**Supporting Figure 1. Control human iPSC characterization and validation**

A) Control hiPSCs express stem cell markers. Control hiPSC were immunostained for OCT4, SSEA4, SSEA3, Tra-1-60 and Dapi. Scale bar is 50μm. B) Control hiPSC colony stained positive for alkaline phosphatase. Scale bar is 50μm. C) Chromosomal assessment of control hiPSCs. D) Pluripotency validation. HiPSCs were differentiated into embryoid bodies for 2 weeks. Embryoid bodies were immunostained for alpha smooth muscle actin (α-SMA), GATA4 and class III beta-tubulin (βIII-tubulin), markers of mesoderm, endoderm, and ectoderm, respectively. Scale bar is 50μm.

**Supporting Figure 2. Titin-mEos3.2 hiPSC pluripotency validation**

Titin-mEos3.2 hiPSCs were immunostained for OCT4, SSEA4, SSEA3, Tra-1-60 and Dapi. Scale bar, 100 μm.

**Supporting Figure 3. Titin-mEos3.2 and myomesin colocalize at the M-band in hiPSC-CMs**

Day 30 titin-mEos3.2 hiPSC-CMs were fixed and immunostained for M-band protein, myomesin. Green, titin-mEos3.2; red, myomesin. A) Whole cell 2-D STORM images of titin-mEos3.2 (left panel), myomesin (middle panel), and merged images (right panel). Scale bar is 10 μm. B) 3-D STORM projections of regions of interest (ROIs corresponding to the boxed areas in the right panel of A) spanning two neighboring sarcomeric M bands.

**Supporting Figure 4. CHX reduces global protein synthesis**

Day 30 hiPSC-CMs were pretreated with vehicle or CHX (10 μg/mL) for 30 min in culture. Cells were then treated with a metabolic probe O-propargyl-puromycin (OPP), OPP+ CHX or no OPP for 1 hour in culture. Cells were then fixed and treated with 5-FAM-Azide for OPP probe detection.  Fluorescence was detected (excitation/emission, 485nm/535nm) via Promega Glomax reader. Fluorescence intensity was normalized to no OPP to account for background. Data presented as MEAN ± SEM, \*, p< 0.05 vs. control, groups compared by ANOVA (n=3-4).

**Supporting Figure 5. Fixed titin-eGFP expressing cardiomyocytes does not display reversible photobleaching.**

A) Representative FRAP images (pre-bleach, bleach, and recovery) of mouse neonatal cardiomyocytes expressing titin-eGFP. Scale bar is 5 μm. B) Quantification of FRAP images. FRAP images were acquired every 30 min for 9 hours. Pixel intensities of the ROI, cell and background were acquired. Background was subtracted from ROI and whole cell. Images were normalized to pre-bleach intensities. Data was fitted into a one-phase association equation to extrapolate a reversible photoswitching rate (n=1). C) Mobile fraction of fixed titin-eGFP expressing cardiomyocyte.