**Mesenchymal stromal cell-derived extracellular vesicles restore thymic architecture and T cell function disrupted by neonatal hyperoxia**

Monica Reis1,2, Gareth R. Willis1,2, Angeles Fernandez-Gonzalez1,2, Vincent Yeung1,2, Elizabeth Taglauer1,2, Margaret Magaletta3, Teagan Parsons3, Alan Derr3, Xianlan Liu1,2,Rene Maehr3, Stella Kourembanas1,2, and S. Alex Mitsialis1,2

Affiliations:

1Division of Newborn Medicine & Department of Pediatrics, Boston Children’s Hospital, Boston, Massachusetts, USA

2Department of Pediatrics, Harvard Medical School, Boston, Massachusetts, USA

3Program in Molecular Medicine, Diabetes Center of Excellence, University of Massachusetts Medical School, Worcester, MA, USA

Correspondence and requests for reprints should be addressed to: Dr. Stella Kourembanas, Boston Children’s Hospital, 300 Longwood Avenue, Division of Newborn Medicine, Boston, MA 02115

Tel: 617-919-2355. Fax: 617-730-0260. E-mail: stella.kourembanas@childrens.harvard.edu

**Supplementary Tables:**

**Supplementary Table S1: List of reagents used in 10x Genomics single cell RNA sequencing experiments.** Summary of extra kits and reagents, suppliers and product numbers used for single cell RNA sequencing experiments.

|  |  |  |
| --- | --- | --- |
| **Kits & Extra Reagents** | **Supplier** | **Product Number** |
| **ChromiumTM Single Cell 3' GEM, Library & Gel Bead Kit v3, 4 rxns** | 10x Genomics | 1000092 |
| **ChromiumTM i7 Multiplex kit, 96 rxns** | 10x Genomics | 120262 |
| **ChromiumTM Chip B Single Cell Kit, 16 rxns** | 10x Genomics | 1000074 |
| **Nuclease-free Water** | Thermo Fisher Scientific | AM9937 |
| **Low TE Buffer (10mM Tris-HCl, pH = 8.0, 0.1 mM EDTA)** | Thermo Fisher Scientific | 12090-015 |
| **Ethanol, Pure (200 Proof, anhydrous)** | Sigma | E7023-500mL |
| **SPRIselect Reagent Kit** | Beckman Coulter | B23318 |
| **Glycerin (glycerol), 50% (v/v) Aqueous Solution** | Ricca Chemical Company | 3290-32 |
| **Qiagen Buffer EB** | Qiagen | 19086 |
| **Agilent High Sensitivity DNA bioanalyzer Chip** | Agilent | NA |
| **NextSeq 500/550 Hi Output Kit V2.5 75-cycle** | Illumina | 20024906 |

**Supplementary Table S2: Quality control for each obtained transcriptome using the 10x Genomics single cell sequencing platform.** The table shows the number of cells, unique molecular identifiers (UMIs) and genes/cell in each sample after data filtering.

|  |  |  |  |
| --- | --- | --- | --- |
|   | **Total Cells** | **Unique Molecular Identifiers (UMIs)**  | **Genes/cell** |
| **PN7** | **NRMX** | 9430 | 3316 | 1060.6 |
| **HYRX** | 16364 | 3572.1 | 1585.5 |
| **HYRX+MEx** | 6728 | 7430.9 | 2416.4 |

**Supplementary Tables S3: Genes differentially expressed (DE) between pair-wise group combinations HYRX vs NRMX and HYRX+MEx vs HYRX for each T cell cluster identified in PN7.** Tables show Log2 (Fold Changes [FC]) and false discovery rate (FDR). Genes that displayed a Log2(FC) > 1 and <-1 and FDR <0.05 are considered significant. UMAP for clusters herein identified are shown in **Fig. S8**.

**Supplementary Tables S4: Genes differentially expressed between the pair-wise group combinations HYRX vs NRMX and HYRX+MEx vs HYRX for each non-T cell cluster identified in PN7.** Tables show Log2 (Fold Changes [FC]) and false discovery rate (FDR). Genes that displayed a Log2(FC) > 1 and <-1 and FDR <0.05 are considered significant. UMAP displaying these clusters are shown in **Fig. 7b**.

**Supplementary Table S5: Number of upregulated and downregulated genes in non-T cell clusters.** Table shows the number of DEGs in the two relevant pair-wise combinations, HYRX vs NRMX and HYRX+MEx vs NRMX.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Comparisons** | **C01** | **C02** | **C04** | **C06** | **C05** | **C07** | **C09** | **C10** | **C11** |
| **#Genes** | **Total genes** |  | 11601 | 10855 | 12460 | 12647 | 16880 | 13456 | 12243 | 10883 | 11335 |
| **Upregulated genes** | **HYRX vs NRMX** | 106 | 90 | 205 | 209 | 14 | 28 | 11 | 15 | 38 |
| **HYRX+MEx vs HYRX** | 44 | 379 | 738 | 557 | 339 | 208 | 10 | 3 | 26 |
| **Downregulated genes** | **HYRX vs NRMX** | 18 | 42 | 46 | 56 | 52 | 49 | 1 | 2 | 26 |
| **HYRX+MEx vs HYRX** | 1 | 0 | 9 | 23 | 6 | 1 | 5 | 5 | 7 |