Supplemental Material

Detailed Methods

Cell culture

Mouse CPCs are isolated, maintained and lenti virally engineered to stably over express either eGFP alone (CPCe) or eGFP and human Pim-1 kinase (CPCeP) together as bicistronic transcripts by previously established methods[1]. For label release and retention assays cells were plated at 250 cells/mm^2. Cells were labeled with 10 μmol/L BrdU for one generation or with 1 μmol/L BrdU for longer labeling period. BrdU concentration used to label DNA replication is well below typical concentrations used to track chromosomal activities such as DNA repair (30-100 μmol/L).

Cell cycle analysis and treatments

Cells were fixed in 70% ethanol and stored overnight at -20°C. Cell cycle distribution was analyzed by staining with propidium idodie (PI) RNase staining buffer (BD) at 37°C for 3 hours using BD FACSCanto and data processed by FlowJo software. Cells were incubated with 2.5 mmol/L thymidine for indicated times in full medium to block in G1-S phase. Cells were released by trypsinizing and washing twice with PBS and plated in full medium for synchronized progression of cell cycle. 2 μmol/L cytochalasin B was used whenever necessary to block in cytokinesis. For mitotic shake-off cells were grown at 70% confluence, blocked at S phase by 2.5 mmol/L thymidine blocking for 24 hours, released in thymidine free media for 18 hours. Loosely adherent mitotic cells were collected by gently knocking the plate against a hard surface once in every 1.30 hours for up to 5-6 times after thymidine release and cells were fixed in 70% ethanol.

Cell cycle analysis of CPCs indicated most of the cells complete mitosis 12-18 hours after thymidine removal. Thymidine block for 30 hours and releasing immediately in cytochalasin B for up to 24 hours blocks cells in first cytokinesis. Release in thymidine free medium for first 24-30 hours followed by cytochalasin B for another 30 hours block cells in second cytokinesis. These conditions are followed to block cells in first and second mitosis during chase period in BrdU free media to see asymmetric DNA segregation.

BrdU staining

Cells were fixed in 70% ethanol, stored at 4°C until use. Cells were denatured with 2N HCl/0.2% Triton-X100 for 1 hour at RT, then neutralized with 0.1 mol/L TrisCl pH 8.5 and blocked with 10% horse serum. For detecting BrdU, mouse-anti-BrdU (clone B44, BD # 347580) antibody was used at 1:100 dilution, 1 hour at RT followed by donkey-anti-mouse IgG-549 (1:150) as secondary antibody. Cells were mounted in Vectashield containing 1:1000 of Sytox-blue nuclear dye and scanned using Leica SP2 confocal microscope. Images were acquired using 20X lens with 2X electronic zoom for BrdU measurement and 15X electronic zoom for high resolution single cell panels. Differential interference contrast (DIC) images were acquired to confirm binucleated cells.

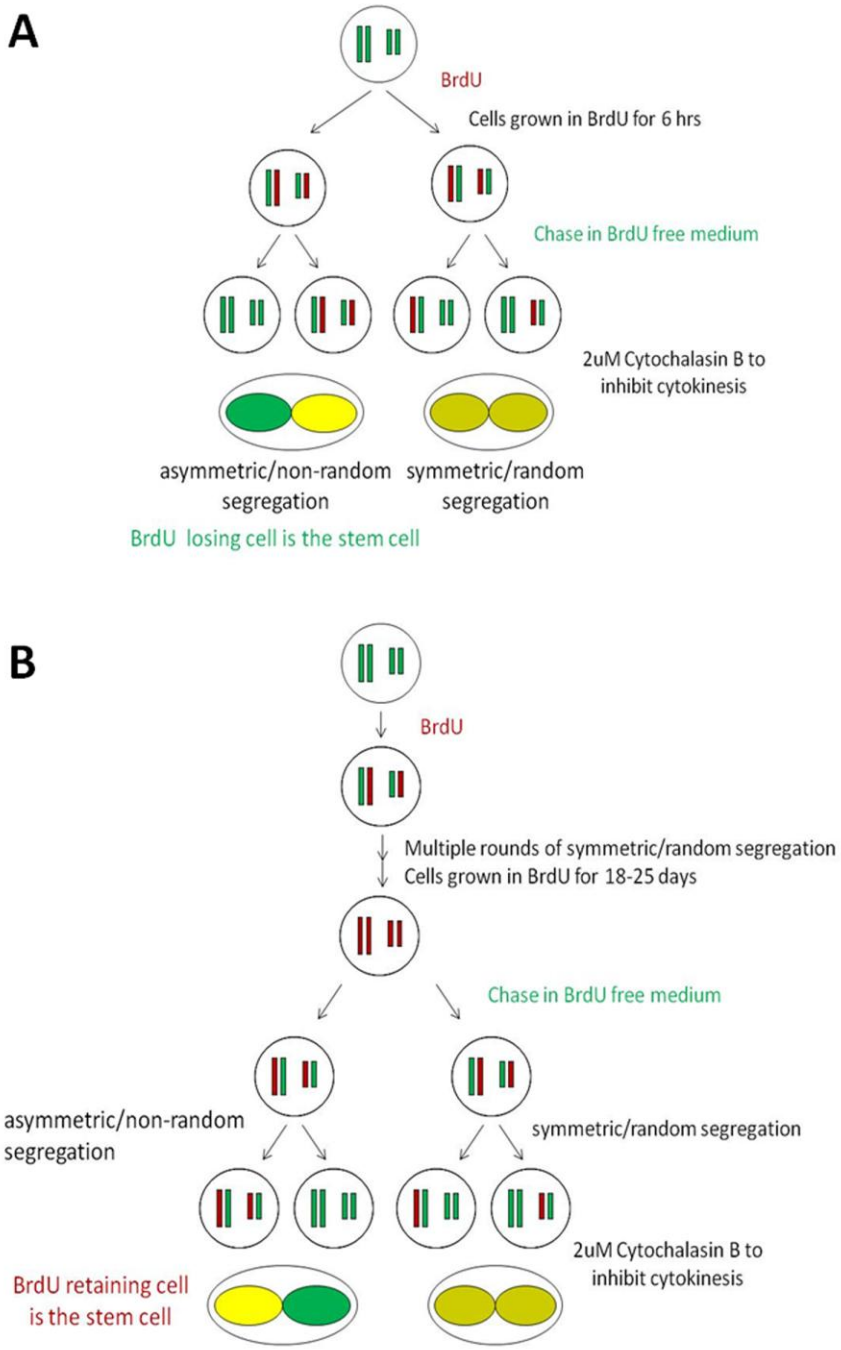
Image processing and density measurements

All images and panels were identically processed. ImageJ software was used for BrdU intensity measurements. Threshold levels of the single scan images were set using auto adjust then the image color was inverted. Mean gray value (d) of two daughter nuclei of a binucleated cell was measured separately and multiplied by their corresponding area (A). Fraction of BrdU intensity of individual daughter nuclei to the total ($T= A_1*d_1 + A_2*d_2$) was plotted ($D_1= A_1*d_1/T$ versus $D_2=1-D_1$).

Method of deriving tumor free Pim-1 CPCs

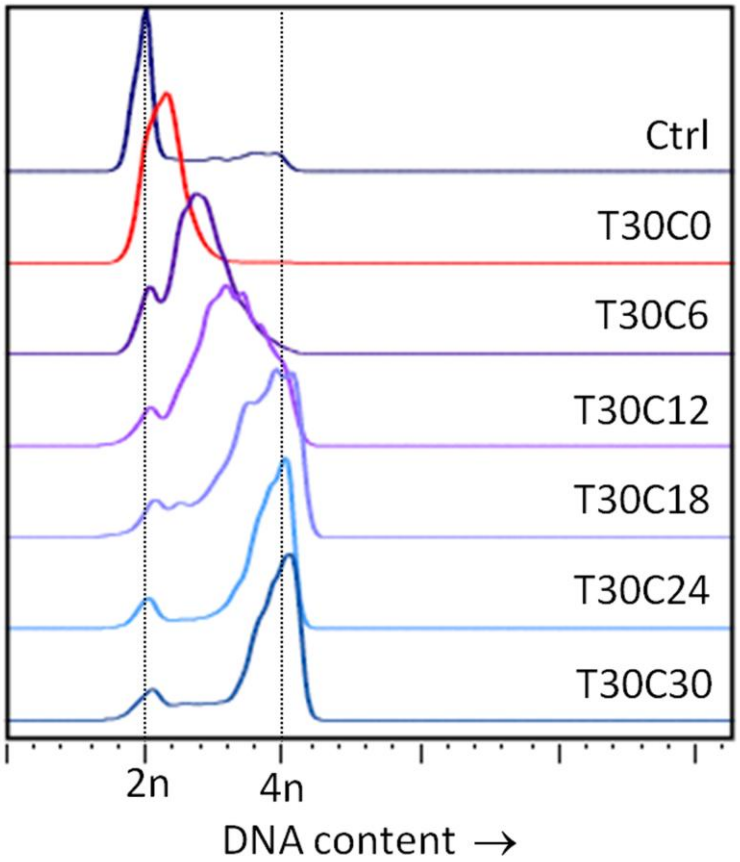
Recent results from our lab indicate that Pim-1-mediated proliferation of CPCeP is transient. Increased proliferation of CPCeP observed at early passages diminishes to normal levels with repeated cell passage without loss of Pim-1 expression (manuscript in preparation). We are also exploring alternative non-lentiviral based methods for Pim-1 gene delivery including episomal minicircle vectors and cell penetrant proteins.

Online Figure I



Online Figure I. Experimental design for label release and retention assay. A, In label release assay, cells are labeled for 6hrs and then chased in BrdU free media for 24hrs followed by a cytochalasin B treatment to induce binucleated cells. By retaining the unlabeled 'immortal strands', the stem cell will lose the BrdU labeled chromosomes. **B,** In the label retention assay cell are labeled for up to 15-25 days and chased for two consecutive cells divisions. Stem cells will retain the BrdU labeled immortal strands for ever while the daughter cells dilute the label.

Online Figure II



Online Figure II. Cell cycle distribution of CPCs with drug treatments. Establishing CPCs' cell cycle kinetics is necessary to successfully block at specific mitosis. Cell cycle distribution is determined by propidium iodide staining and flow cytometry. Population distribution of DNA content is plotted to examine different phases of cell cycle. CPCs grown without any drugs or treatment showed that more than 70% are in G1 and 5% of cells in G2 phase of cell cycle (Ctrl). Thymidine treatment for 30 hours blocked almost all cells in G1-S phase boundary (T30C0). Cells grown with thymidine (T) are washed and plated immediately with medium containing 2μM cytochalasin B (C) and fixed at six hour intervals. Cytochalasin blocks cells in cytokinesis preventing from further G1 phase, creating binucleated cells (cells with 4n DNA content). Six hours after thymidine removal, cells progressed to S phase (T30C6) and by 12 hours a small percentage of cells entered G2 phase. Eighteen hours after thymidine removal the majority of cells entered G2/M phase and by 24 hours more than 85% cells completed mitosis but are binucleated (T30C24). Analysis at thirty hours after thymidine removal and cytochalasin treatment confirmed that cells are permanently blocked in G2.

Supplementary references

1. Fischer KM, Cottage CT, Wu W, Din S, Gude NA, Avitabile D, Quijada P, Collins BL, Fransioli J, Sussman MA. Enhancement of myocardial regeneration through genetic engineering of cardiac progenitor cells expressing pim-1 kinase. *Circulation*. 2009;120:2077-2087