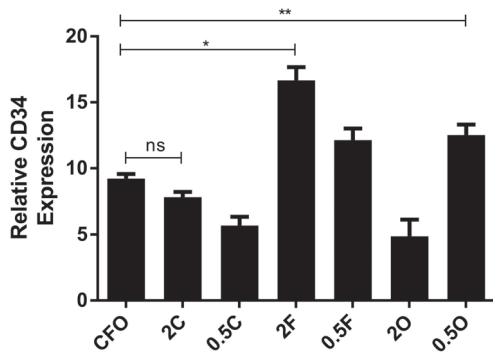
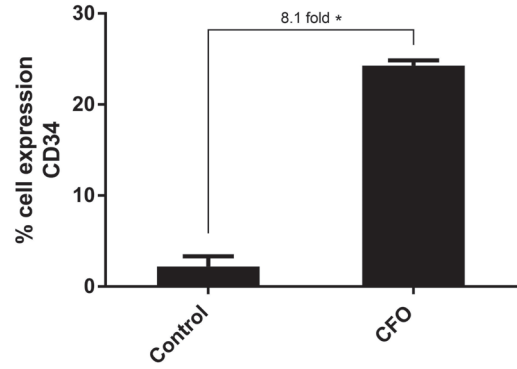


Supplementary Figure 1

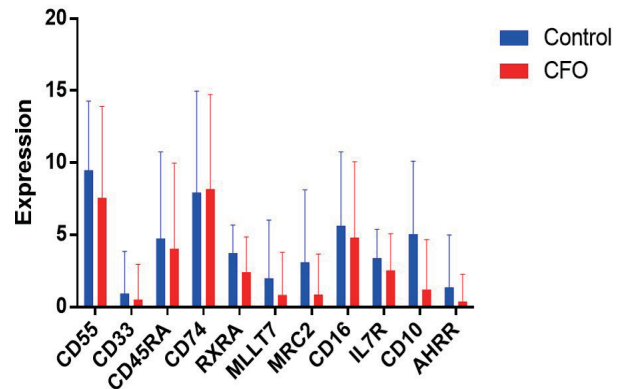
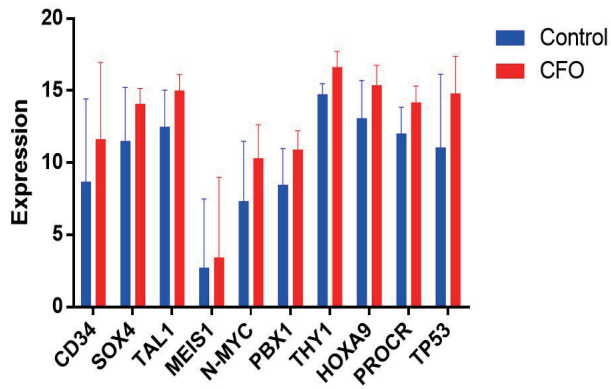
a



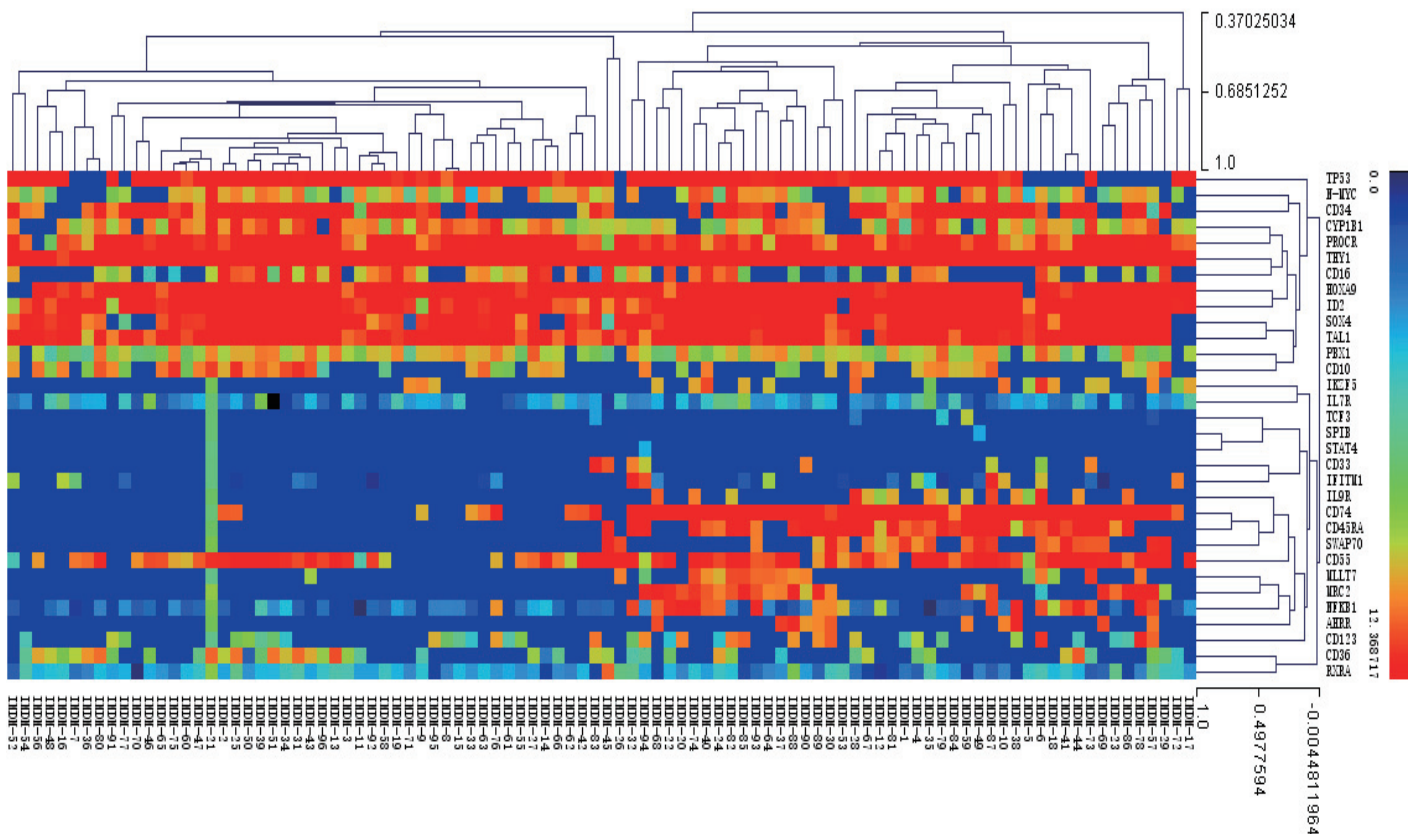
b



c



d

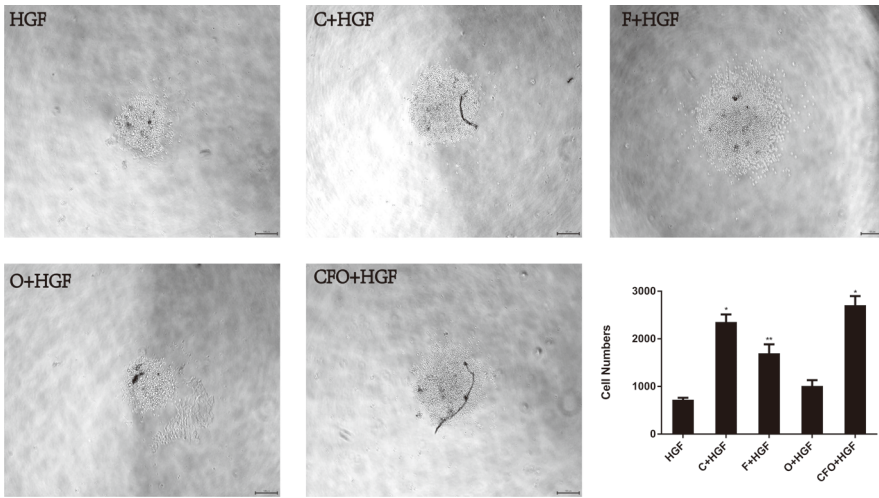


Supplementary Figure S1 (Related to Figure 2 and Figure 3): **CFO maintains human HSPCs self-renewal.**

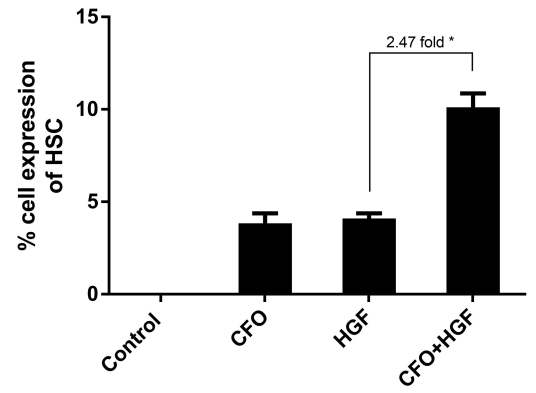
(a) Small molecule concentration adjustment. Experiments with double or half concentration of C, F and O determined the optimal combination for C (10 μ M), F (20 μ M) and O (5 μ M). $**P < 0.01$, $*P < 0.05$. Data represents the mean \pm s.d. ns, non-significant. (b) Quantification of the percentage of human CD34-positive cells showing *CD34* expression. Human CD34-positive cells exposed to CFO combination were assayed for surface protein expression of *CD34* by flow cytometry. $**P < 0.01$. Data represents the mean \pm s.d. (c) Relative expression of related genes in control and CFO. HSPC related markers such as *CD34* and *HOXA9*, differentiated genes such as myeloid (*CD33*, *CD45RA*), erythroid (*RXRA*, *MLLT7*) and lymphoid (*IL7R*, *CD10*) were detected in control and CFO combination. Data represents the mean \pm s.d. (d) Gene expression profile of control. Control were randomly picked to perform single cell qRT-PCR assay with selected genes from early human haematopoietic lineages. IMDM supplemented with serum substitute as control. C, CHIR-99021; F, Forskolin; O, OAC1.

Supplementary Figure 2

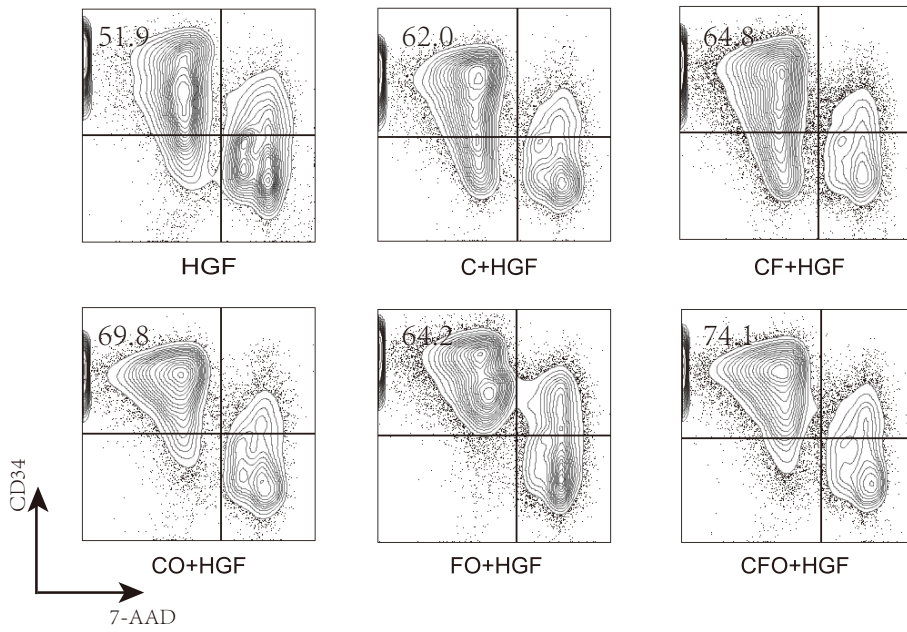
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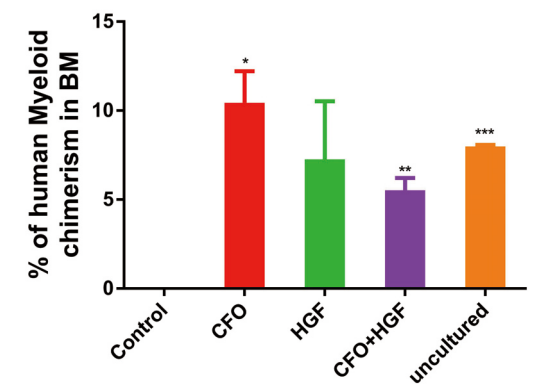
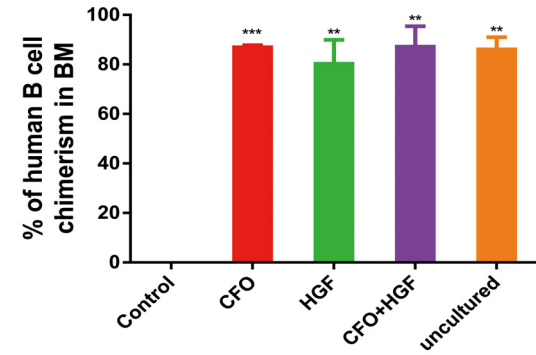
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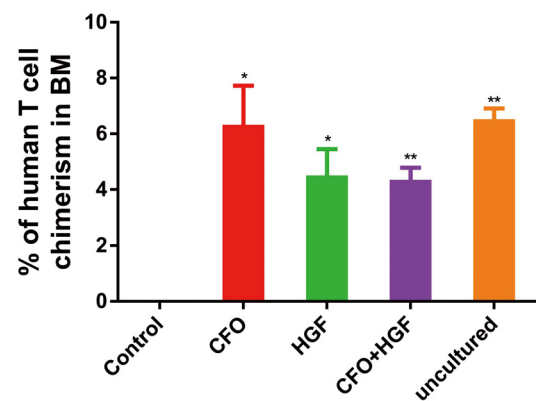
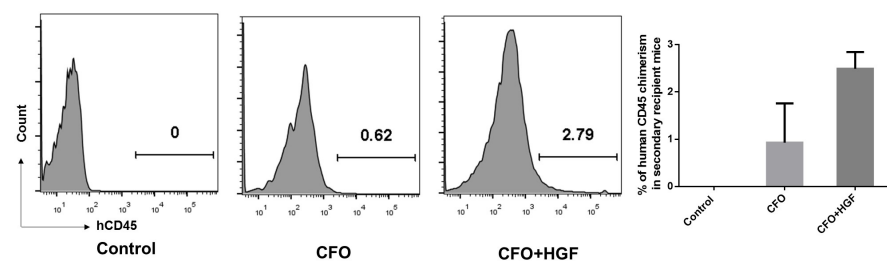
b



d



e

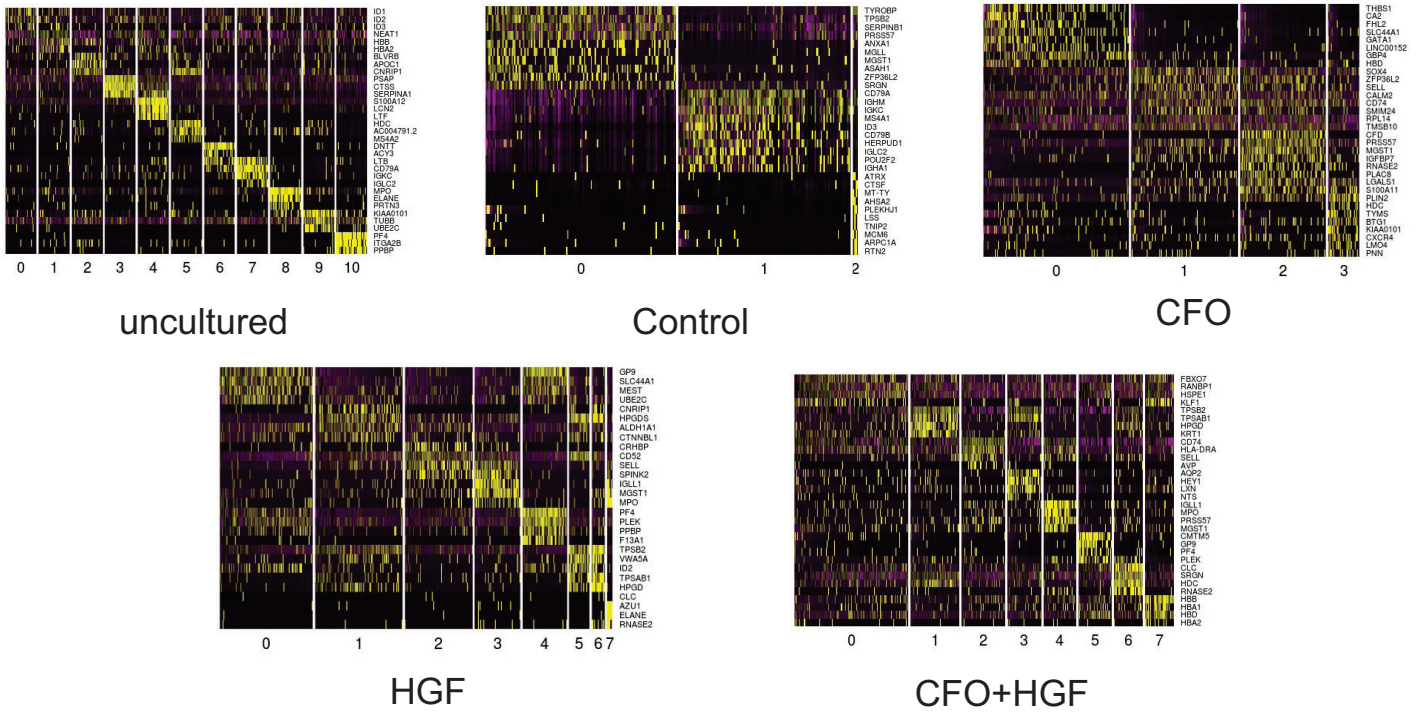


Supplementary Figure S2 (Related to Figure 4 and Figure 5): **Combination of CFO and HGF increases functional human HSPCs.**

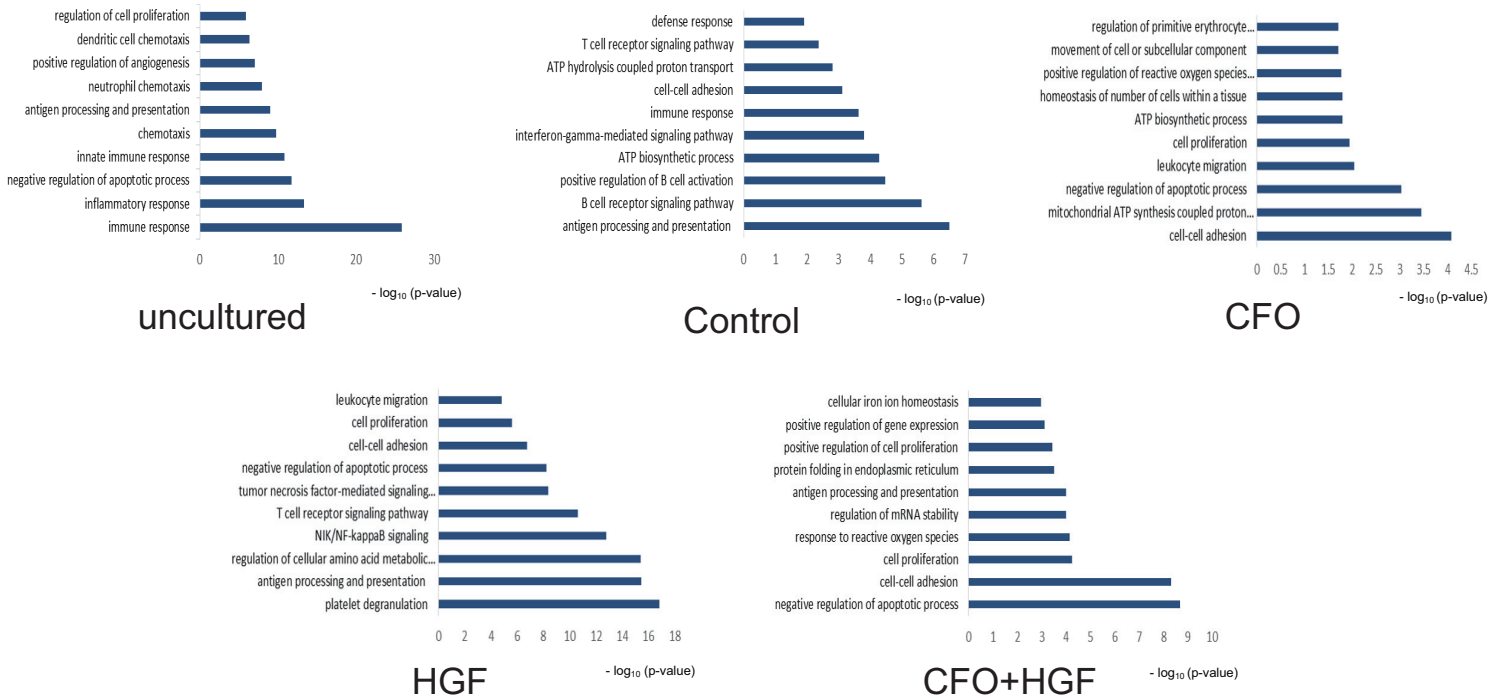
(a) Cell morphology and numbers. Numbers of human CD34-positive cells exposed to C, F, O and CFO on the basic medium with haematopoietic growth factors (HGF) were calculated (scale bar, 100 μm). $**P < 0.01$, $*P < 0.05$. Data represents the mean \pm s.d. (b) Phenotype of HSPCs. Human CD34-positive cells exposed to C, FO, CF, CO and CFO on the basic medium with HGF were assayed for surface protein expression of *CD34*. 7-AAD was assayed for dead cells. (c) Quantification of the percentage of human HSPCs showing *CD34* and *CD90* expression. Human CD34-positive cells exposed to CFO, HGF and CFO + HGF were assayed for surface protein expression of *CD34* and *CD90* by flow cytometry. $*P < 0.05$. Data represents the mean \pm s.d. (d) Chimerism of human myeloid cells, T cells and B cells in B-NSG mice with the injection of control cells, Human CD34-positive cells exposed to CFO, HGF, CFO + HGF and uncultured cells after 8-week post-transplantation. $***P < 0.001$, $**P < 0.01$, $*P < 0.05$. Data represents the mean \pm s.d. (e) Representative flow cytometric analysis of secondary engraftment in the bone marrow of B-NSG mice. Human engraftment was assessed as the percentage of human CD45⁺ cells. Data represents the mean \pm s.d. C, CHIR-99021; F, Forskolin; O, OAC1; HGF, stem cell factor (SCF) and thrombopoietin (TPO).

Supplementary Figure 3

a



b

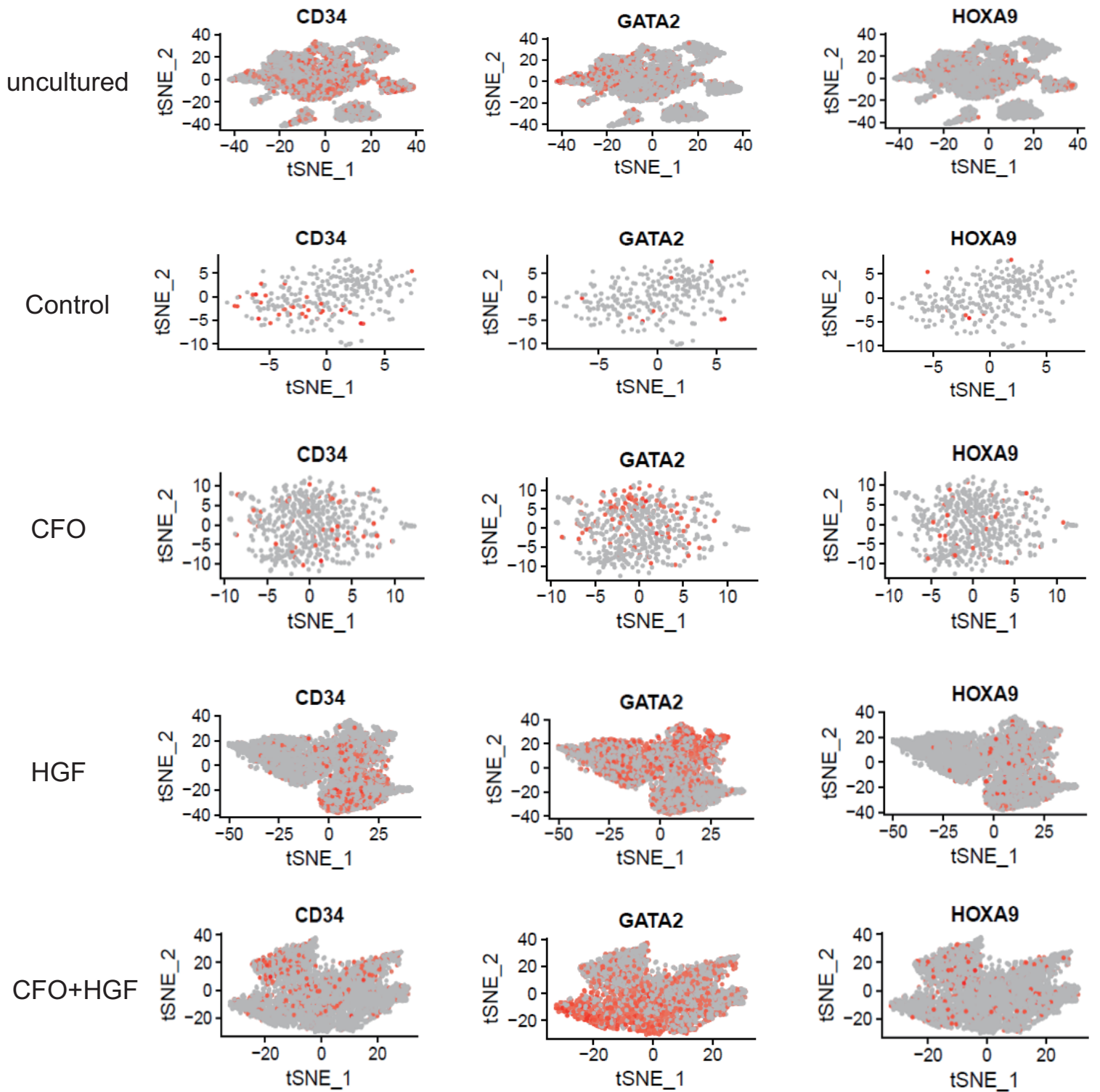


Supplementary Figure S3 (Related to Figure 6): Single cell RNA-seq identify the mechanism of action.

(a) Heatmap of 5 samples: uncultured human CD34-positive cells, control cells, cells with the treatment of CFO, HGF and CFO + HGF. Heatmap reveals specific gene expression modules associated with each cluster. (b) Gene Ontology analysis. GO analysis revealed gene function and relationships in above 5 samples. C, CHIR-99021; F, Forskolin; O, OAC1; HGF, stem cell factor (SCF) and thrombopoietin (TPO).

Supplementary Figure 4

a



Supplementary Figure S4 (Related to Figure 6): Single cell RNA-seq identify the mechanism of action.

(a) T-Distributed Stochastic Neighbor Embedding (t-SNE) analysis of *CD34*, *GATA2* and *HOXA9* in 5 samples: uncultured human CD34-positive cells, control cells, cells with the treatment of CFO, HGF and CFO + HGF. Red plots represent highly expressed cells. C, CHIR-99021; F, Forskolin; O, OAC1; HGF, stem cell factor (SCF) and thrombopoietin (TPO).