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King Abdullah University of
Science and Technology

# Deep characterization of the cellular diversity in the bone marrow microenvironment

Jin Ye[1], Itziar Cenzano Armendáriz[3],Xabier Martinez-de-Morentin[2], Miren Lasaga-Goyeneche[2], Isabel Calvo Arnedo[3], Nuria Planell Picola[2], Amaia Vilas-Zornoza[4], Larisa Morales-Soto[1], Patxi San-Martin[4], Borja Saez-Ochoa[3], Felipe Prosper[4], Jesper Tegner[1], David Gomez-Cabrero[1,2,5*]

[1]Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology, Thuwal, 23955, Saudi Arabia
[2]Navarrabiomed, Complejo Hospitalario de Navarra (CHN), Universidad Pública de Navarra (UPNA), IdiSNA, Pamplona, Spain
[3]Centro de Investigacion Medica Aplicada and IDISNA, Pamplona, Spain
[4]Clinica Universidad de Navarra, Pamplona, Spain
[5]Mucosal and Salivary Biology Division, King's College London Dental Institute, London, SE1 9RT, United Kingdom

# BACKGROUND

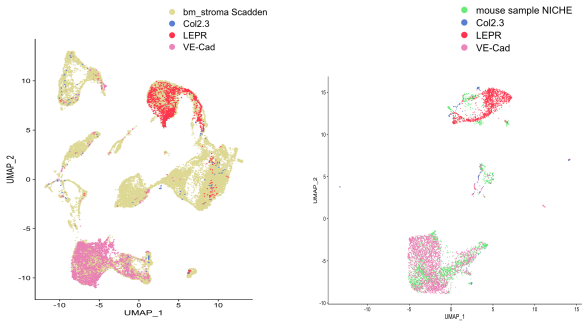
Hematopoiesis is tightly controlled by the bone marrow microenvironment, yet its cellular composition remains partially unresolved. To fully understand the regulation of hematopoiesis, it is essential to generate a complete taxonomy of the bone marrow microenvironment. Such a goal has been partially accomplished recently with the use of scRNA-seq technologies by several laboratories in parallel. However, in each of these studies, a different strategy for cell-type characterization was used; furthermore, the number of cells used did not exclude the existence of additional sub-types.

## Datasets

To facilitate the identification of cell subtypes, cellular states and differentiation trajectories, we integrated three datasets (publicly available and own generated) separately for two well-defined populations, which are mesenchymal (MSCs) and endothelial cells (ECs). To verified the findings in human, 4 human bone marrow niche scRNA-seq samples were collected in addition.

# METHODS

1) Pairwise integration and select target cells
2) Integrate separately and perform clustering
3) Cluster evaluation with bootstrapping strategy, cell cycle phase, batch effect removal
4) Biological annotation considering markers, functional analysis and cytokines




Scadden & Aifnatis | Aifantis & Pamplona

# CLUSTER EVALUATION

1 select 2 clusters, remove 20% cells as training dataset

2 Compute DEGs between 2 clusters.

3 Train a random forest classifier with top 10 DEGs from each cluster

4 Applied the classifier to the 20% training dataset

**Result:**

For each cell in the training dataset to which cluster it assigned

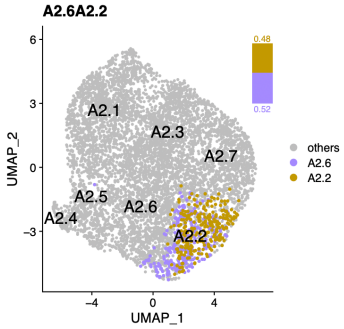
x5 To cover all cells in the 2 clusters

**Result:**
Each cell from the 2 clusters correctly/not-correctly assigned

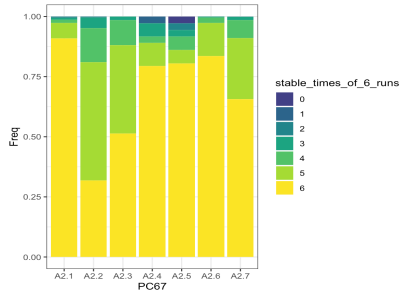
x10

Repeat for all cluster pairs

Type 1:
For every pair of clusters, a cell pertains to which one most of the time?

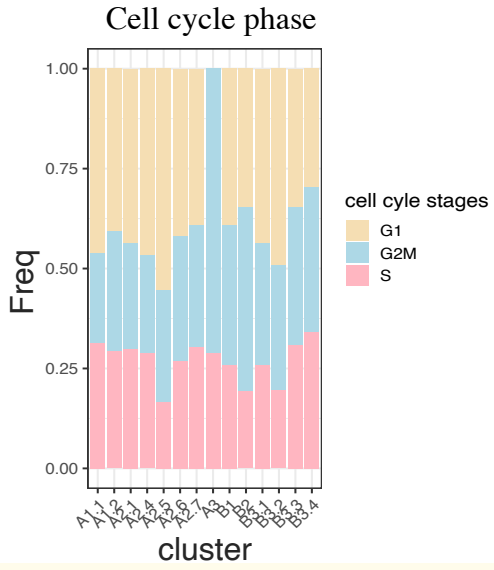


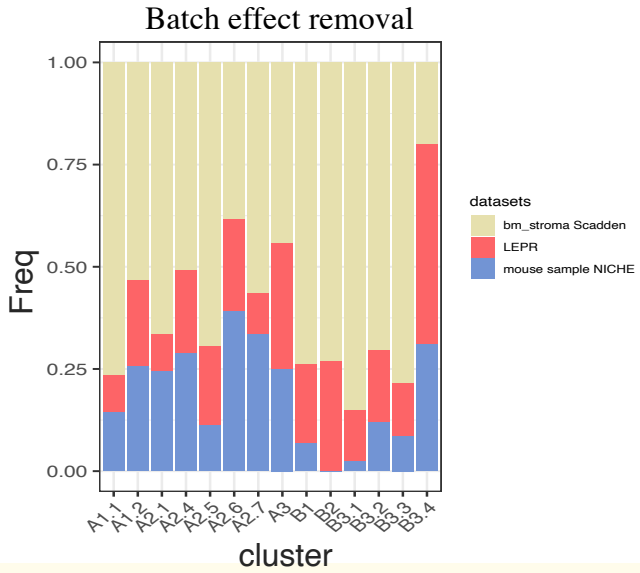

Type 2:
For each cluster, how many times out of all pair comparisons the cluster was the dominant cluster for all cells within it?

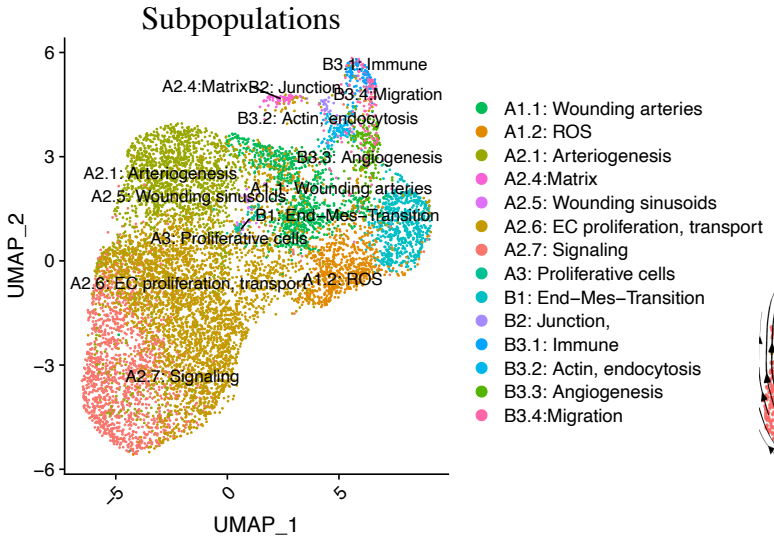


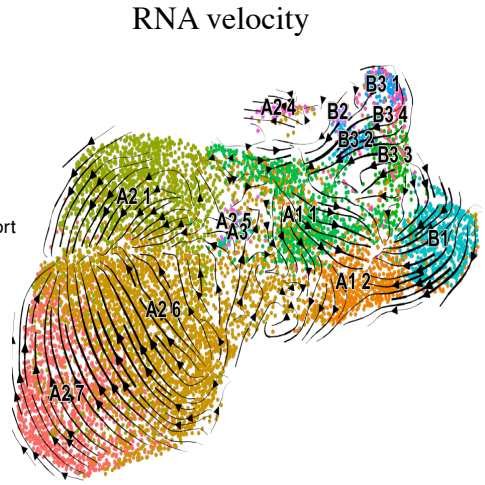
# RESULTS

## Result 1: **Mouse ECs.** Integration of independent experimental datasets defines new murine EC states.

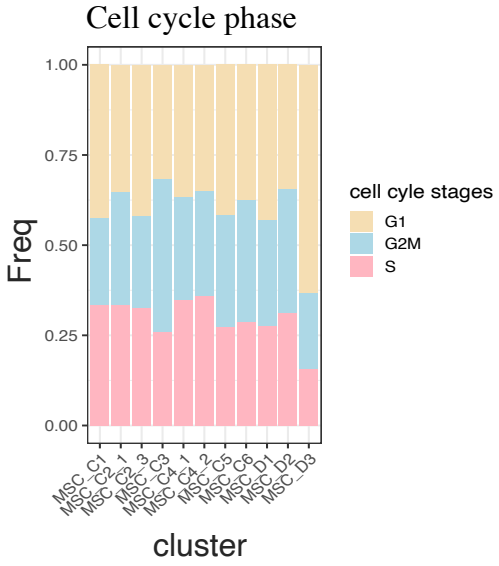


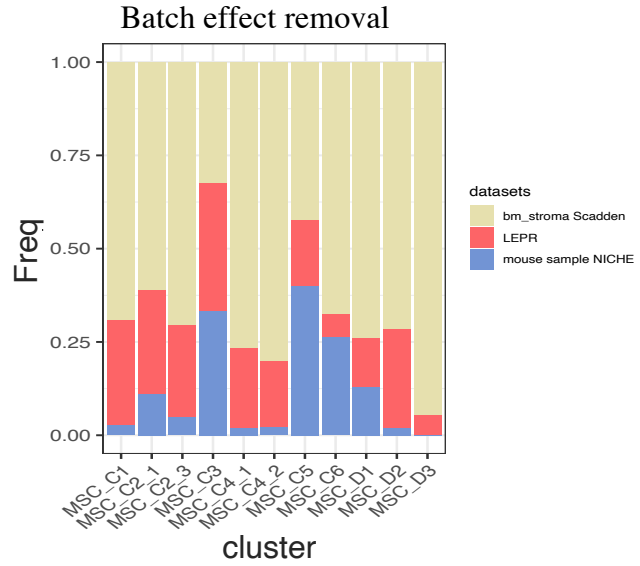



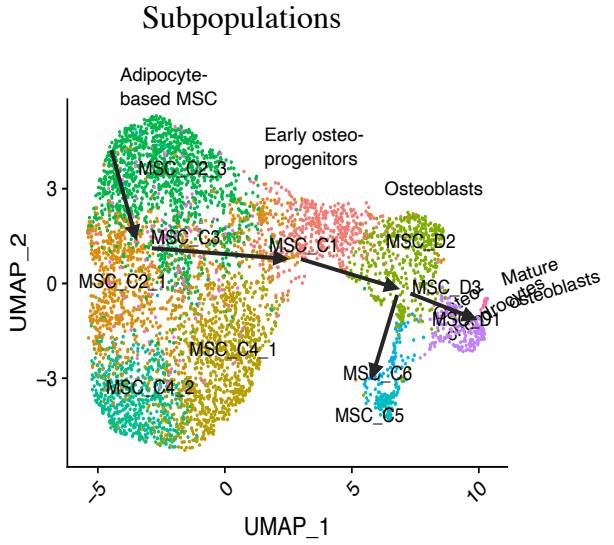



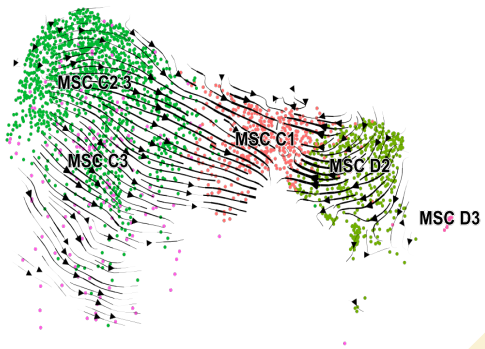




## Result 2: Mouse **MSCs.** RNA velocity predicts the differentiation trajectories from MSCs toward osteogenic lineages.

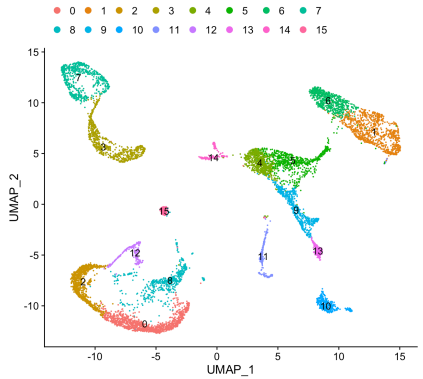


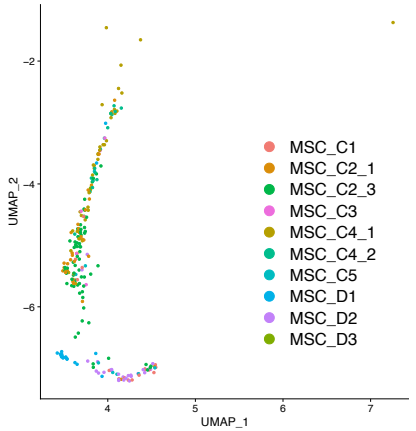



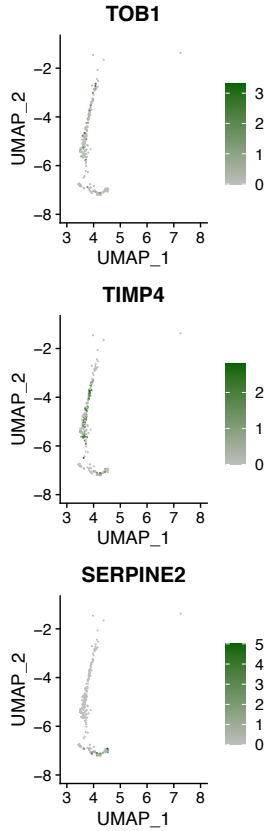
# RESULTS




Four human niche samples




Predicted MSC populations in human from Seurat label transfer.




Gene expression in human MSCs

Result 3: **Human**

Conservation of composition and lineage differentiation patterns in the human BM mesenchymal compartment.

# CONCLUSIONS

- For each population, by leveraging on multiple-data-set integration, we identified and characterized previously unrecognized cell types and intermediate cell states.
- We evaluated the statistical robustness of the novel subpopulations by adapting an existing bootstrapping strategy.
- We examined whether the newly identified subtypes are conserved in the human bone marrow microenvironment.
- As an example: similarities between species were identified in the differentiation patterns of mesenchymal stem cells.

# REFERENCES


- Tasic, Bosiljka, et al. "Adult mouse cortical cell taxonomy revealed by single cell transcriptomics." *Nature neuroscience* 19.2 (2016): 335-346.
- Baryawno, Ninib, et al. "A cellular taxonomy of the bone marrow stroma in homeostasis and leukemia." *Cell* 177.7 (2019): 1915-1932.
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jin.ye@kaust.edu.sa