

Expanded View Figures

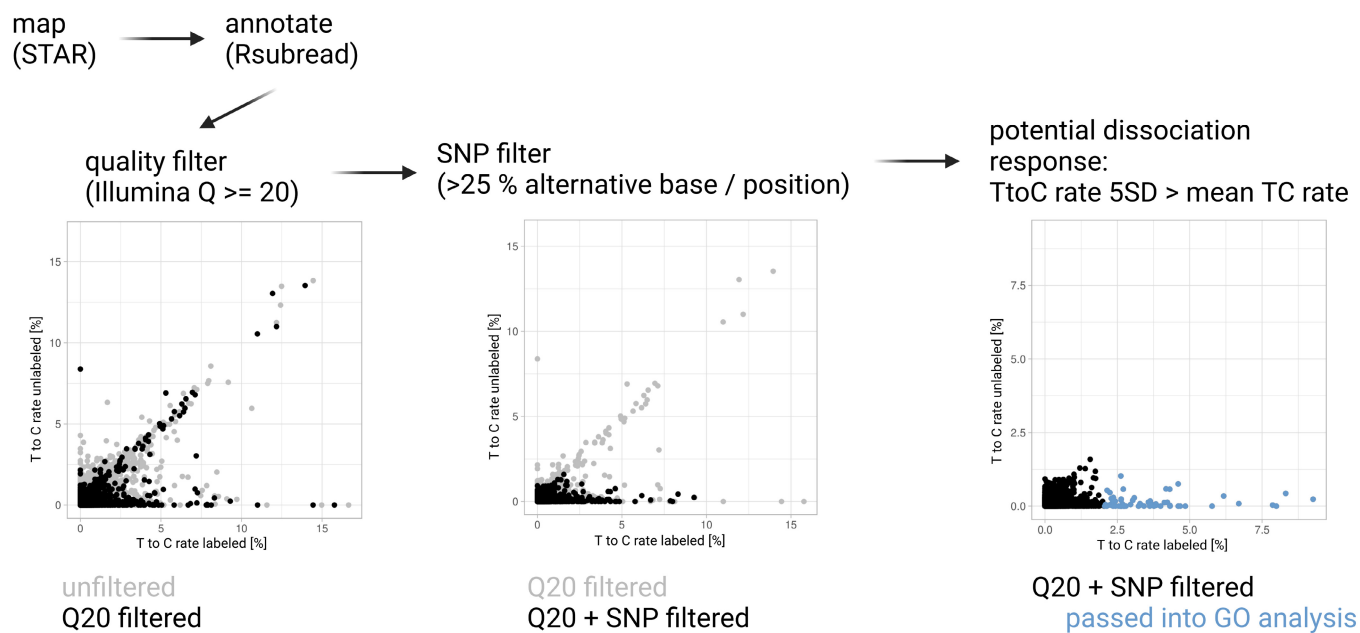


Figure EV1. Computational workflow for bulk data.

Reads are mapped using STAR, annotated using Rsubread and passed through quality and SNP filtering steps before selecting genes for GO analysis based on a T-to-C conversion rate at least 5 standard deviations larger than the mean.

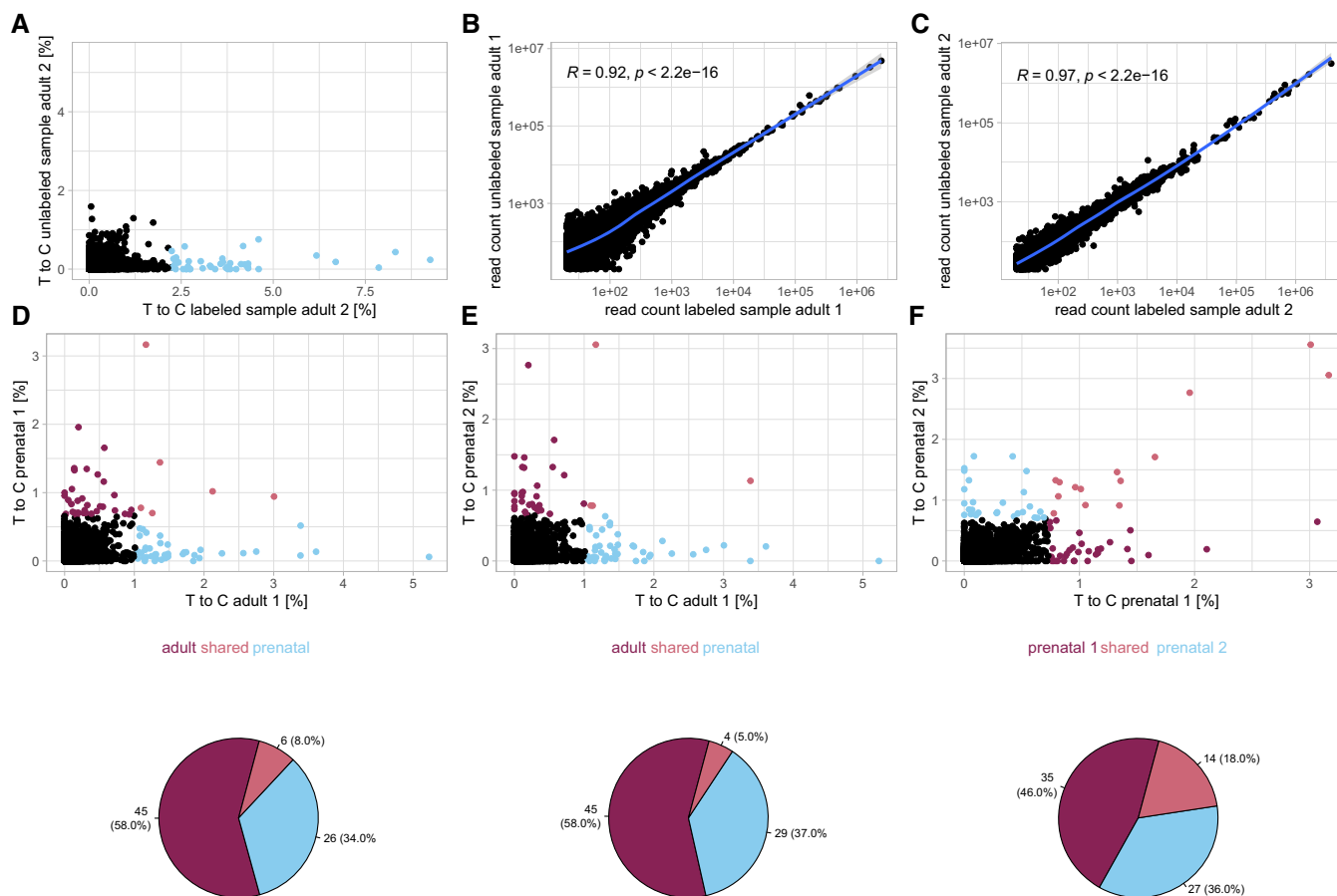


Figure EV2. Biological replicates for cardiomyocyte dissociation experiments.

- A Adult mouse cardiomyocytes, replicate 2, T-to-C rates per gene in labeled (4sU added) and unlabeled (no 4sU added) sample. Blue ($\sim 1\%$ of genes): T-to-C rate ≥ 5 standard deviations.
- B, C Read count correlation between labeled and unlabeled adult mouse cardiomyocyte samples. Blue lines show sliding window average (smoothed conditional mean) and 95% confidence interval. R values calculated through Pearson correlation.
- D, E T-to-C rates per gene in prenatal vs. adult cardiomyocytes. Genes with T-to-C rate ≥ 5 standard deviations in one or both samples are highlighted.
- F T-to-C rates per gene in prenatal replicate 1 vs. prenatal replicate 2. Genes with T-to-C rate ≥ 5 standard deviations in one or both samples are highlighted.

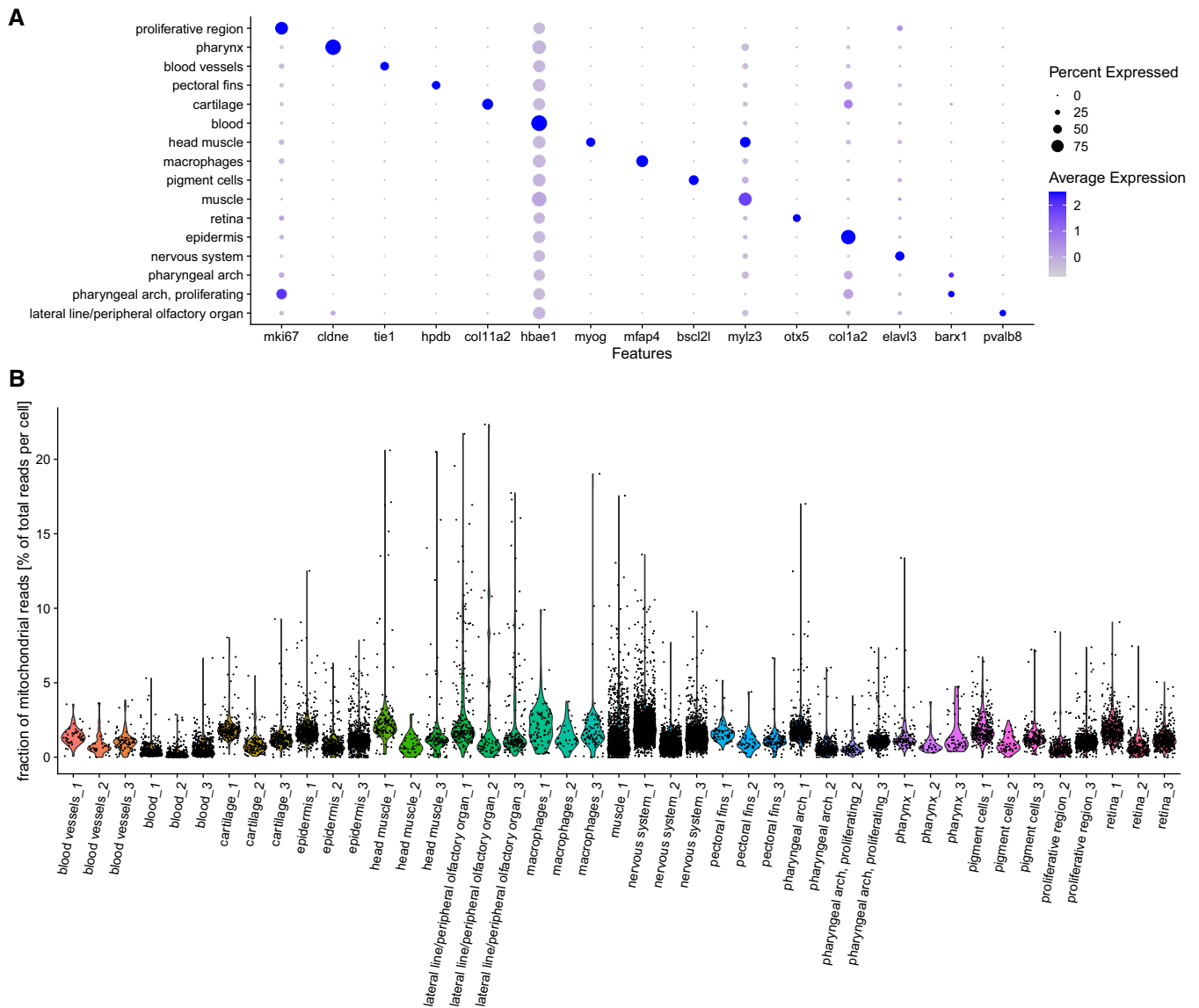


Figure EV3. Characterization of single cell zebrafish data.

- A Marker gene expression in 48 hpf zebrafish cells, data from all three samples is merged.
- B Fraction of mitochondrial reads per cell, cell types are separated by sample of origin.

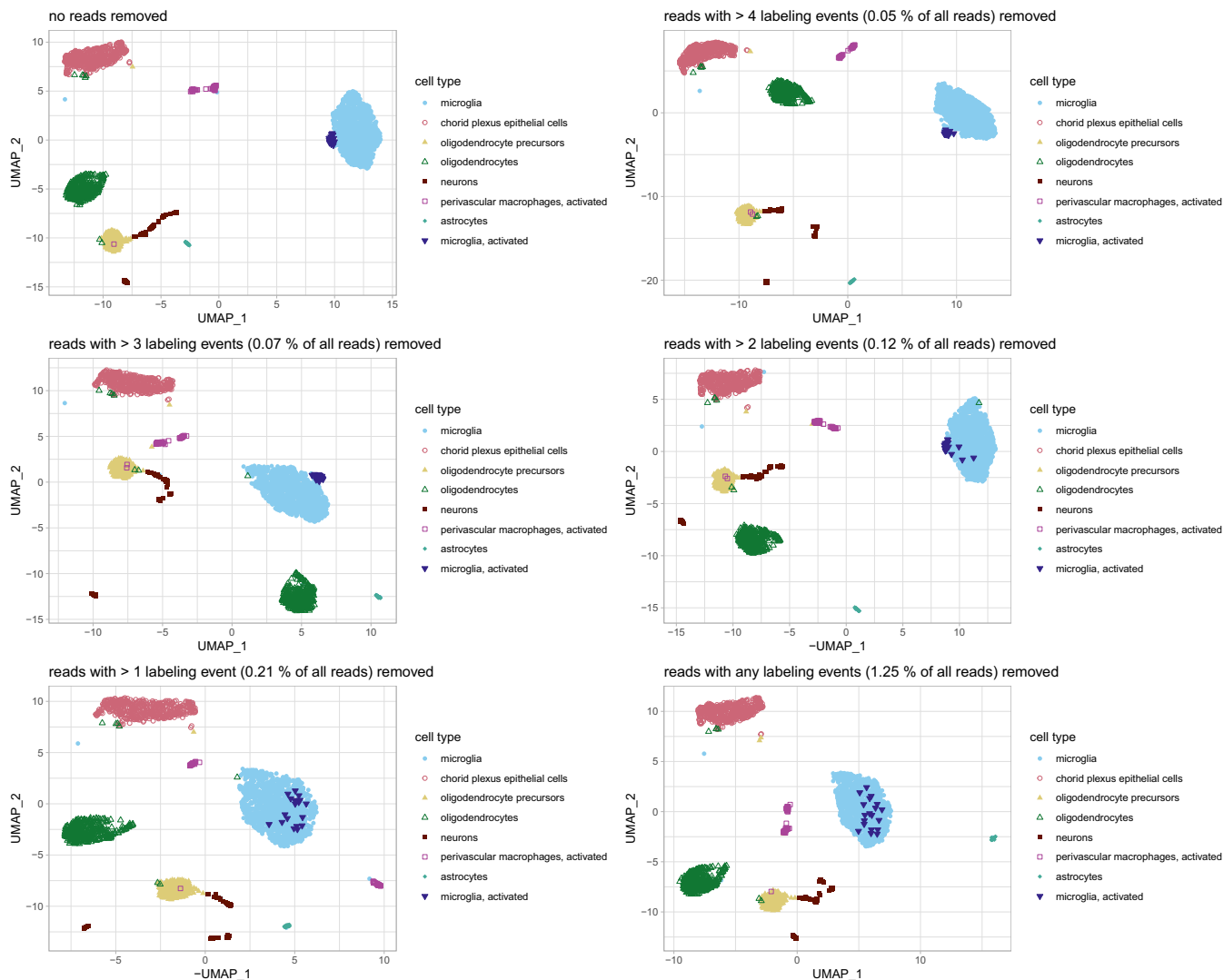


Figure EV4. Dependence of clustering results on labeled transcripts.

Gradual removal of labeled UMIs leads to the activated microglia cluster merging with the non-activated microglia.