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Supporting Material

Non-invasive detection and imaging of molecular markers in live cardiomyocytes derived from human embryonic stem-cells

Flavius C Pascut, Huey T Goh, Nathan Welch, Lee D Buttery, Chris Denning, and Ioan Notingher

Culture and differentiation of hESCs

hESCs were routinely passaged by incubating with 0.05% trypsin for 1 minute at 37°C and then tapping the flasks to liberate single cells or preferably small clumps of cells. To prepare the conditioning medium, ouse embryo fibroblasts (MEFs) strain CD1, 13.5 days post coitum were mitotically inactivated with mitomycin C (MMC) (10 µg/ml, 2.5 hours) and seeded at 6 x 10^4 cells per cm^2. The next day, inactivated MEFs in a T75 flask were incubated with 25 ml of Dulbecco's modified Eagle's medium- Ham's F-12 medium (DMEM-F12) supplemented with 15% KnockOut Serum Replacement, 100 µM β-mercaptoethanol (β-ME), 1% nonessential amino acids (NEAA), 2 mM GlutaMAX, and 4 ng/ml bFGF for 24 hours, at which time conditioning medium was harvested and made ready for use by supplementing with an additional 4 ng/ml bFGF. Each flask of inactivated MEFs was used to condition the medium for 7 consecutive days .

Embryoid body (EB) formation was performed by forced aggregation of defined numbers of hESCs. On day 0 of differentiation, EB formation was initiated by seeding V-96 plates with 3,000 cells in conditioned medium and centrifuging at 950g (~2,800 rpm) for 5 min at room temperature. EBs were maintained in V-96 plates for 4 days in conditioned medium. EBs were then transferred to an untreated 90mm dish in D-FBS (DMEM supplemented with 20% fetal bovine serum, 100 µM β-ME, 1% NEAA, and 2mM GlutaMAX) where they were maintained in suspension for 6 days to allow further differentiation before transfer to untreated U-96 well plates (one EB/well) in D-FBS.

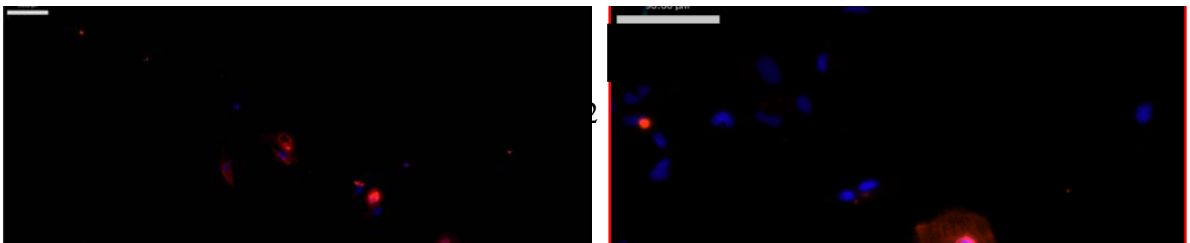

Fig. S1: low magnification immuo-fluorescence image of heterogeneous cell populations as derived from differentiated hESCs. Cells were stained for cell nuclei (DAPI, purple) and cardiac phenotype (α-actinin, red).