

# Cord Blood Hematopoietic Stem Cell Function is Affected by In Vitro Oxygen

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## Abstract

**Background and Objectives:** Expansion of CB-HSC *in vitro* under cleanroom conditions exposes the cells to atmospheric air which contains oxygen at far higher levels than *in vivo*. Exposure of HSC to room air oxygen has been shown to increase damaging intracellular reactive oxygen species, radically reducing cell yields and reducing the ability of CB-HSC to engraft the host. This effect has been termed Extraphysiologic Oxygen Shock/Stress (EPOSS). We previously showed that room air O<sub>2</sub> incubation affects primary human B cell migration. Our purpose with this study is to determine if room air incubation affects CB-HSC migration in response to CXCR4 stimulation.

**Materials and Methods:** We used Pro-Ox 110 gas controllers and C-chamber incubator sub-chambers (BioSpherix, Parish, NY) to control the incubation oxygen in a standard CO<sub>2</sub> incubator. Primary human CD34+ CB-HSC were incubated in the chambers at 5% CO<sub>2</sub> and 1% (hypoxic), 5% (physioxic), or 19% (extraphysiologic room air) oxygen and cell function was assessed as migration across transwell membranes in response to CXCL12.

**Results:** Preliminary data showed that migration in response to CXCL12 was affected in an O<sub>2</sub>-dependent manner, with a trend toward greater numbers of CB-HSC migrating at higher oxygen levels. This is consistent with HSC to homing to and remaining in the low-oxygen stem cell niche in bone marrow.

**Conclusions:** Exposure of CB-HSC to room air oxygen directly affected cellular migration in response to CXCL-12. Protection of CB-HSC from room air should be provided *in vitro*.

## Background

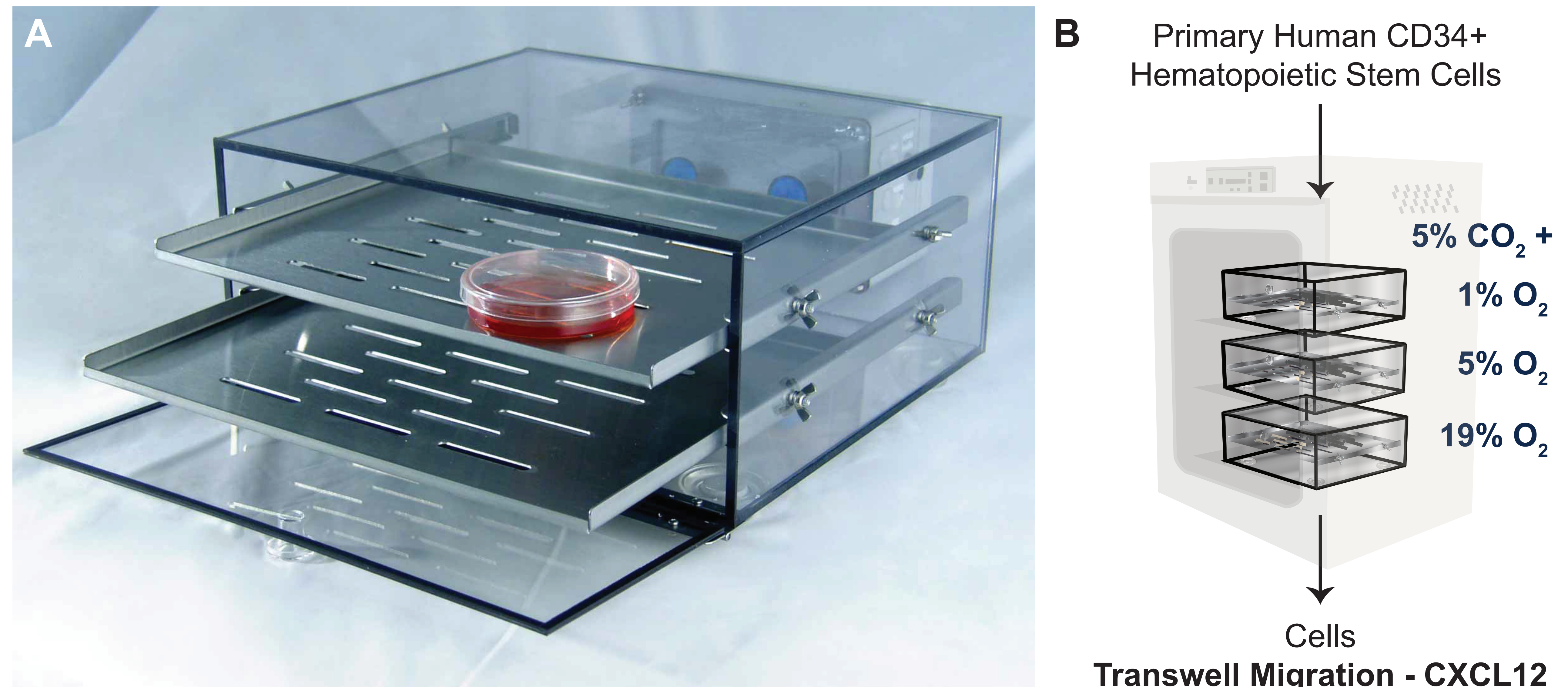
- Exposure of CB-HSC to room air oxygen during isolation procedures has been shown to be detrimental to cell yield and engraftment in bone marrow<sup>1</sup>
- We previously showed that primary human B cells and B cell lines are affected by cell culture oxygen levels without affecting cell viability<sup>2</sup>
- Physiologic oxygen *in vitro* is increasingly important as a driver of immune cell behavior and a critical consideration in the new field of Immunometabolism<sup>3</sup>

## Objectives

Assess human CD34+ cord blood hematopoietic stem cell CXCR4 function at different oxygen levels:

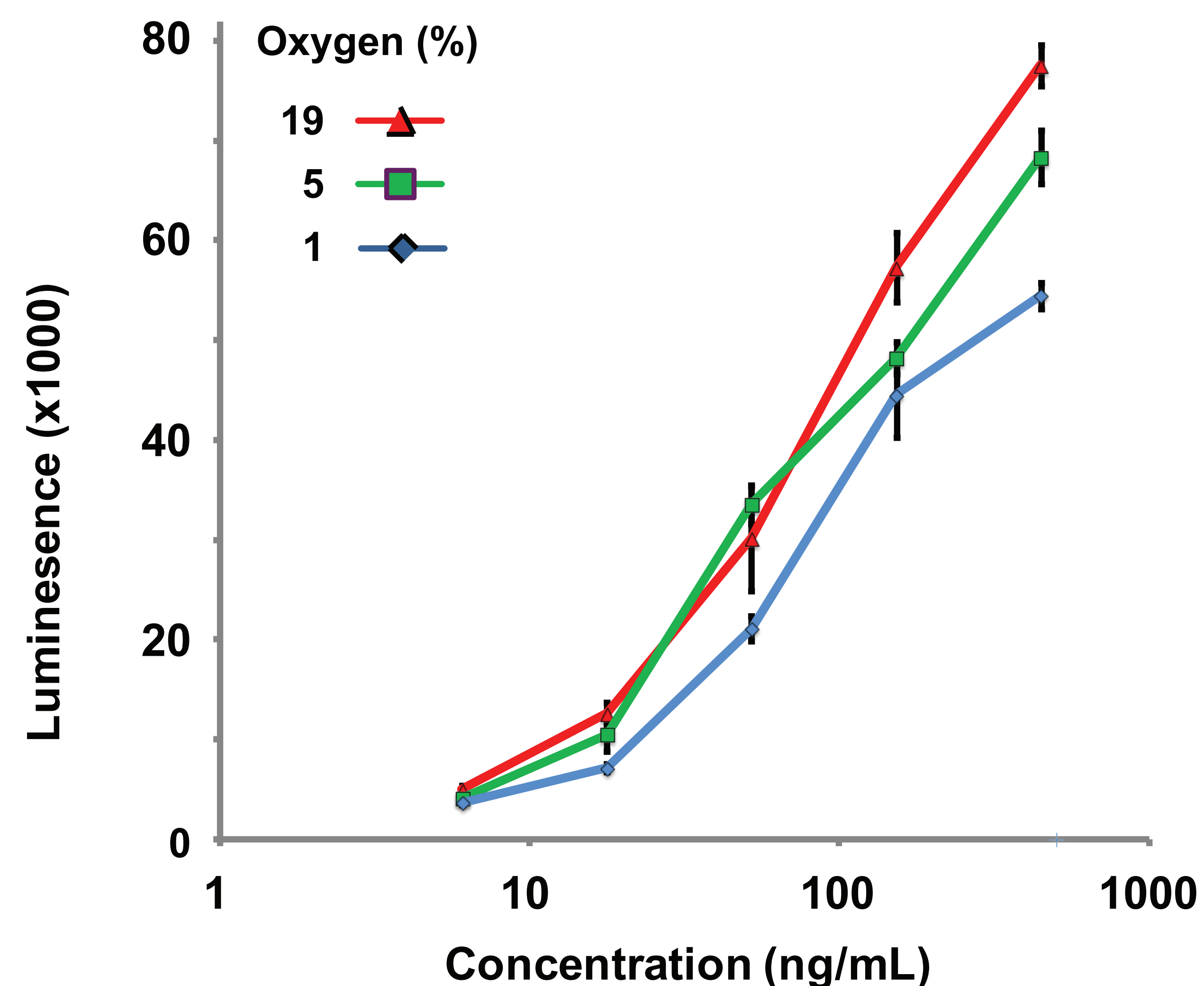
- 1% Hypoxic or Low Physioxic
- 5% Normal Physioxic Range
- 19% Extraphysioxic (Traditional Room Air Culture)

## Experimental Design



**Figure 1. Experimental Design.** (A) Individual C-Chambers incubation subchambers with Pro-Ox controllers were used to provide a controlled oxygen environment for each cell culture inside a standard CO<sub>2</sub> incubator (B) Cells were incubated in 5% CO<sub>2</sub> plus O<sub>2</sub> at 1%, 5%, or 19% (room air incubator oxygen). All media were pre-equilibrated in the subchambers to the proper oxygen level before use. Cells were put into a standard chemotaxis assays with a chemiluminescent read-out.

## Results



**Figure 2. Modulation of CB-HSC Migration by Culture Oxygen Levels.** Human cord blood CD34+ HSC (StemCell Technologies, USA) were thawed and placed into culture at 37°C, 5% carbon dioxide plus 1%, 5%, or 19% oxygen in RPMI 1640 plus 10% FBS (Gibco, USA) overnight. Incubator subchambers (C-Chambers and Pro-Ox gas controllers, BioSpherix) were used to control multiple oxygen levels in the same CO<sub>2</sub> incubator. The next day, cells were transferred to the upper chambers of 96-well PC 5 um transwell plates (Corning) in triplicate. Media containing the indicated concentrations of human CXCL12, the ligand for CXCR4, (R&D Systems) were added to the lower chambers and the plates were incubated for an additional hour at the indicated oxygen conditions. After incubation, the upper chambers are removed and 100 ul of CellTiter-Glo (Promega) was added to the lower chamber to assess the cells that had migrated. Luminescence was measured utilizing a Bio-Tek Synergy HT plate reader. An oxygen-dependent migration of CB-HSC in response to CXCL12, with fewer CB-HSC cells migrating at lower oxygen levels. This is consistent with a model in which CB-HSC cells migrate to and remain in the low-oxygen bone marrow niche.

## Conclusions

Human CB-HSC are affected functionally by room air oxygen, displaying reduced migration  
This is consistent with CB-HSC migrating to and staying in low oxygen niches  
Culturing CB-HSC in room air may produce results that do not translate to physiologically relevant O<sub>2</sub> levels

## References

- 1 Mantel, C. R. et al. Enhancing Hematopoietic Stem Cell Transplantation Efficacy by Mitigating Oxygen Shock. *Cell* 161, 1553-1565, doi:10.1016/j.cell.2015.04.054 (2015).
- 2 Henn, A. D. et al. Cell Culture Oxygen and Signaling: Supraphysiologic *in vitro* oxygen signals through the HIF-CXCR4 pathway in human B cells. (Poster) *Immunology* 2017 (2017).
- 3 Zenewicz, L. A. Oxygen Levels and Immunological Studies. *Frontiers in immunology* 8, doi:10.3389/fimmu.2017.00324 (2017).