



Figure S4. Ordering cells by cell cycle progression and examining changes in population frequencies

(A) A large proportion of the transcriptome is correlated with cell cycle status. Shown is the average expression of genes (rows) along the inferred cell cycle progression (columns, cells ordered according to cell cycle trajectory), as calculated with a sliding window of 150 cells, for genes with a significant upregulation (top) or downregulation (bottom) in any window of cycling cells, compared to the non-cycling cells [color bar, $\log_2(\text{fold change relative to G0})$]. (B) Ordering cells by cell cycle progression. Shown is the same plot as in Figure 2D with edges connecting adjacent cells based on the inferred cell cycle ordering. As described in Methods, the ordering was defined by dividing the cells into four regions (marked by shaded boxes) and ordering cells within each region by the apparent direction of cell cycle progression (marked by gray arrows). Non-connected cells were not included in the cell cycle ordering due to ambiguities of their ranking. (C) The relative frequency of cells from each population (y axis; cell type and age; colored curves) along the inferred cell cycle progression (x axis), shown with a sliding window of 100 cells. Relative frequency was defined as the \log_2 -ratio of the frequency in a specific window of cells divided by the average frequency across all windows. (D) Cell cycle progression analysis of cells and an additional biological replicate of LT-HSC and ST-HSCs. Cells were ordered according to their inferred cell cycle progression (see Figure S3A) and the average expression of G1/S, S and G2/M genes (Whitfield et al. 2002) was calculated with a sliding window of 11 cells. (E) Cell cycle distribution changes with age. For each cell type, including biological replicates of LT-HSC and ST-HSCs derived from different set of mice, shown is the \log_2 of the ratio between percentages of old cells divided by the percentage of young cells along the inferred cell cycle progression (with a sliding window of 150 cells). Shaded colors reflect the inferred cell cycle phases.