

***In vitro* metabolic zonation through oxygen gradient on a chip**

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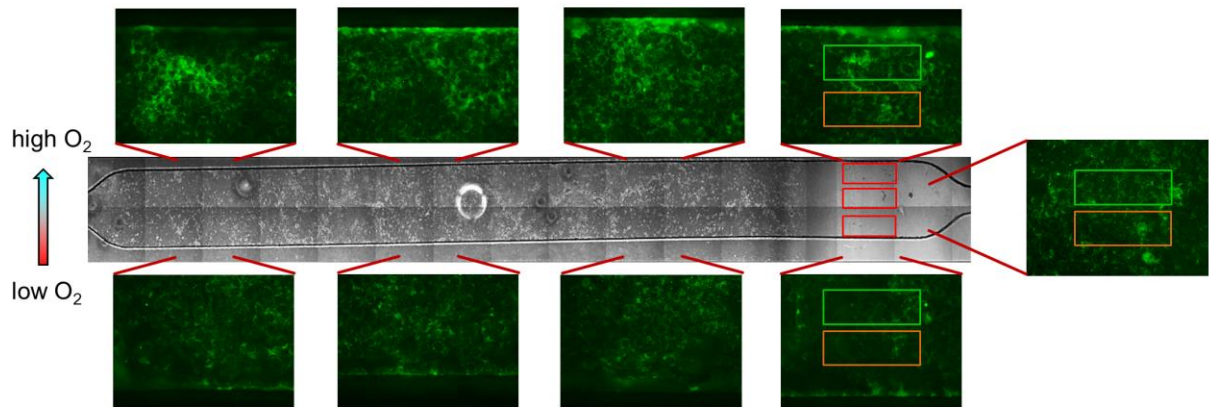
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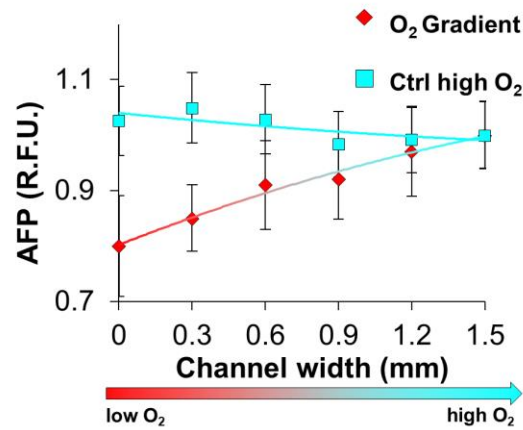
¶ First co-authorship: Federica Tonon and Giovanni Giuseppe Giobbe contributed equally to this work.

Supplementary Figures

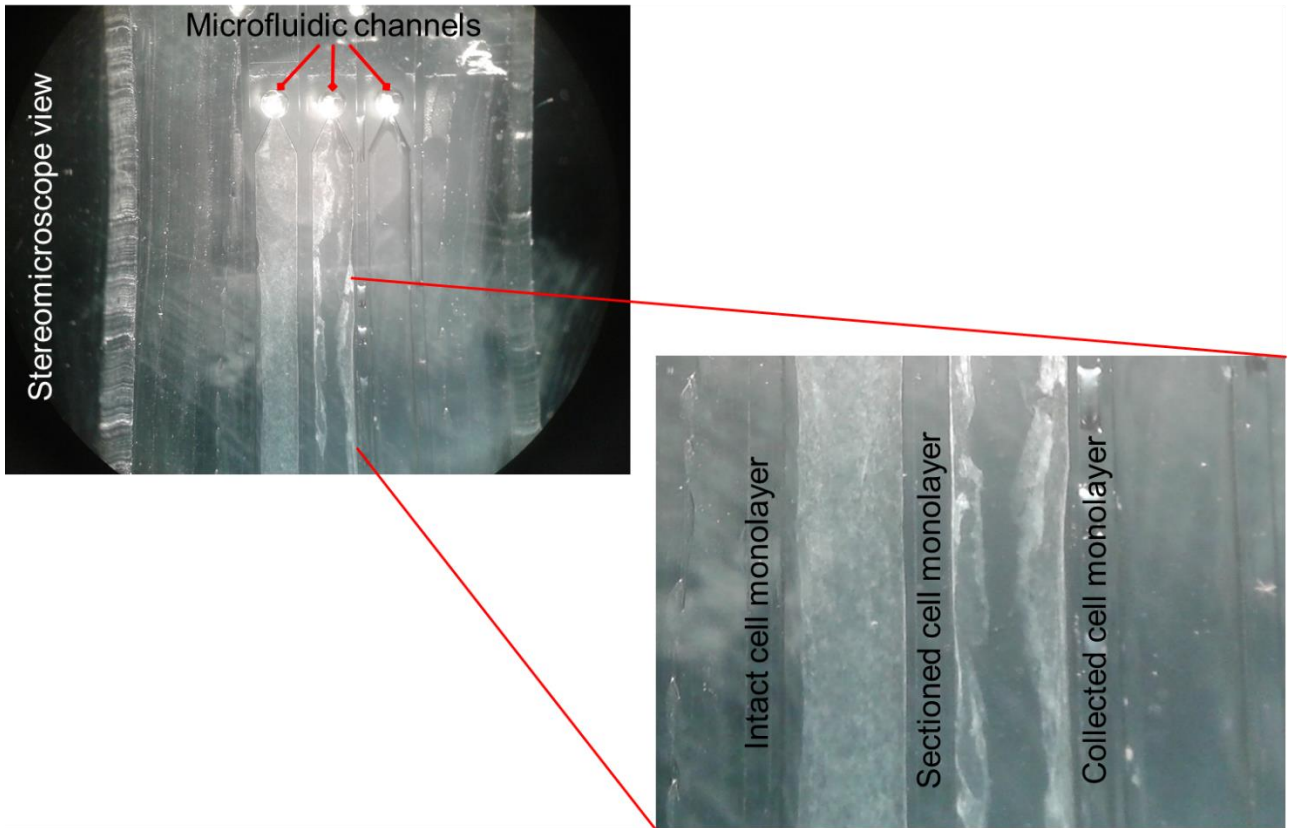
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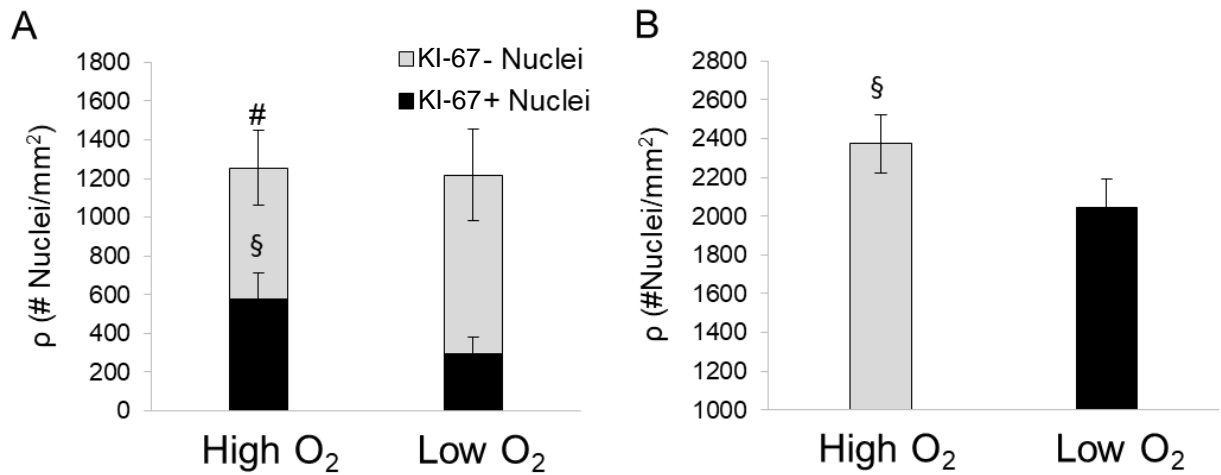
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Supplementary Figure 1. (A) Alpha-fetoprotein immunostaining (green) at the different sides of the microfluidic channel with oxygen gradient applied from day 1 (hESCs) to day 8 (hepatic endoderm) of differentiation, and the 6 regions of interest (ROI) selected for fluorescence quantification. (B) Relative fluorescence unit plot of control chip not exposed to gradient and cultured at standard high O₂ (21%) and gradient chips that shows differential expression of alpha-fetoprotein across the gradient.



Supplementary Figure 2. Stereomicroscope manual picking of the two sides of the hESC-hepatocyte monolayer within the microfluidic channel for RNA collection.



Supplementary Figure 3. Evaluation of the proliferative potential of hepatocyte-like cells differentiated and grown on plate or on a microfluidic device. (A) Quantification of KI-67 immunostaining in hepatocyte-like cells obtained differentiating hESCs grown on a plate at low (<5%) or high (21%) oxygen. Cells differentiated at high O₂ show a higher amount of KI-67 positive nuclei compared to cells grown at low oxygen. Data are shown as mean \pm SD, n=25. p \S < 0.0001 respect to KI-67+ nuclei at low (<5%) oxygen and p# < 0.05 respect to KI-67 – nuclei at low (<5%) oxygen. (B) Nuclei counting of hepatocyte-like cells obtained from hESC grown on a microfluidic device. On the side with higher O₂, more nuclei were observed compared to the other side of the chamber (low O₂). Data are shown as mean \pm SD. n=55. p \S < 0.0001 respect to low O₂.