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Non-biased analysis of ACL tissue identifies sex-based differences in gene expression and a novel ligament progenitor population with distinct differentiation capacity

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Disclosure Information

- We have nothing to disclose



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Introduction

- The rate of anterior cruciate ligament (ACL) tears has increased over the past 20 years
 - ~68.6 per 100,000 person-years
- Female tear rates are 2-8x higher than male counterparts
- Following ACL reconstruction, retear rates are similar between sexes
 - Suggests an intrinsic difference in native male and female ACL tissue biology
- **Hypothesis:** There are sex differences in the composition of the ACL at the cellular and transcriptomic level that can help explain the increased female ACL tear rates



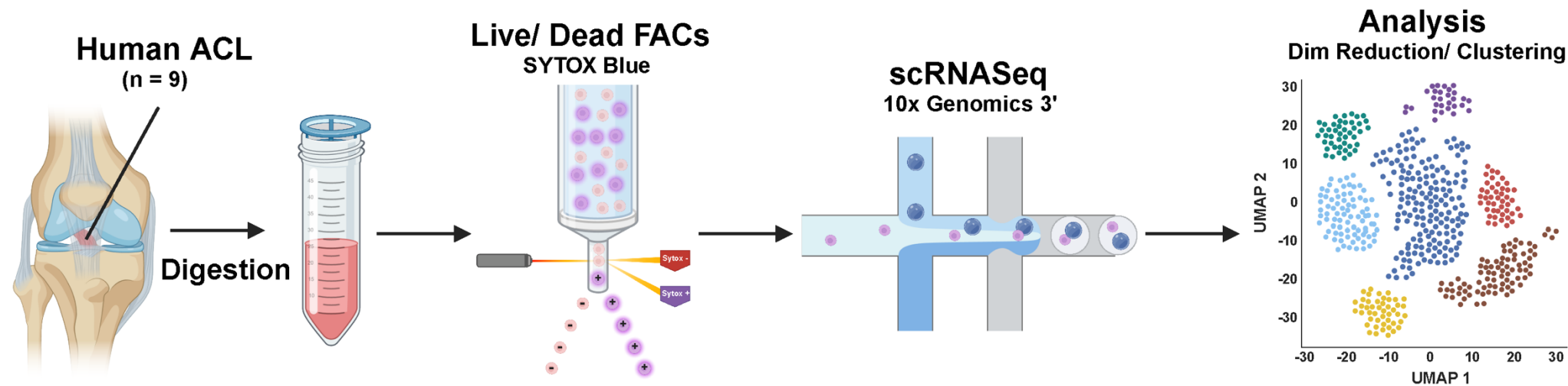
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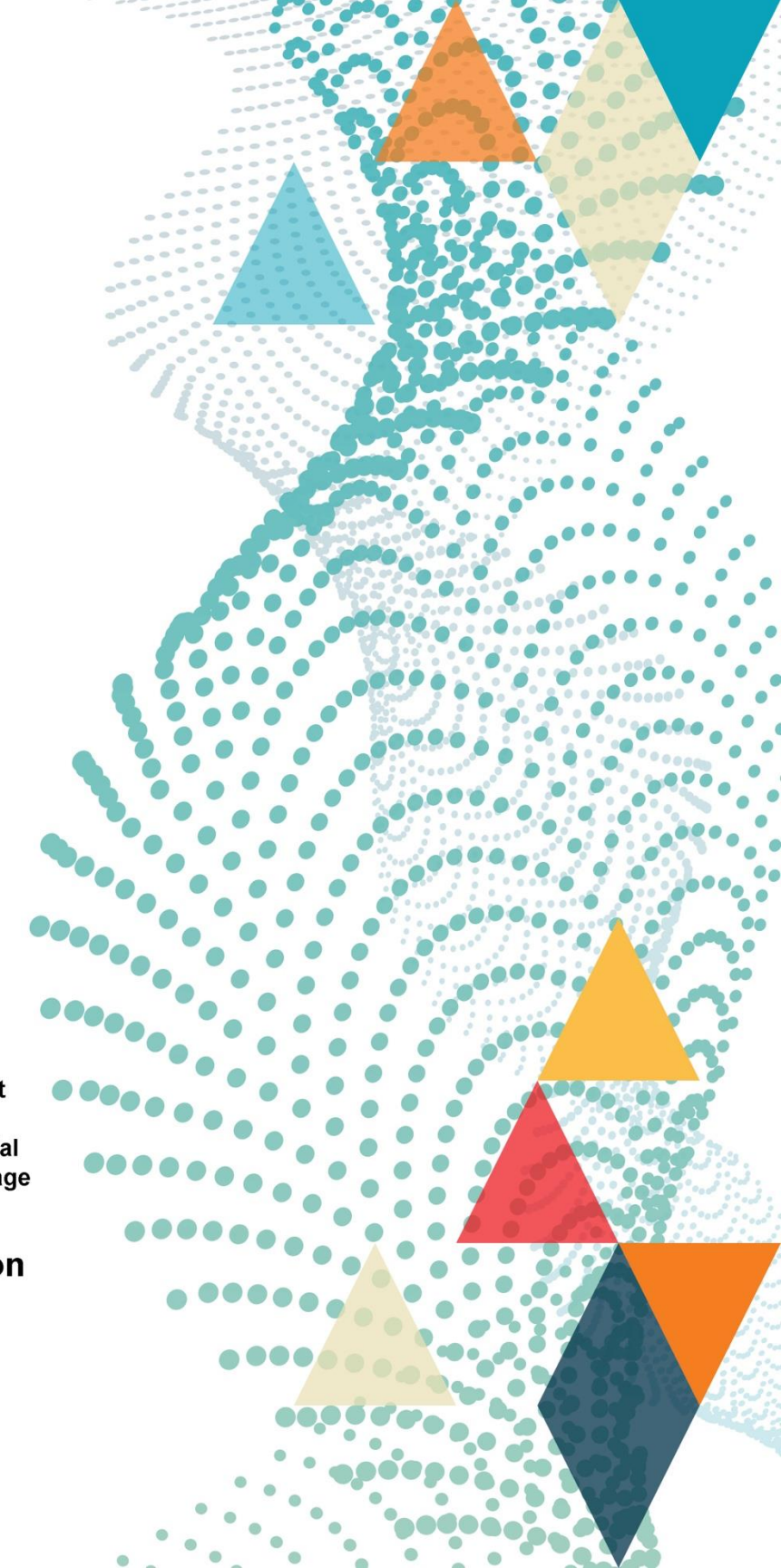
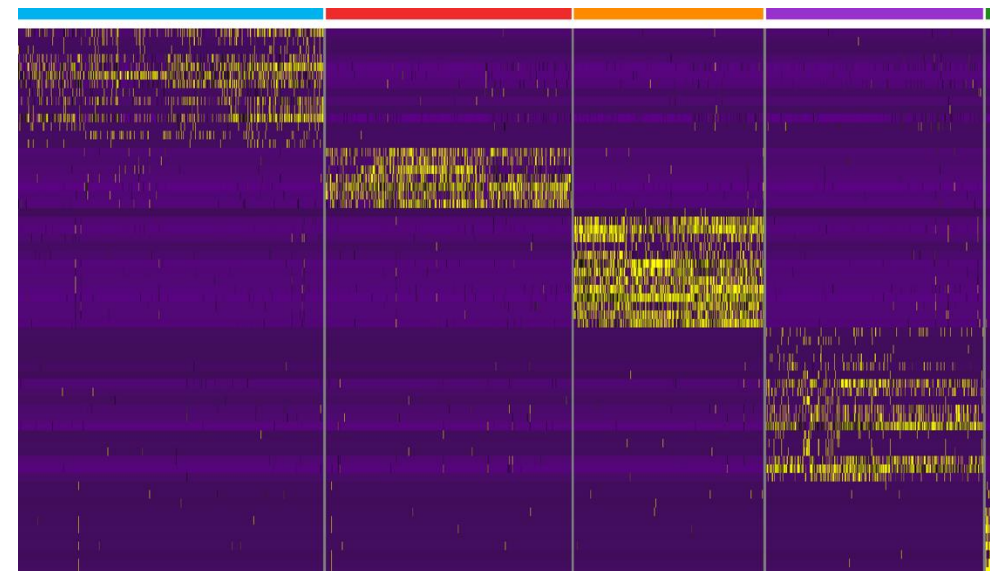
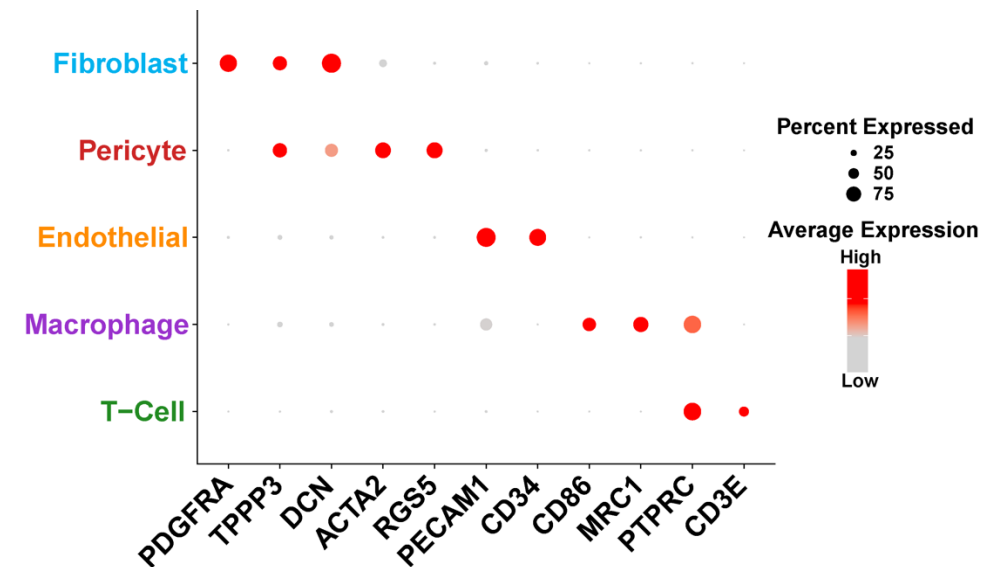
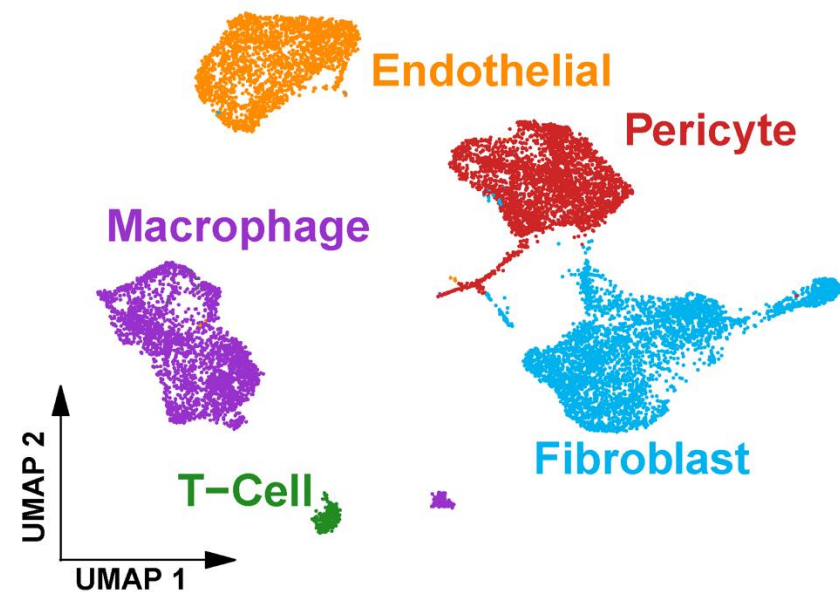
Methods – Isolation and scRNA-seq of ACL cells

- Human ACL tissue from 4 males and 5 females
- Flow cytometry used to isolate live cells
- Single-cell RNA-seq performed via 10x Genomics
- Data processed using CellRanger, Freemuxlet, and Seurat



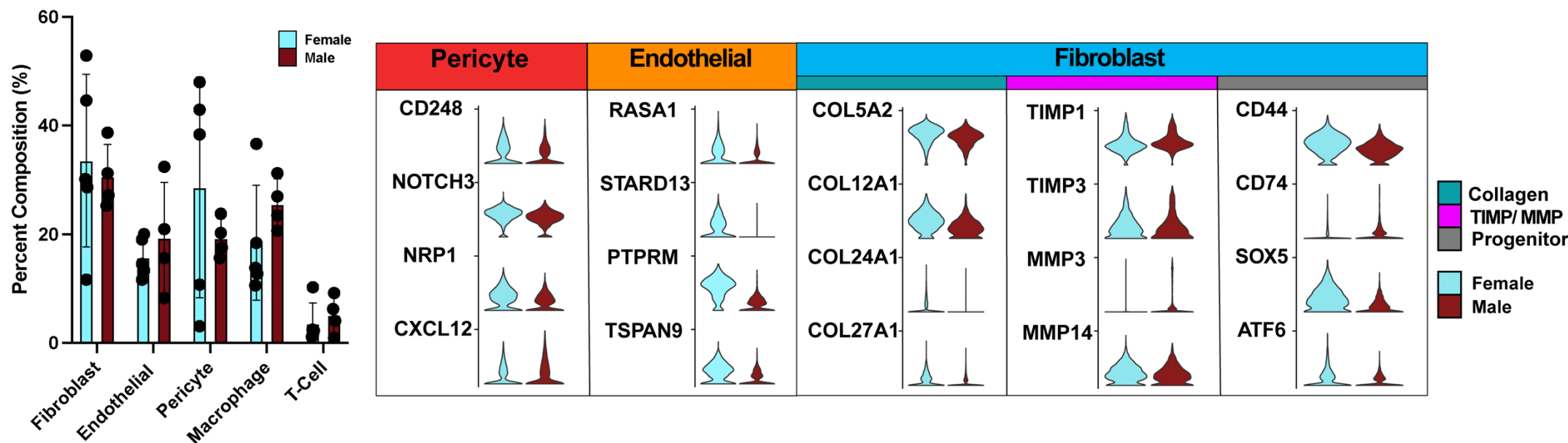
Results – ACL cell heterogeneity

- Five distinct celltypes identified
 - Using expression of known canonical gene markers



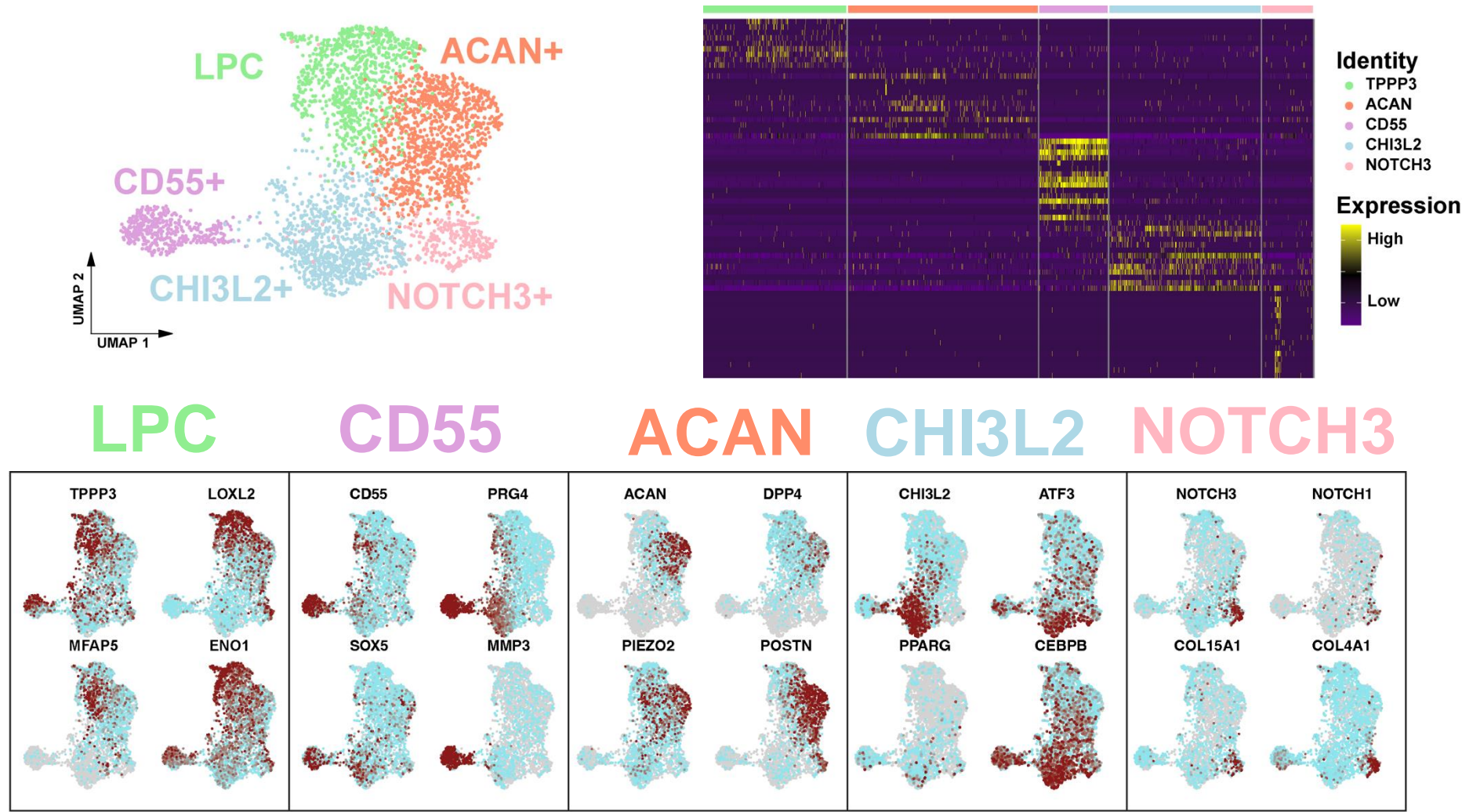
Results – Sex-based differences in ACL cells

- Similar cell type composition between male and female samples
- Differential gene expression observed in specific cell types across sexes



Results – ACL fibroblast heterogeneity

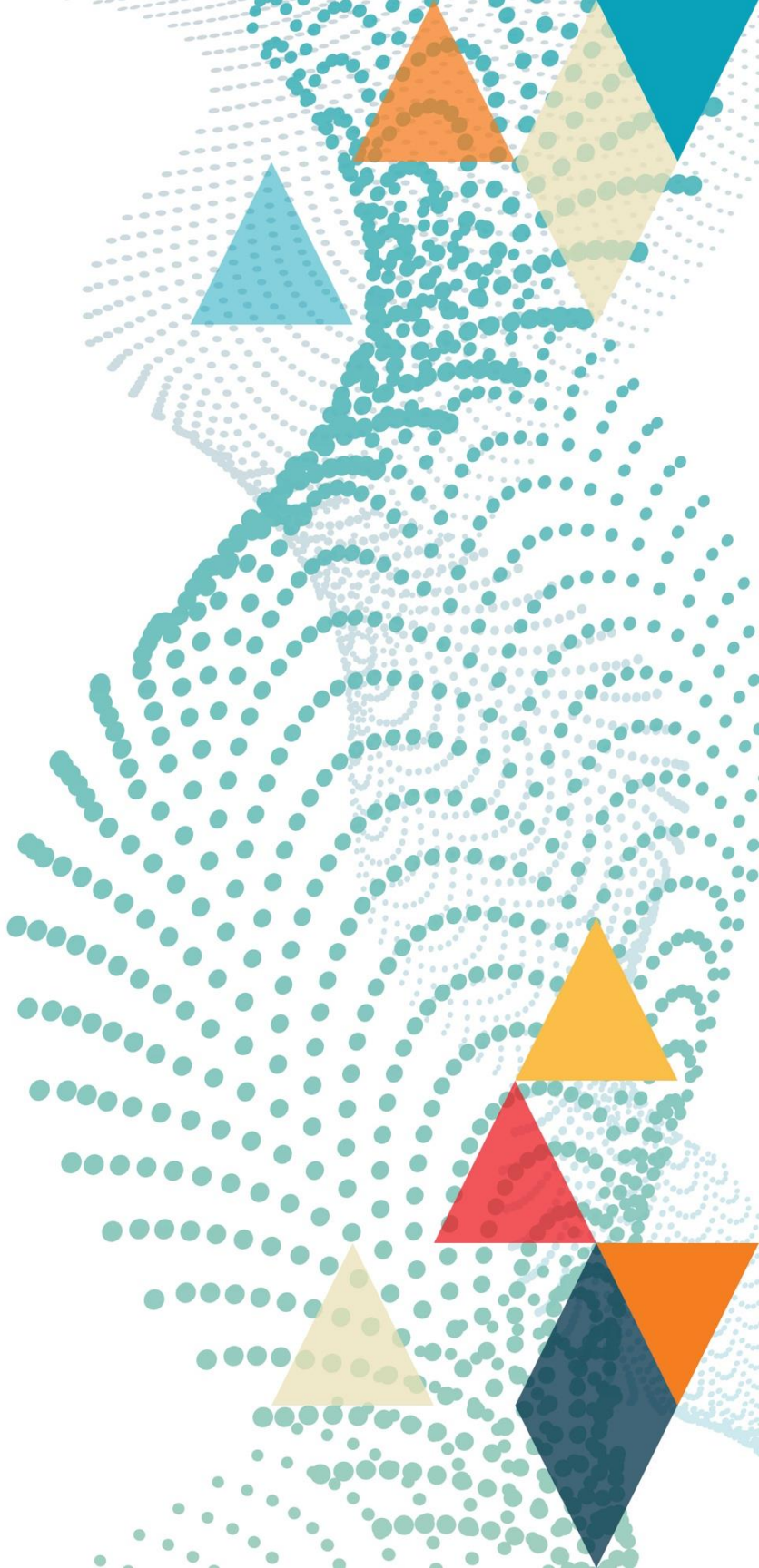
- ACL fibroblasts contain five unique subpopulations
 - Including a PDGFR α + / TPPP3+ ligament progenitor cell (LPC) population



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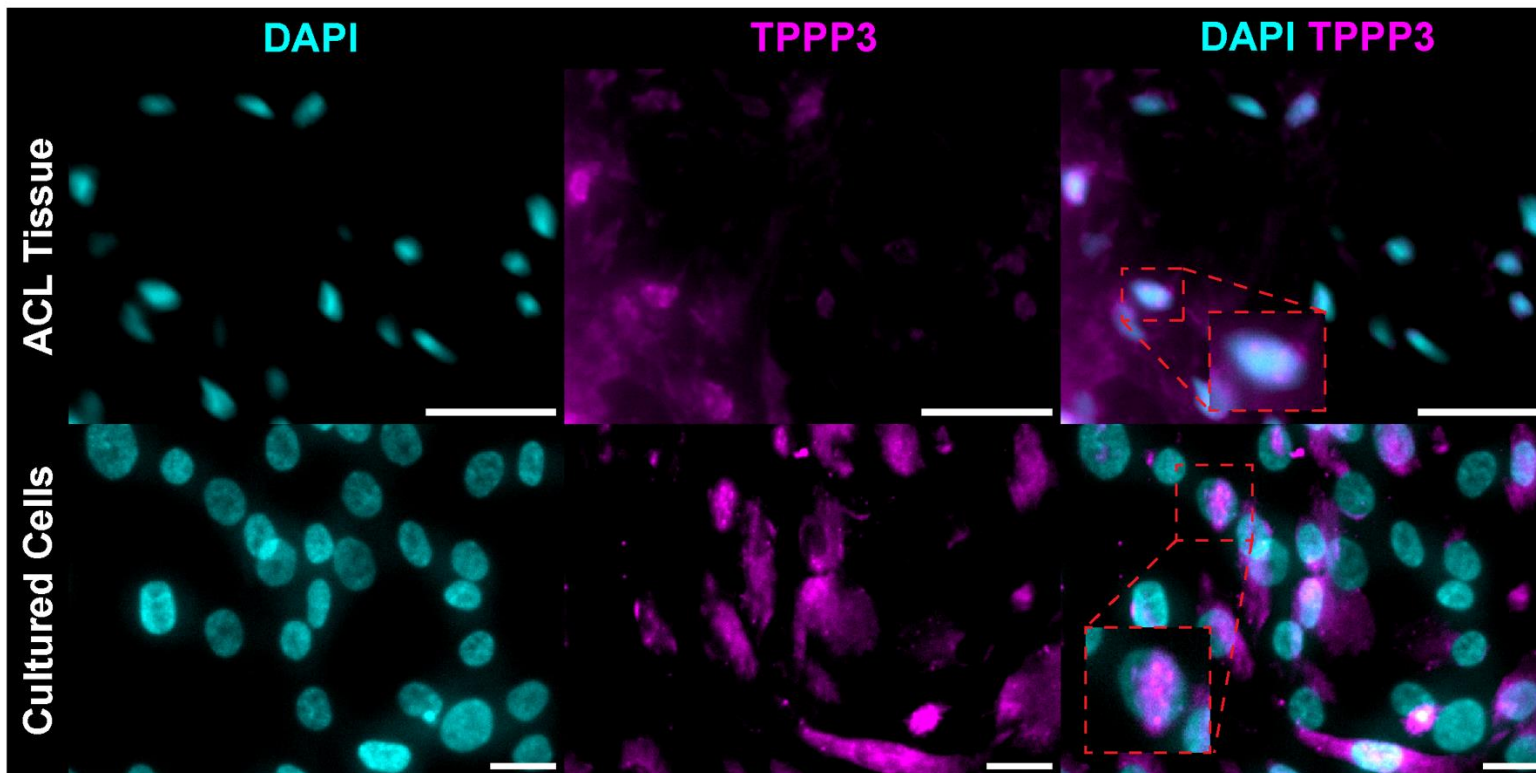


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Results – In vitro characterization of TPPP3 expression in ACL

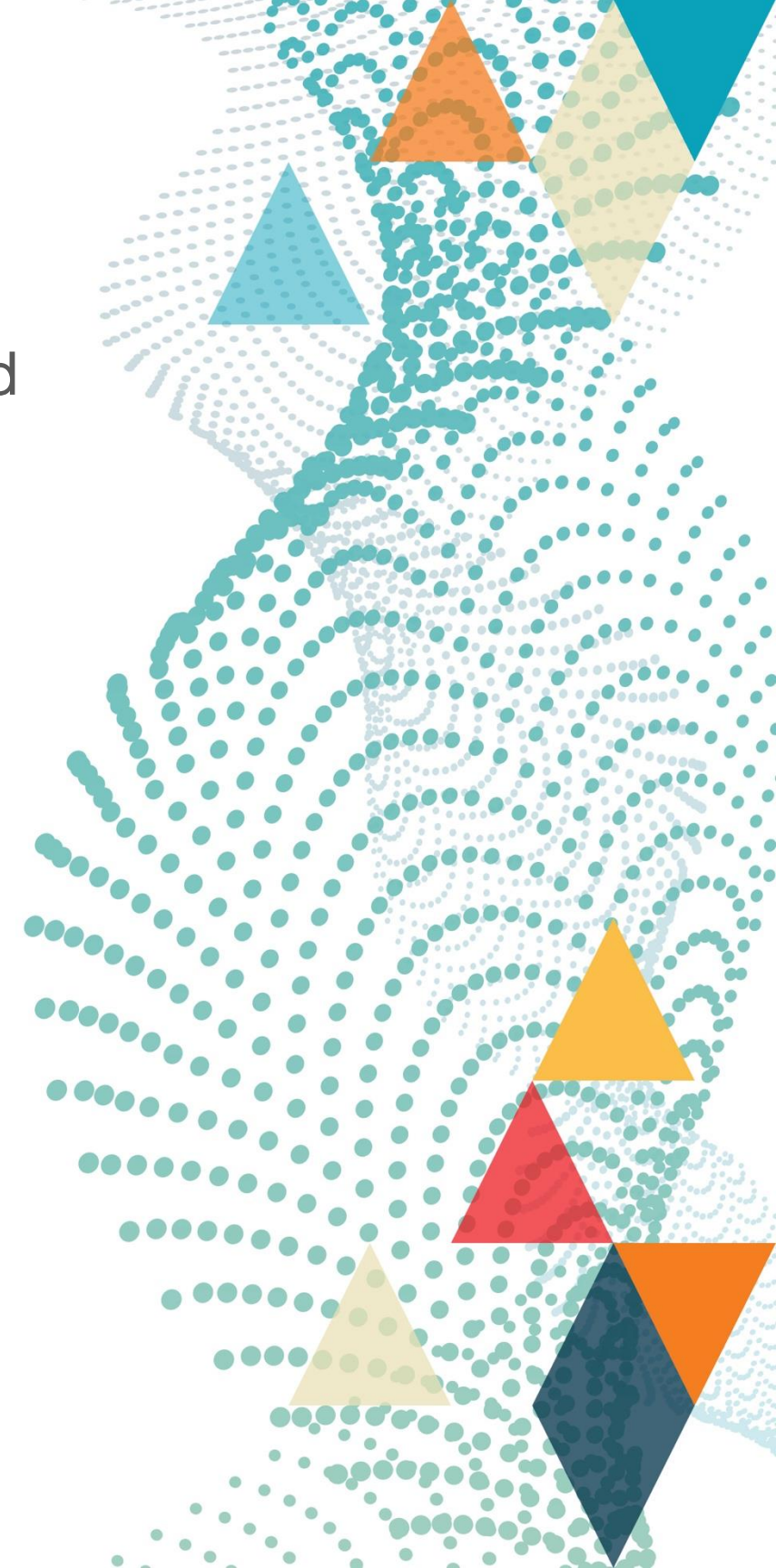
- TPPP3 expression detected by IHC staining in ACL tissue and cultured PDGFR α ⁺ ACL cells



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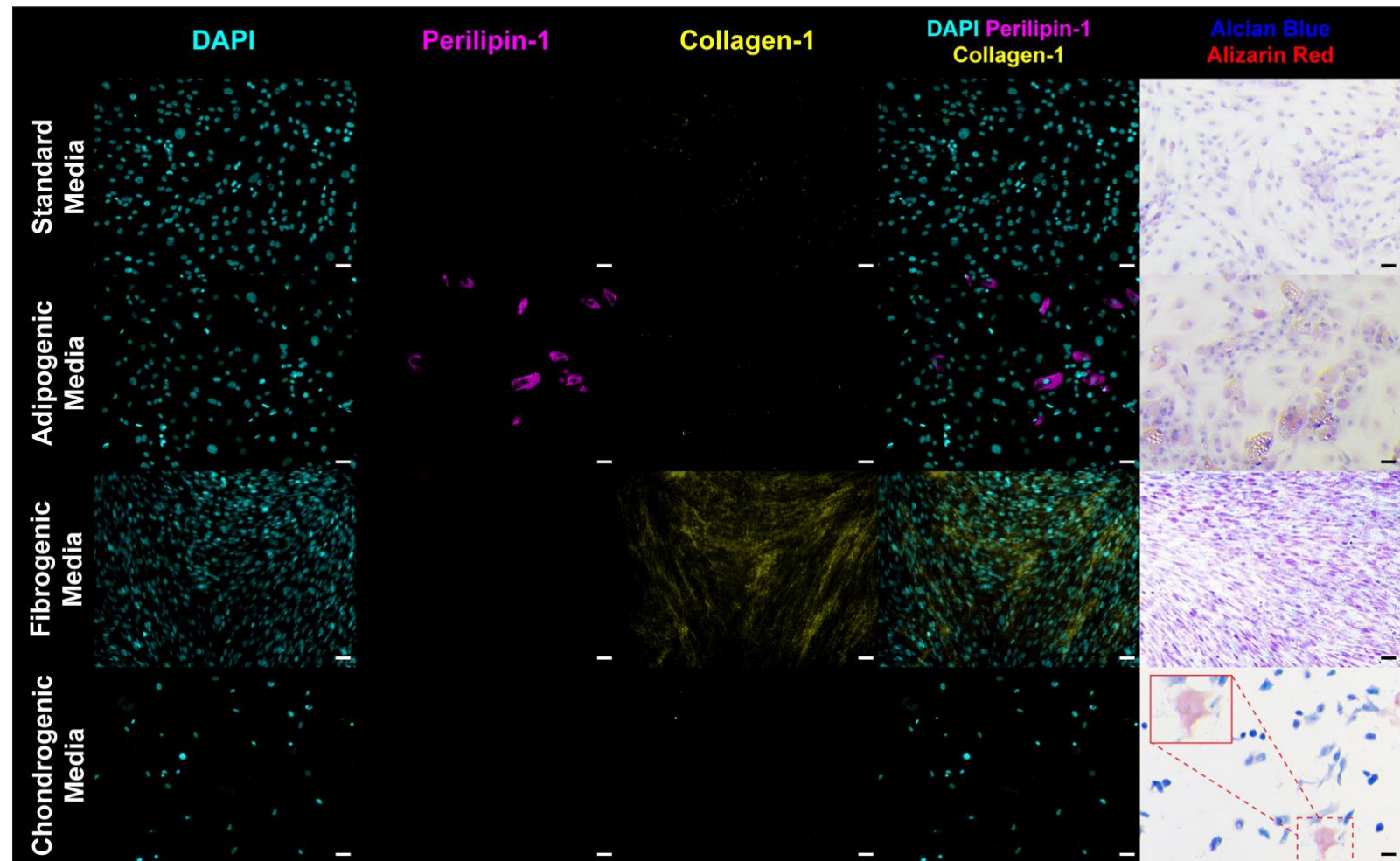
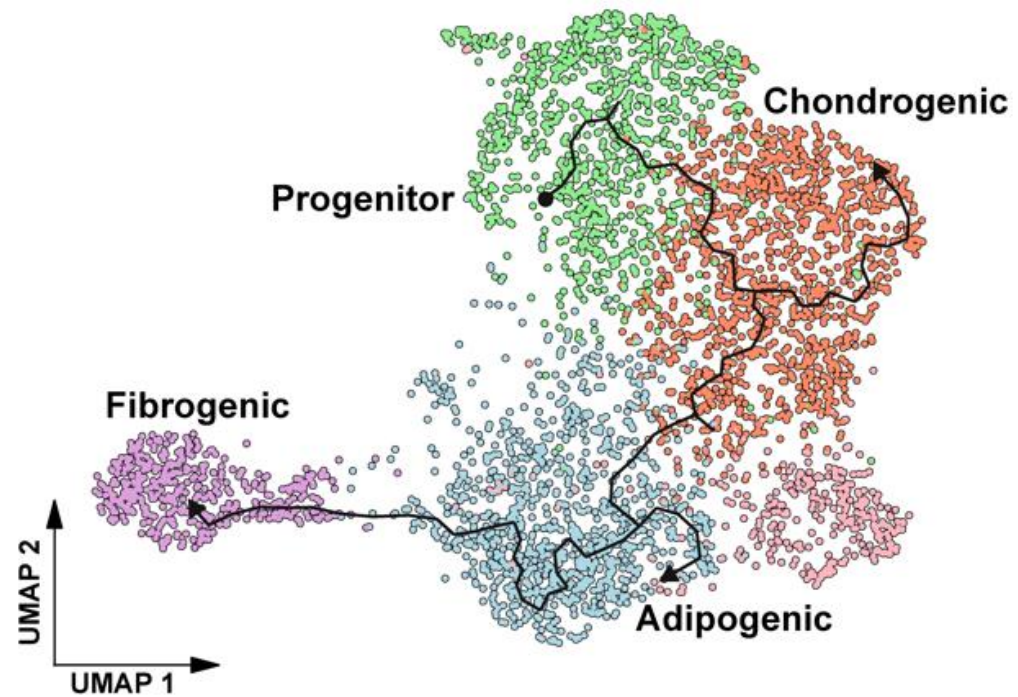


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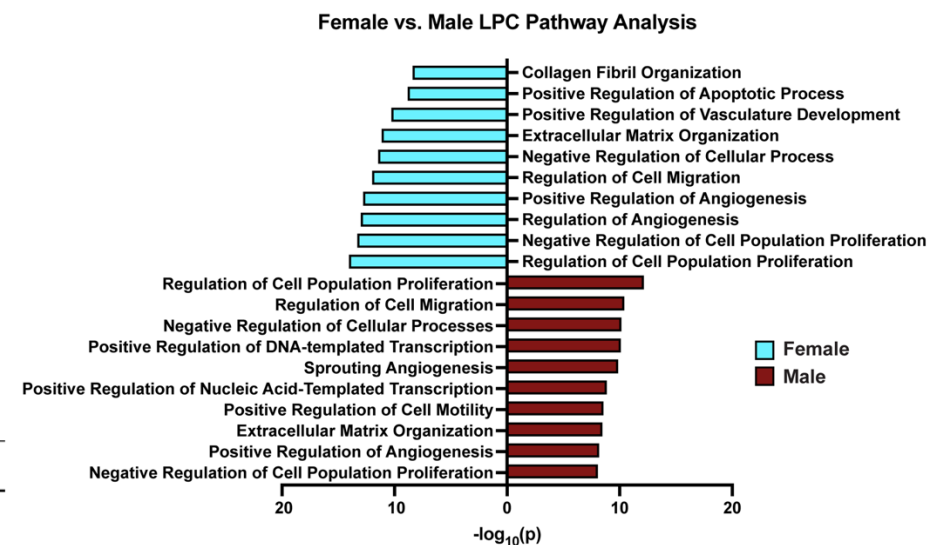
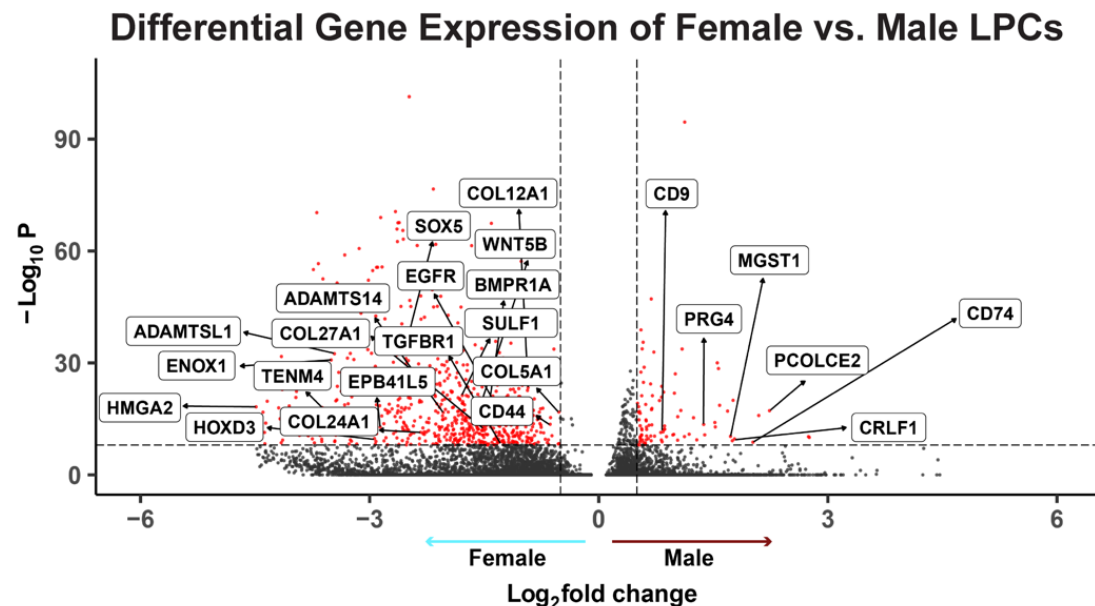
Results – Functional Capacity of LPCs

- LPCs show multipotent differentiation ability
- Evidence for fibrogenic, adipogenic, and chondrogenic potential



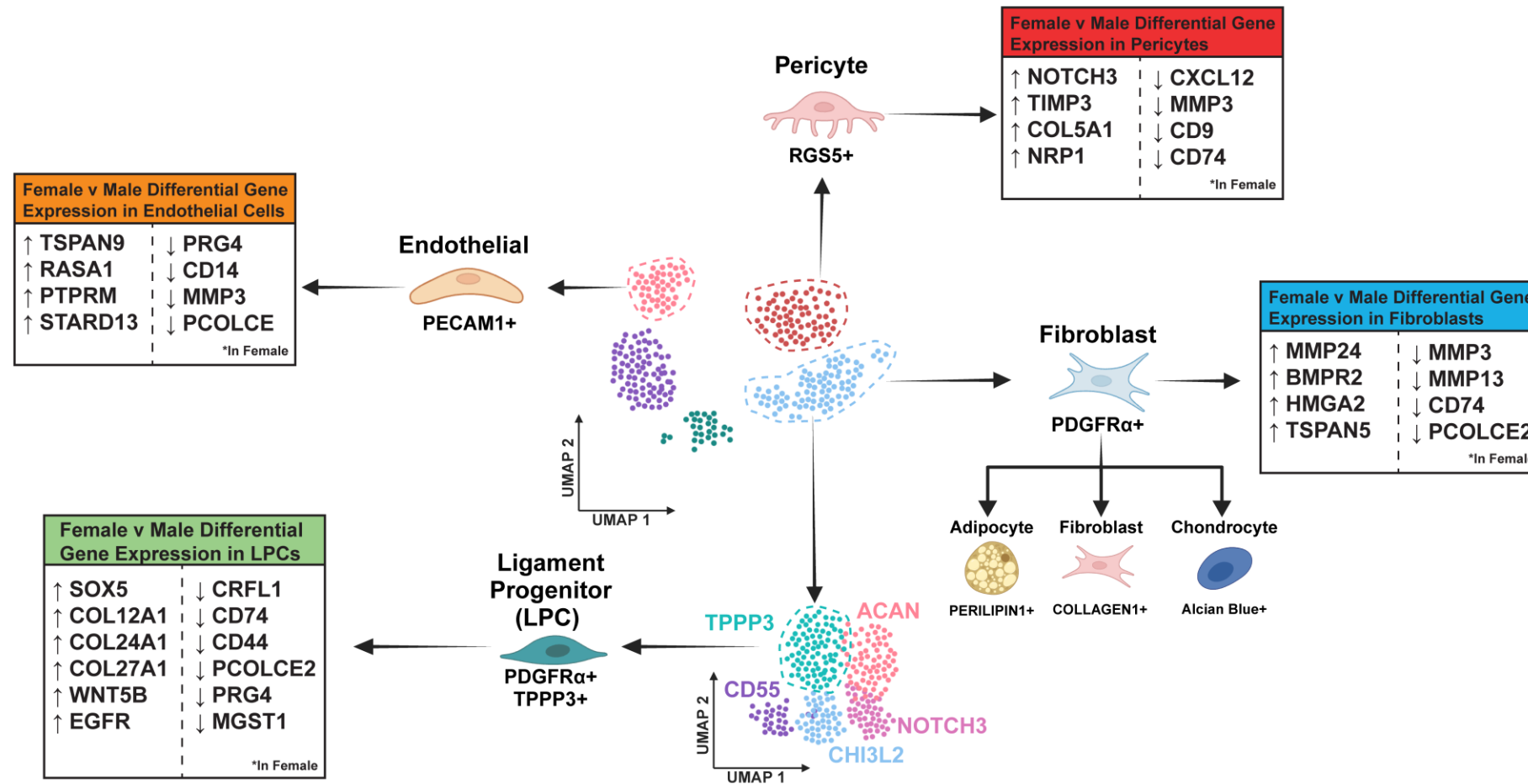
Results – Sex-based differences in LPCs

- ECM and collagen-related gene expression differs by sex
 - Upregulation of rupture-associated genes in females
 - Upregulation of ECM integrity and stemness-associated genes in males
- Key biological pathways differ by sex, including
 - Angiogenesis, collagen organization, apoptosis, and cell proliferation



Characterization of ACL cell populations and sex-based differences

- Provides evidence for a cell-intrinsic explanation for increased ACL ruptures in females



References

- Sanders TL, Maradit Kremers H, Bryan AJ, et al. Incidence of Anterior Cruciate Ligament Tears and Reconstruction. *Am J Sports Med*.
- Novaretti JV, Astur DC, Casadio D, et al. Higher Gene Expression of Healing Factors in Anterior Cruciate Ligament Remnant in Acute Anterior Cruciate Ligament Tear. *Am J Sports Med*.
- Posthumus M, September A V, O'Cuinneagain D, van der Merwe W, Schwellnus MP, Collins M. The association between the COL12A1 gene and anterior cruciate ligament ruptures. *Br J Sports Med*.
- Arendt EA, Agel J, Dick R. Anterior cruciate ligament injury patterns among collegiate men and women. *J Athl Train*. 1999;34(2):86-92.
- Beaudry K, De Lisio M. Sex-based differences in Muscle Stem Cell Regulation Following Exercise. *Exerc Sport Sci Rev*. Published online March 6, 2024.
- Gupta AS, Pierpoint LA, Comstock RD, Saper MG. Sex-Based Differences in Anterior Cruciate Ligament Injuries Among United States High School Soccer Players: An Epidemiological Study. *Orthop J Sports Med*. 2020;8(5):232596712091917.
- Jang MK, Kim CH, Seong JK, Jung MH. ATF3 inhibits adipocyte differentiation of 3T3-L1 cells. *Biochem Biophys Res Commun*. 2012;421(1):38-43.
- Harvey T, Flamenco S, Fan CM. A Tppp3+Pdgfra+ tendon stem cell population contributes to regeneration and reveals a shared role for PDGF signalling in regeneration and fibrosis. *Nat Cell Biol*. 2019;21(12):1490-1503.
- Weidenfeld K, Schiff-Zuck S, Abu-Tayeh H, et al. Dormant tumor cells expressing LOXL2 acquire a stem-like phenotype mediating their transition to proliferative growth. *Oncotarget*. 2016;7(44):71362-71377.
- Rosen ED, Hsu CH, Wang X, et al. C/EBP α induces adipogenesis through PPAR γ : a unified pathway. *Genes Dev*. 2002;16(1):22-26.



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