Molecular hallmarks of heterochronic parabiosis at single-cell resolution

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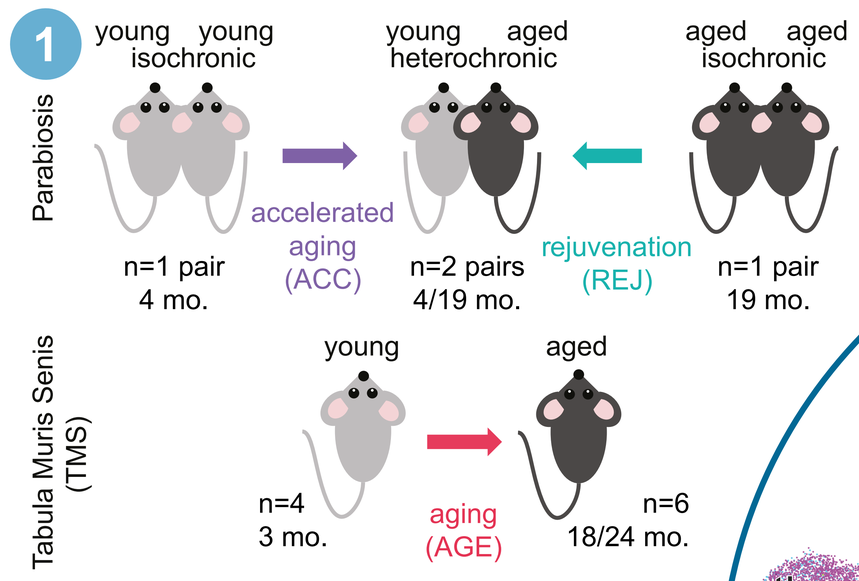
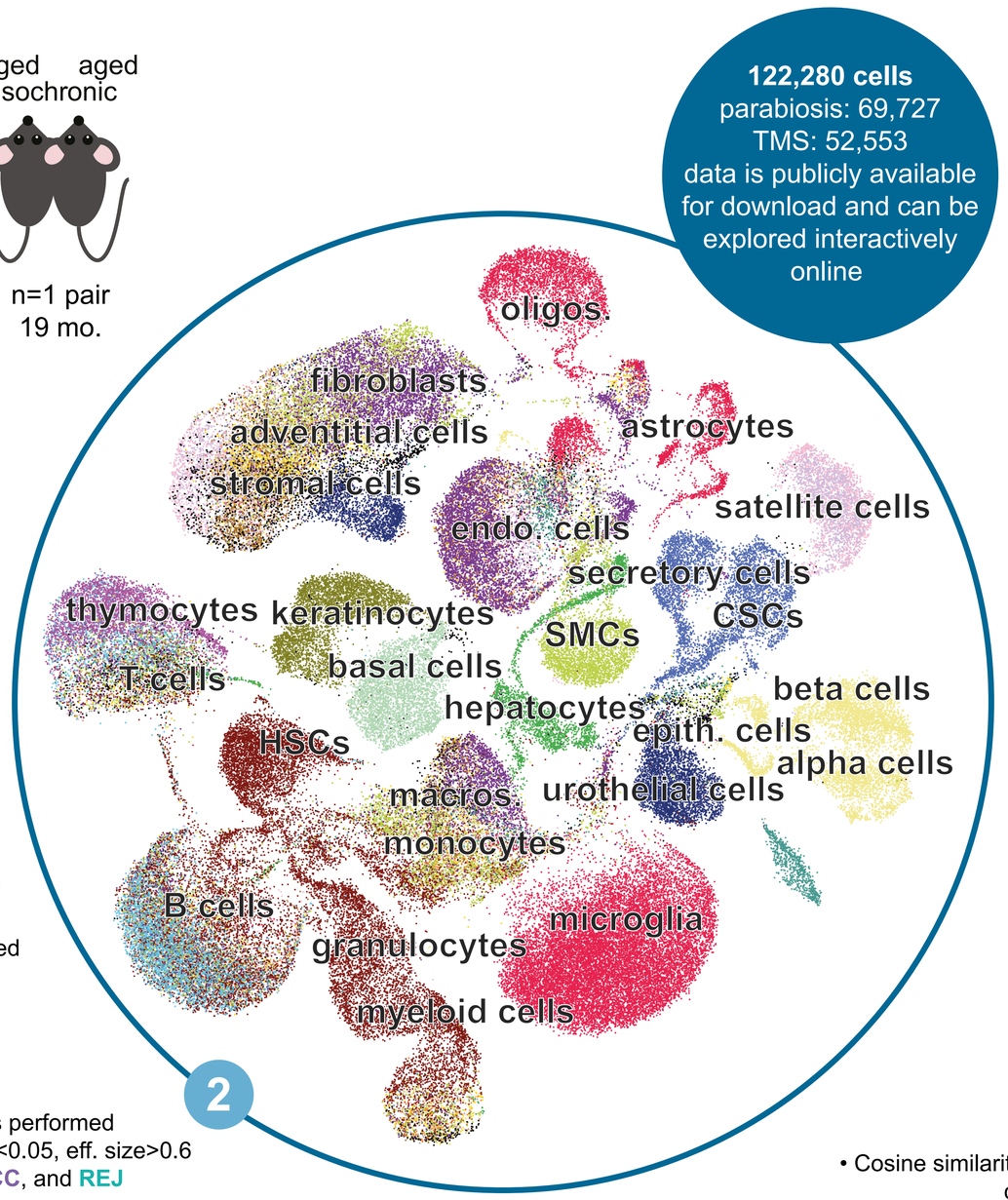
Experiment overview



- Describe heterochronic parabiosis, when a young and aged mouse share a common circulation, across the body **(Fig. 1)**
- Compare parabiosis (**ACC** and **REJ**) to aging (**AGE**)
- Performed Smart-seq2 scRNA-seq of C57BL6/JN male mice following 5 weeks of heterochronic parabiosis
- Targeted cell populations were captured from 20 organs

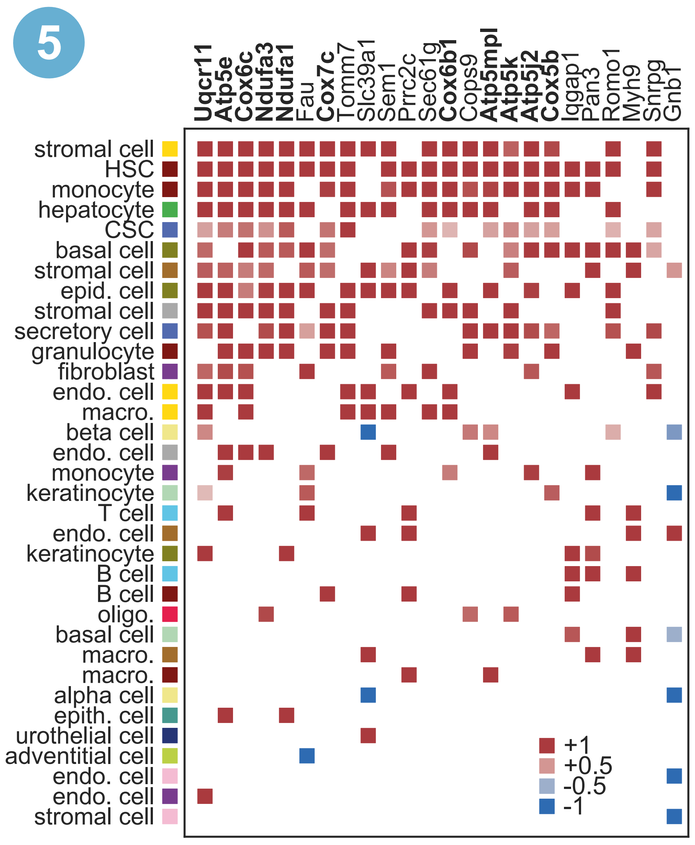
bladder, brain, diaphragm, fat BAT, fat GAT, fat MAT, fat SCAT, heart, kidney, intestine, limb muscle, liver, lung, marrow, pancreas, skin, spleen, thymus, tongue, trachea

- **Fig. 2**: UMAP visualization of the whole single-cell atlas dataset, cells are colored by tissue of origin, major cell types are highlighted (out of 50 in total)



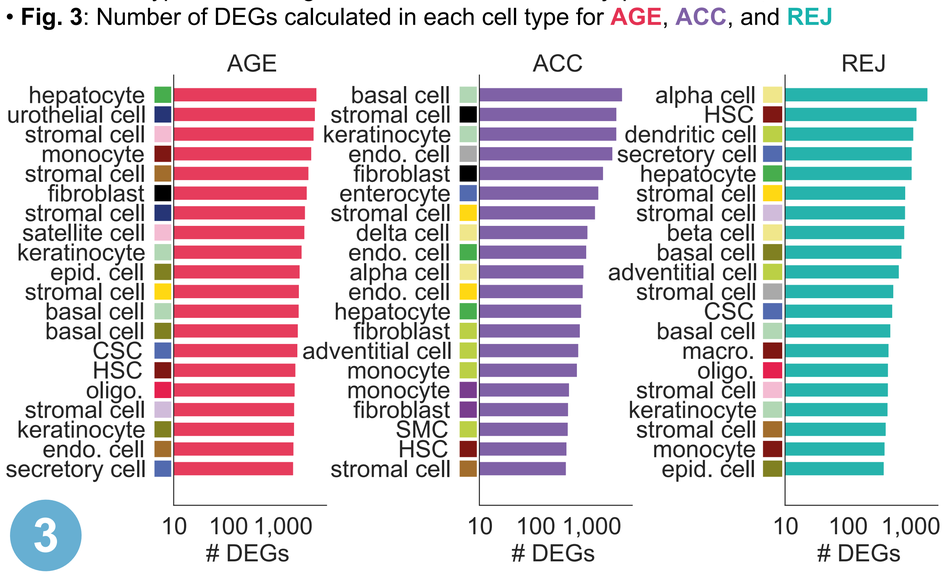
Gene-specific results

- **Fig. 5**: Most frequent DEGs shared across multiple cell types changing in the **opposite direction** with **REJ** and **AGE** heatmap values indicate **REJ** log2-fold changes, genes encoding electron transport chain subunits are highlighted in bold



Cell-type-specific results

- Wilcoxon–Mann–Whitney based differential gene expression was performed in each cell type of each organ, DEGs identified with adj. p-value<0.05, eff. size>0.6
- **Fig. 3**: Number of DEGs calculated in each cell type for **AGE**, **ACC**, and **REJ**

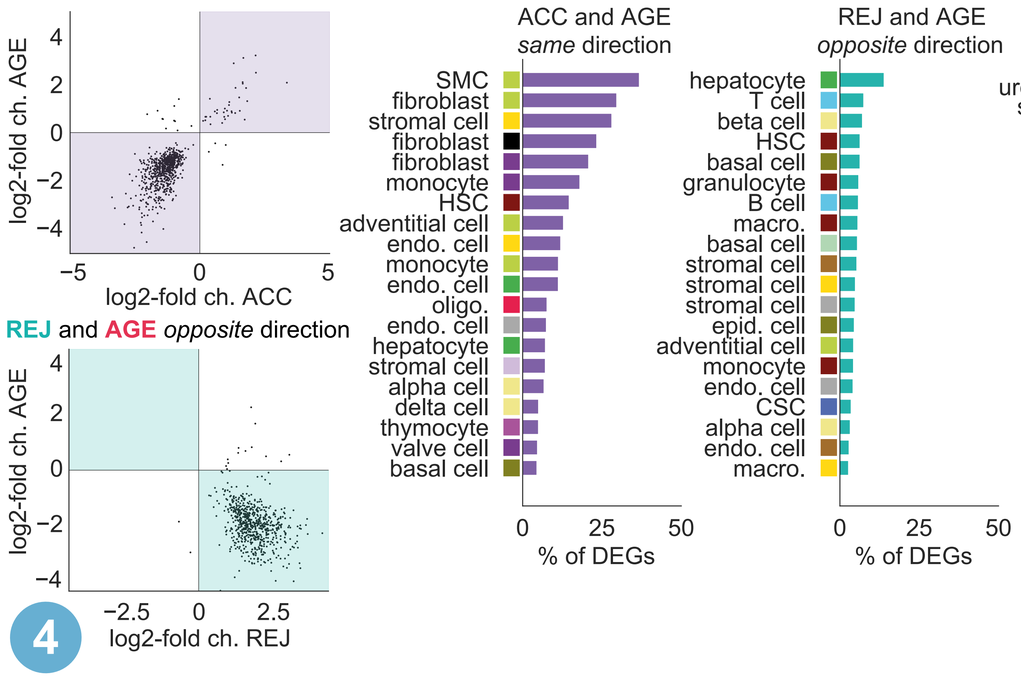


abbreviations: CSC: crypt stem cell, endo. cell: endothelial cell, epid. cell: epidermal cell, epith. cell: epithelial cell, HSC: hematopoietic stem cell, macro.: macrophages, oligo.: oligodendrocytes, SMC: smooth muscle cell

- **Fig. 4:** Calculated consistent overlaps between parabiosis and AGE
- Consistent overlap = |consistent intersection of DEGs| / |union of DEGs|

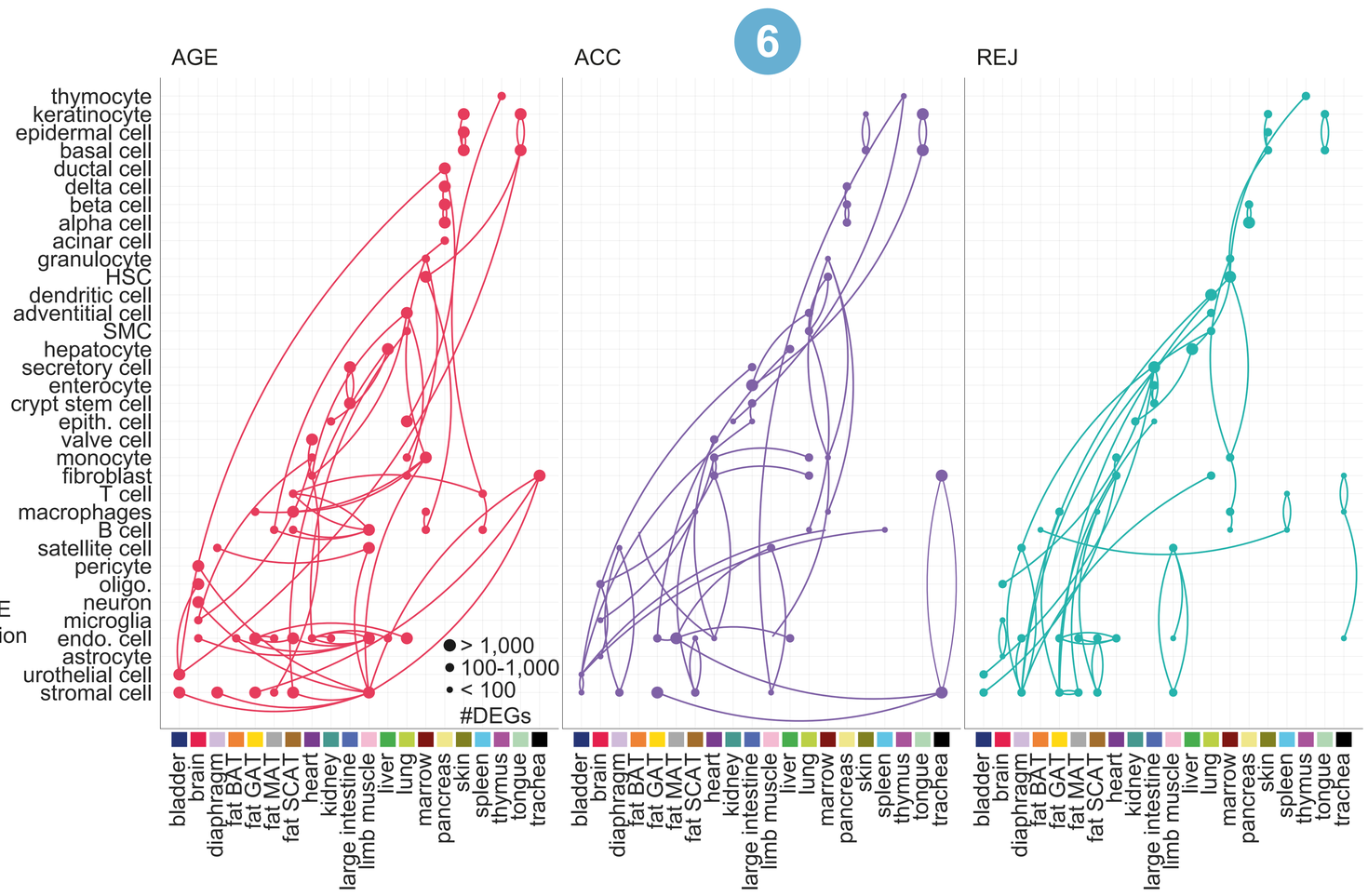
ACC and **AGE** *same* direction

REJ and **AGE** *opposite* direction



Structural results

- Cosine similarities between cell types – separately for **AGE**, **ACC**, and **REJ** – are calculated based on the vectors of gene specific log2-fold changes
- **Fig. 6**: Each cell type is connected to its most similar cell type, cell types of the same tissue are listed vertically, similar cell types with different tissue of origin are listed horizontally



Summary

Our dataset provides a first systematic look into the transcriptomic effects of heterochronic parabiosis at single-cell resolution across the entire organism. Continuous exposure to differentially aged blood alters the transcriptomic landscape across cell types, and we discovered that particular cell types – MSCs, HSCs, and hepatocytes – are especially susceptible to gene expression changes. Whereas the effects of aged blood tend to accelerate normal aging changes, young blood both reverses age-related profiles and initiates new pathways. Systemic rejuvenation of genes encoding components of the electron transport chain is especially notable, as is the reversal of global gene expression loss with age. Together, these findings reveal the details of how aging and parabiosis trigger global, tissue-specific, and cell-type-specific responses across the organism.

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