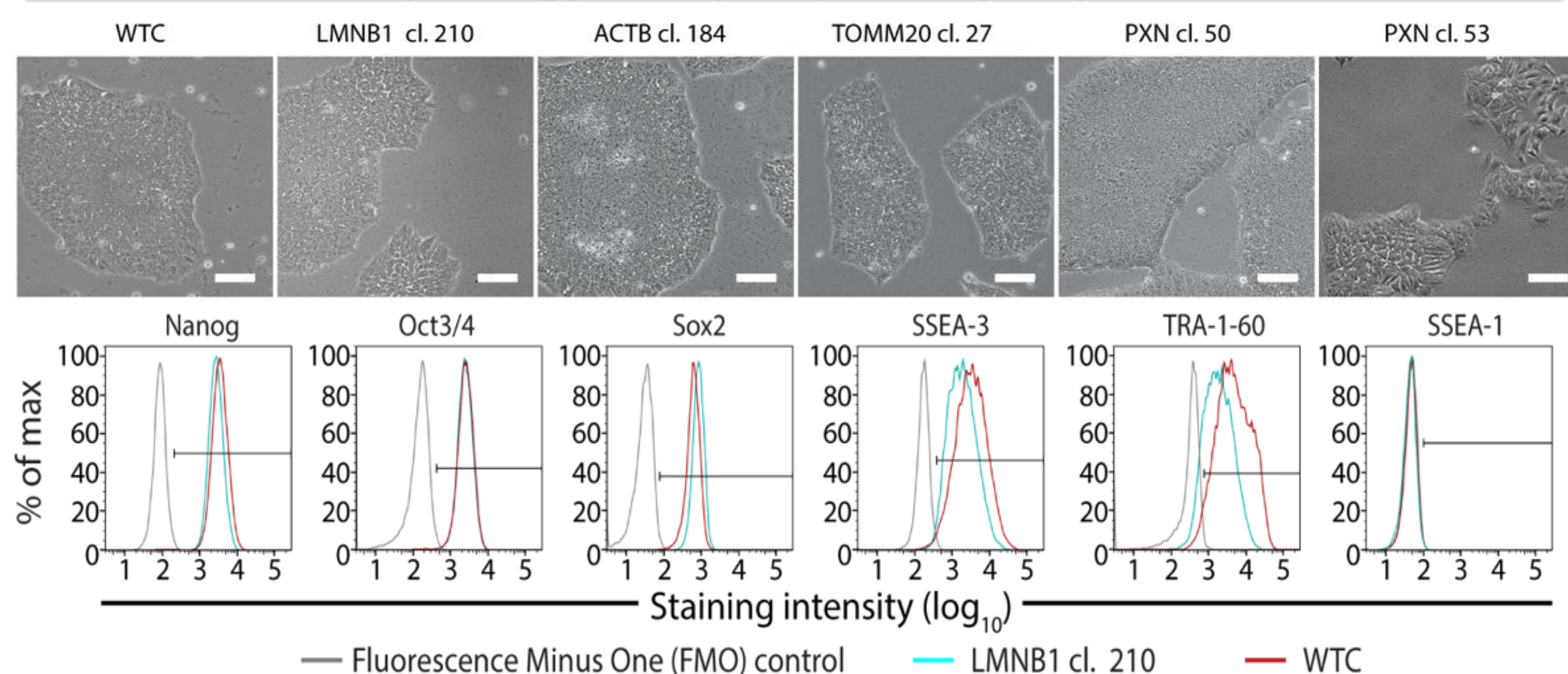
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Introduction

The Allen Institute for Cell Science (AICS) is creating an open source collection of fluorescently tagged human iPSC lines to model cell organization and dynamics of stem cells both in the undifferentiated and differentiated states. Using the WTC human iPSC line and the CRISPR/Cas9 system we have fluorescently tagged 15 target genes representing key cellular organelles, which will be used to characterize the changes in subcellular localization and organization as the stem cell differentiates into cardiomyocytes using live cell fluorescent imaging. Here we present our cardiomyocyte differentiation methods and the quantitative and qualitative assays employed to determine differentiation efficiency including cardiomyocyte protein expression by flow cytometry, immunofluorescent and live cell imaging, and supporting transcriptome profiling by RNAseq We are currently developing optimized methodologies for scalable and efficient production of cardiomyocytes to support our live cell imaging pipeline. We hope that gaining a better understanding of the organization and activities of cardiomyocytes will lead to advances in the development of better disease models, therapies, and regenerative medicine approaches.

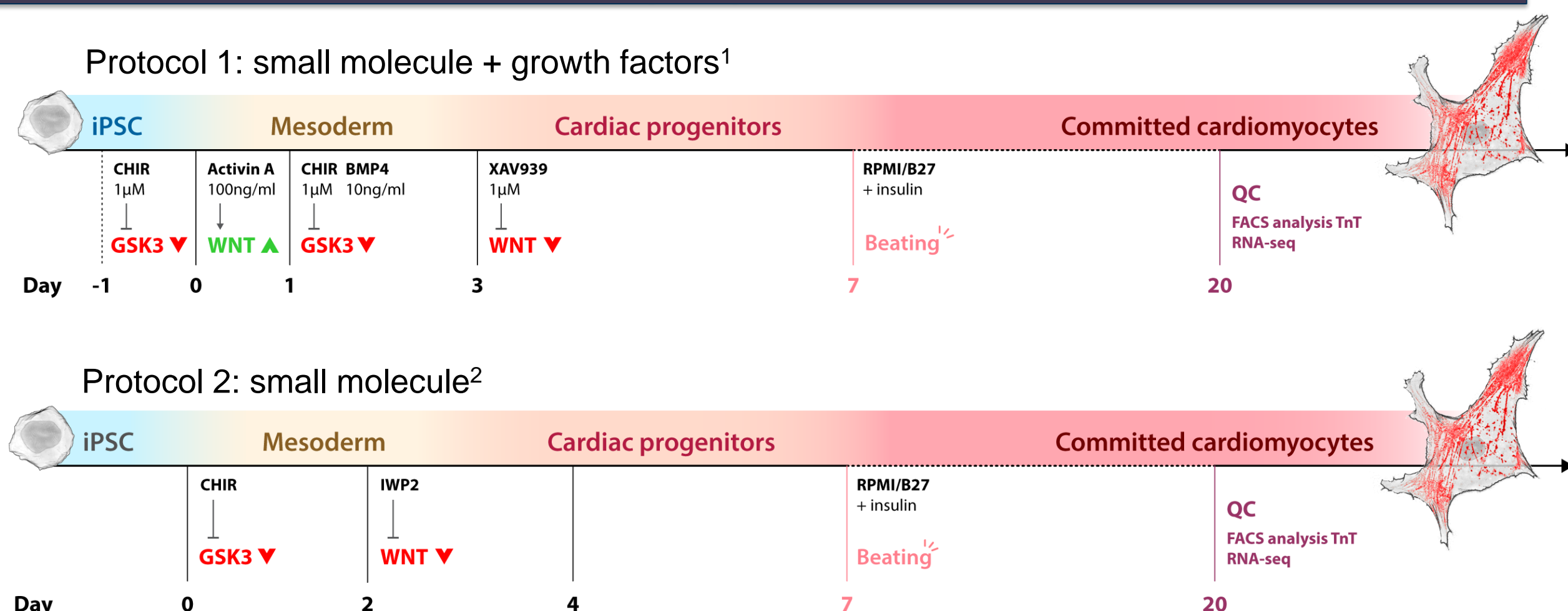## Allen Institute for Cell Science Workflow

iPSCs — Use an established hiPS cell line - WTC

Gene editing — Introduce a fluorescent protein (FP) at endogenous genomic locus of the gene of interest

Clonal line generation — Create a stable cell line expressing the FP

Differentiating — Differentiate the edited line into cardiomyocytes

Live cell imaging — Image **both** the undifferentiated (iPSC) and the differentitated (CM) cells

Quality Control of gene edited clones for healthy colony morphology and pluripotency expression

WTC, LMNB1 cl. 210, ACTB cl. 184, TOMM20 cl. 27, PXN cl. 50, PXN cl. 53

Nanog, Oct3/4, Sox2, SSEA-3, TRA-1-60, SSEA-1

% of max vs Staining intensity (log₁₀)

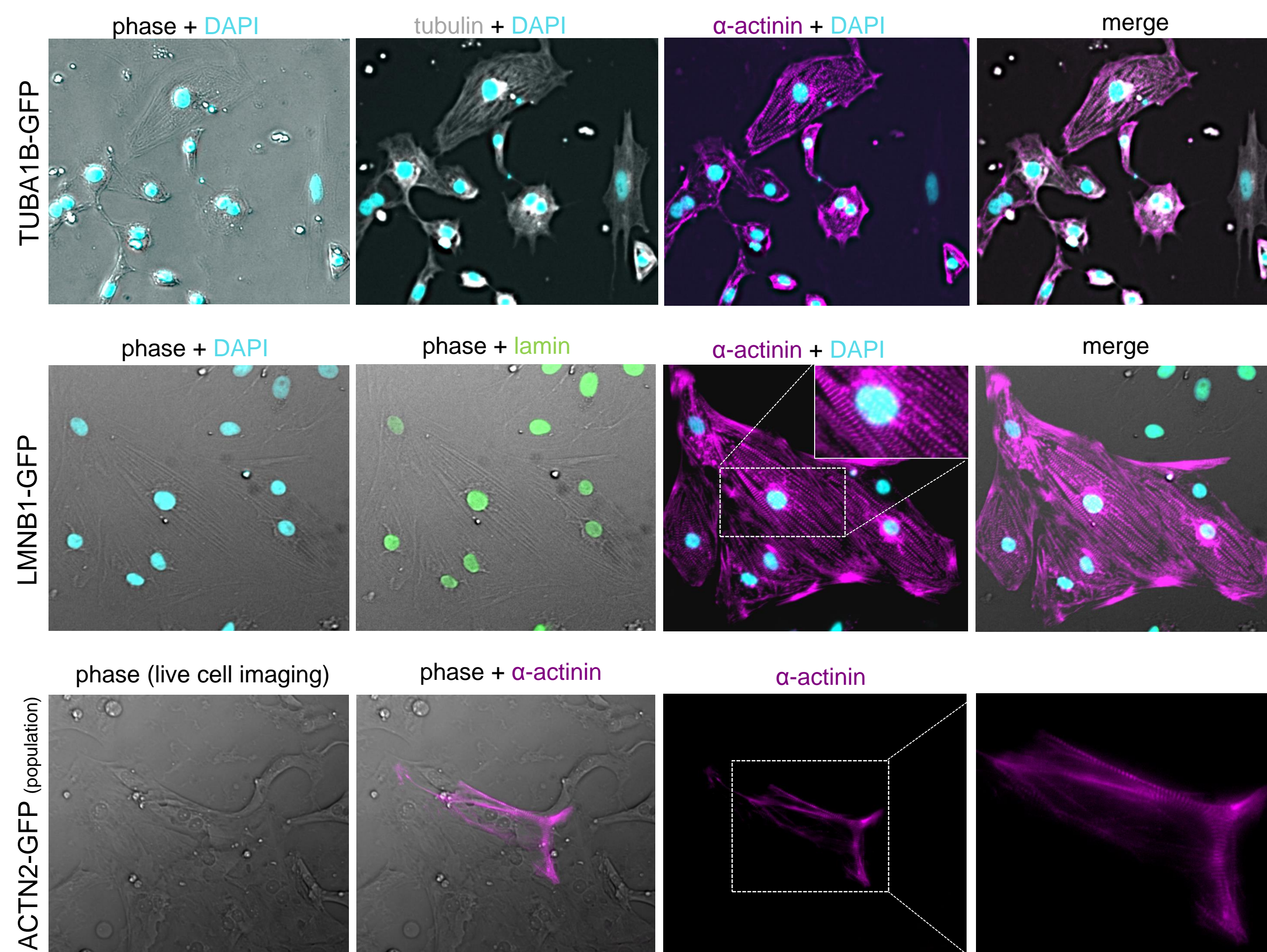— Fluorescence Minus One (FMO) control — LMNB1 cl. 210 — WTC

Edited cell lines undergo an extensive QC process, including morphological and pluripotency analysis.

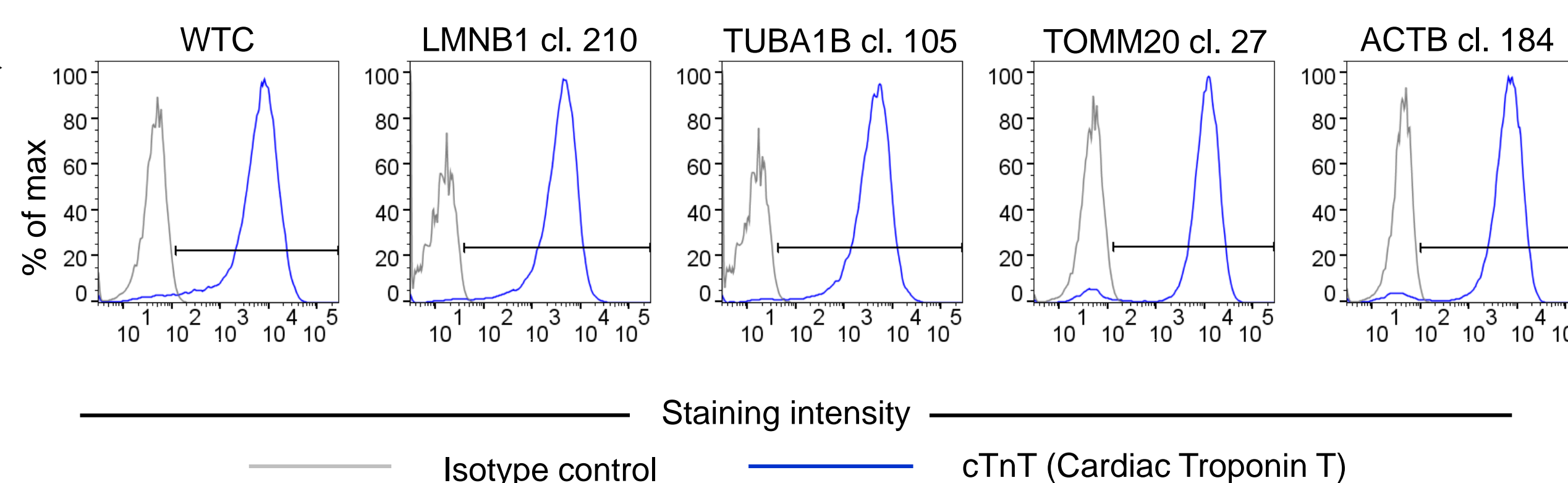## Evaluation of differentiation protocols for robust cardiomyocyte production

Protocol 1: small molecule + growth factors[1]

iPSC — Mesoderm — Cardiac progenitors — Committed cardiomyocytes

CHIR 1µM / GSK3▼ ; Activin A 100ng/ml / WNT▲ ; CHIR BMP4 1µM 10ng/ml / GSK3▼ ; XAV939 1µM / WNT▼ ; RPMI/B27 + insulin / Beating ; QC FACS analysis TnT RNA-seq

Day -1 0 1 3 7 20

Protocol 2: small molecule[2]

iPSC — Mesoderm — Cardiac progenitors — Committed cardiomyocytes

CHIR / GSK3▼ ; IWP2 / WNT▼ ; RPMI/B27 + insulin / Beating ; QC FACS analysis TnT RNA-seq

Day 0 2 4 7 20

Establishing a cardiomyocyte imaging pipeline

TUBA1B-GFP: phase + DAPI | tubulin + DAPI | α-actinin + DAPI | merge

LMNB1-GFP: phase + DAPI | phase + lamin | α-actinin + DAPI | merge

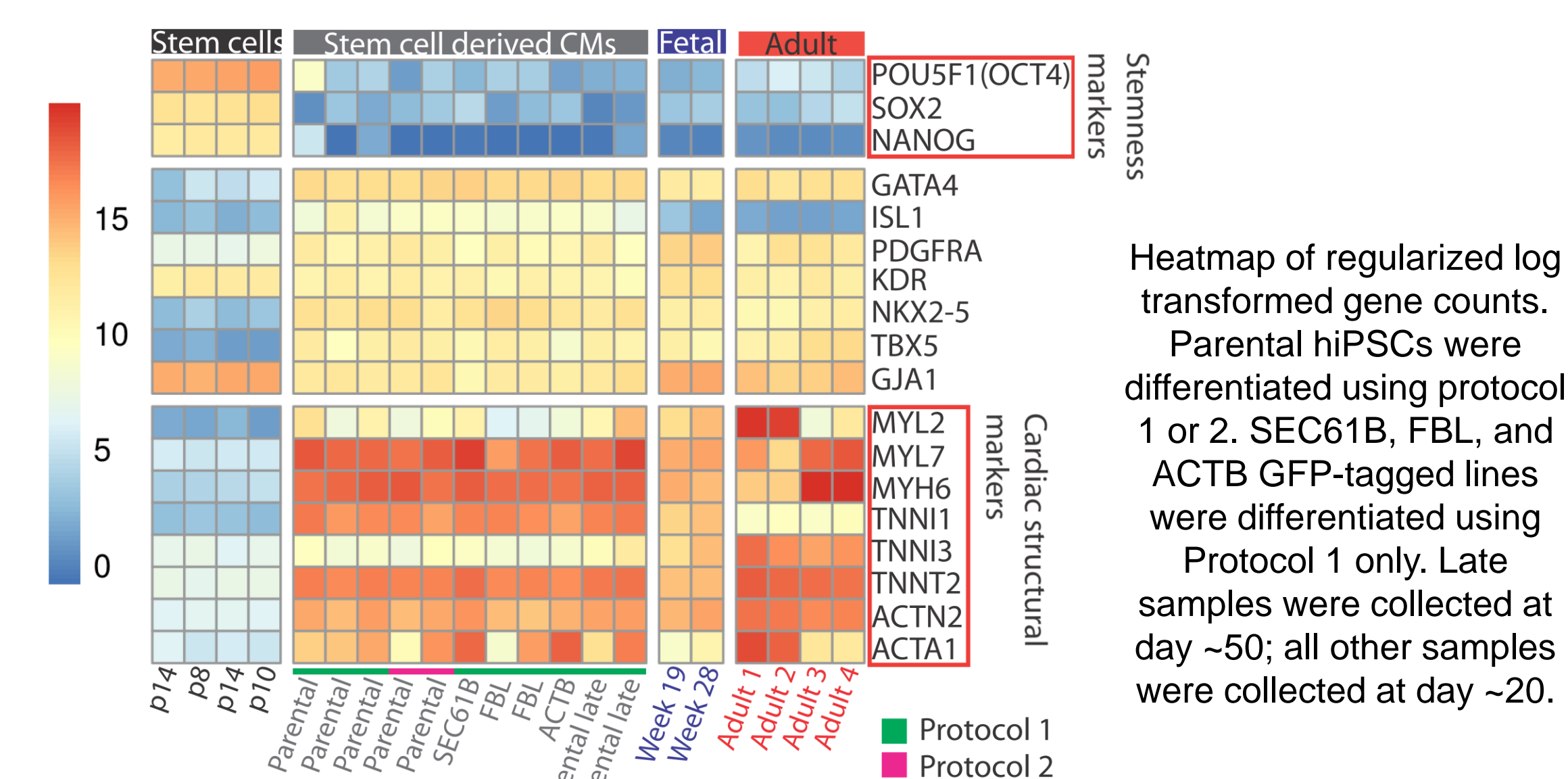ACTN2-GFP (population): phase (live cell imaging) | phase + α-actinin | α-actinin

In the top and middle panels, edited structures are identified by GFP and cardiomyocytes are labeled with α-actinin. The bottom panel represents live cell cardiomyocyte images from the ACTN2-GFP tagged cell line.

Summary of cardiac differentiation experiments

Protein (Gene)	Independent experiments (n)	% cardiac Troponin T	% Experiments with Beating	Initiation of Beating
Unedited	41	70-98	93	d6-d15
Paxillin (PXN)	16	66-98	75	d6-d17
Alpha tubulin (TUBA1B)	12	87-96	75	d6-d20
Nuclear lamin B1 (LMNB1)	10	39-97	80	d7-d12
Tom20 (TOMM20)	12	80-95	75	d7-d13
Desmoplakin (DSP)	10	92-95	50	d7-d15
Beta actin (ACTB)	6	72-96	100	d7-d8
Sec61 beta (SEC61B)	5	91-97	80	d7-d8
Fibrillarin (FBL)	5	64-96	75	d7-d9
Myosin heavy chain IIB (MYH10)	4	75-99	100	d7-d16
Tight junction protein ZO1 (TJP1)	6	75-93	100	d7-d17
Sarcomeric actinin (ACTN2)	7	89-96	100	d7-d8
Troponin I (TNNI1)	7	90	100	d7-d10

WTC | LMNB1 cl. 210 | TUBA1B cl. 105 | TOMM20 cl. 27 | ACTB cl. 184

% of max vs Staining intensity

— Isotype control — cTnT (Cardiac Troponin T)

Establishing a baseline transcriptional profile of WTC-CMs

Stemness markers: POU5F1(OCT4), SOX2, NANOG

Cardiac structural markers: GATA4, ISL1, PDGFRA, KDR, NKX2-5, TBX5, GJA1, MYL2, MYL7, MYH6, TNNI1, TNNI3, TNNT2, ACTN2, ACTA1
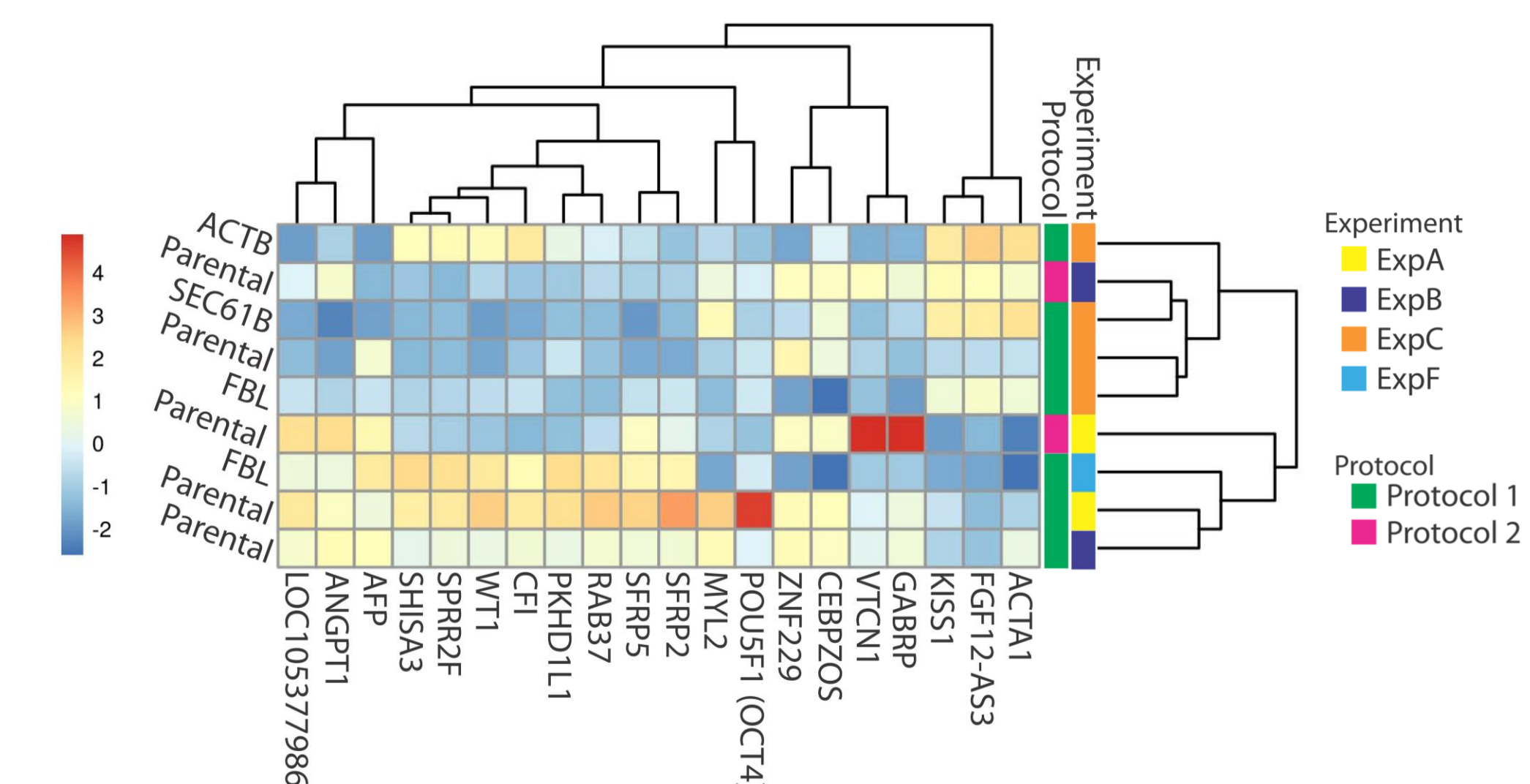
Protocol 1 / Protocol 2

Heatmap of regularized log transformed gene counts. Parental hiPSCs were differentiated using protocol 1 or 2. SEC61B, FBL, and ACTB GFP-tagged lines were differentiated using Protocol 1 only. Late samples were collected at day ~50; all other samples were collected at day ~20.

Integrating transcriptome sequencing into cardiomyocyte QC

PC1: 39% variance vs PC2: 30% variance

Cell Line (protocol): ACTB(1), Parental late CM (1), FBL (1), Parental (2), Parental (1), SEC61B (1)

Experiment: ExpA, ExpB, ExpC, ExpD, ExpE, ExpF
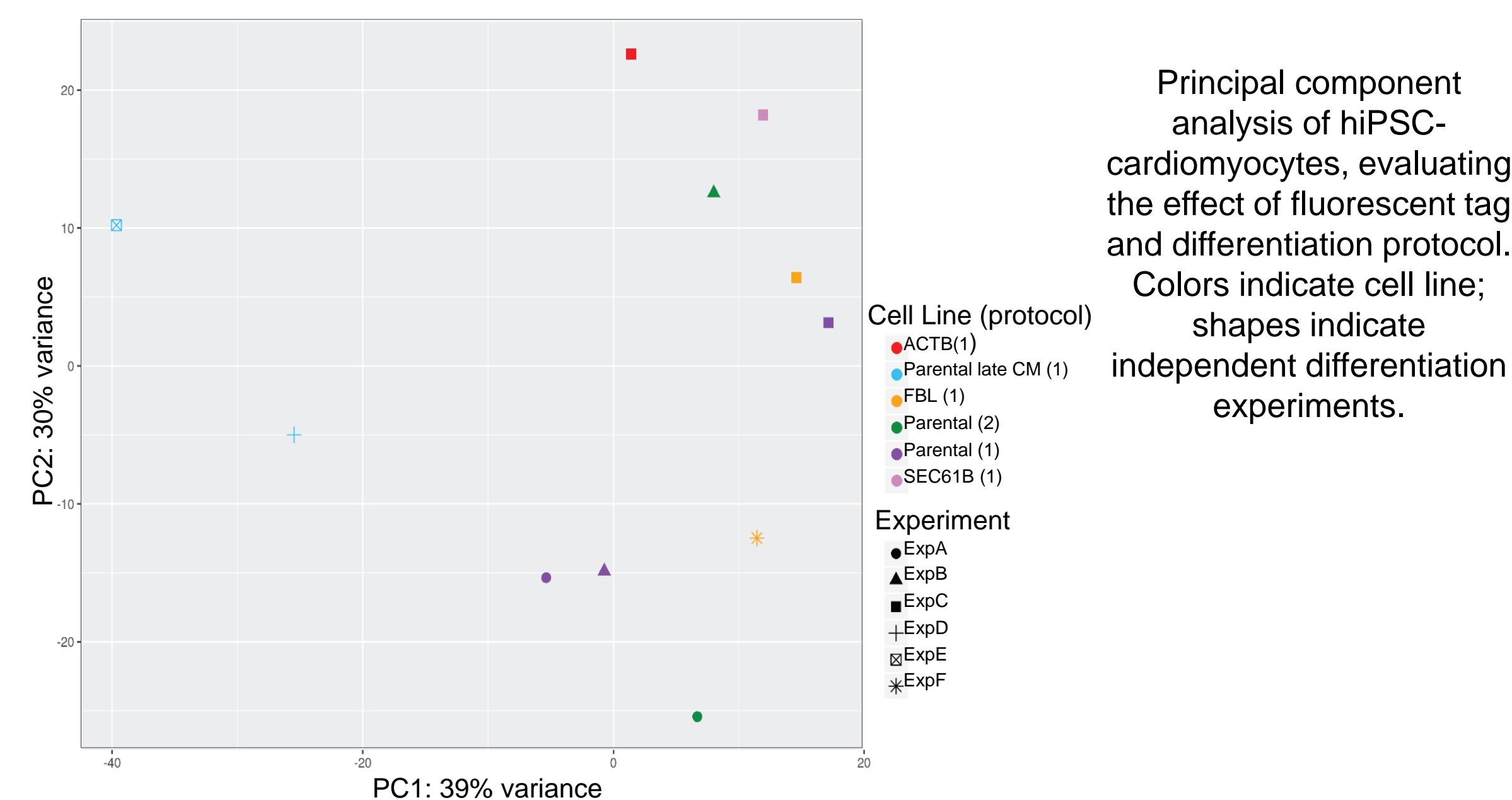
Principal component analysis of hiPSC-cardiomyocytes, evaluating the effect of fluorescent tag and differentiation protocol. Colors indicate cell line; shapes indicate independent differentiation experiments.

Experiment: ExpA, ExpB, ExpC, ExpF
Protocol: Protocol 1, Protocol 2

Hierarchical clustering of 20 most variable genes among parental CMs (Protocol 1 & 2; day ~20) and edited CMs (Protocol 1; day ~20). Heatmap shows regularized log transformed gene counts scaled by column.

Acknowledgements

References
1. Palpant et al. Dev 2015. doi:10.1242/dev.117010
2. Truong A, So P-L. 2015. https://labs.gladstone.org/conklin/files/ips-cm_differentiatiion_protocol_v1.4-06-17-2015.pdf