Protocol

# 1 Procedures

1. To find data suitable for our purpose, we searched the NCBI Gene Expression Omnibus database (GEO), and find the CD31 enriched single-cell RNA-seq of choroidal described by Voigt[[8](#ref-Voigt2019a)], which characterized the arteriole, vein, and choriocapillaris endothelial cells.
2. The choriocapillaris endothelial cells are used to define the potential ligands expressed by the choriocapillaris endothelial cells that might regulator macular degeneration related genes expressed by RPE.
3. The single-cell trajectory was conducted on choriocapillaris endothelial cells among CD31 enriched patients to find the differentially expressed ligands along the trajectory using monocle3[[4](#ref-Qiu2017),[5](#ref-Qiu2017a),[7](#ref-Trapnell2014a)], batch effects were removed using the method described by Haghverdi[[1](#ref-Haghverdi2018)].
4. For the RPE data, we use the bulk RNA-seq data mentioned by Kim[[2](#ref-Kim2018a)], which they find a significant impact on the anti-sense transcripts between the normal and early AMD patients compare to the sense transcripts.
5. This data is used to define the background expressed genes and age-related macular degeneration genes in early AMD.
6. Differential expression analysis was carried out with edgeR-limma[[3](#ref-McCarthy2012),[6](#ref-Ritchie2015)] R/Bioconductor packages between normal and early AMD patients.

# 2 **References**

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