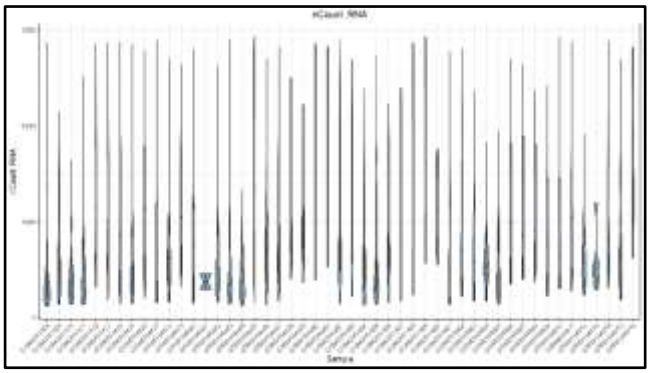
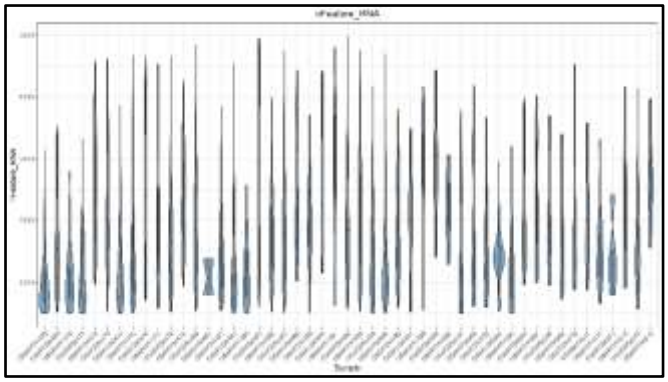
QUALITY CONTROL (Pre and post)



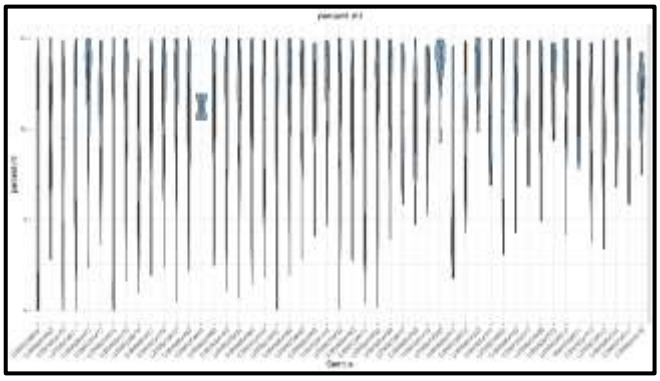
nCount RNA

Most cells have median UMI count



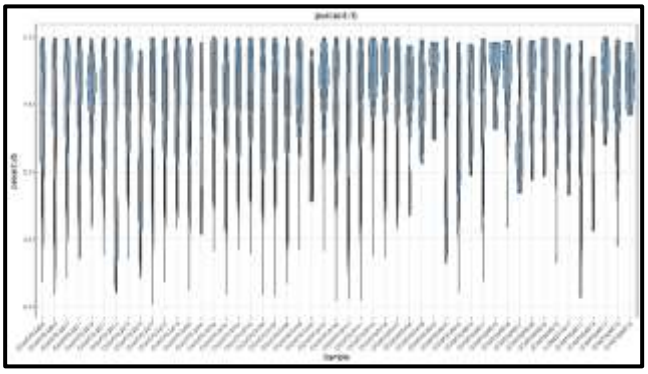
nFeature RNA

Violin plots show most of the samples have 500 -3000 UMI counts meaning the cells are healthy.



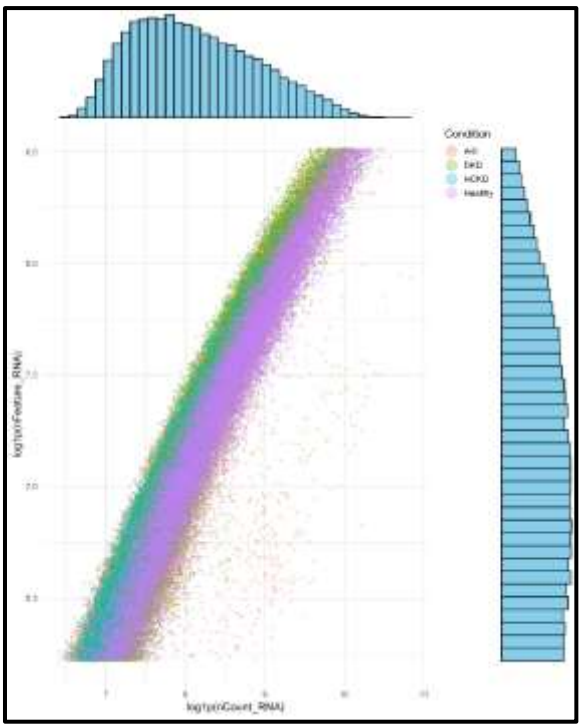
Post QC percent mt violin plot

Most of the samples have less than 15% mitochondrial content, conforming QC filtering was effective

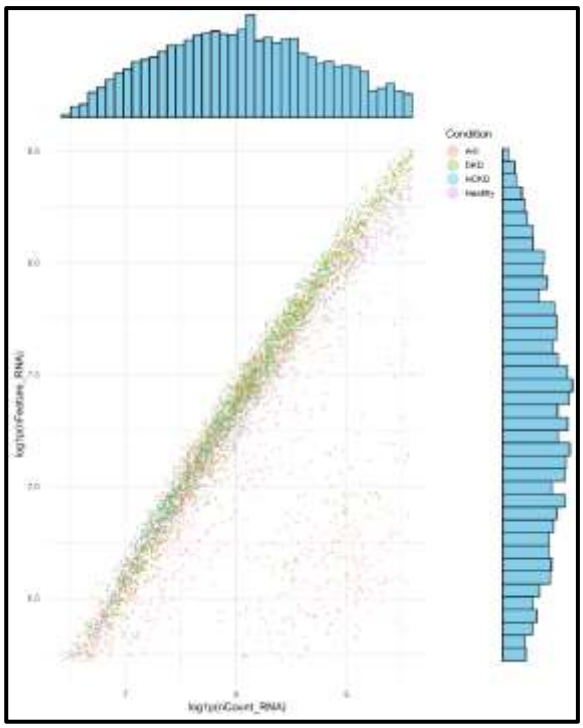


Post QC percent rb violin plot

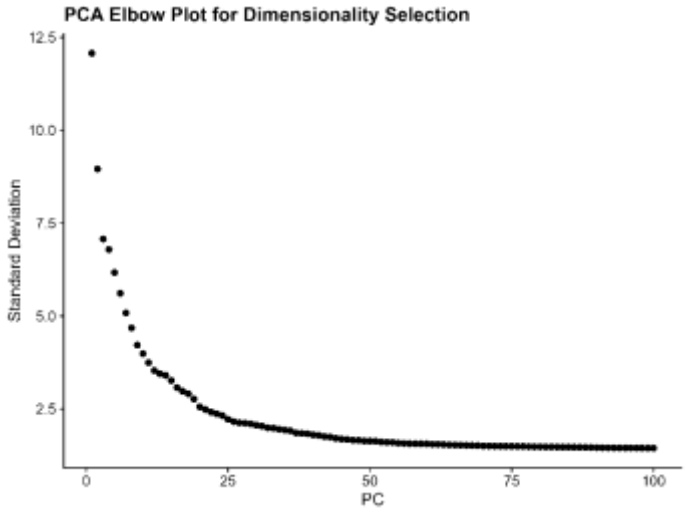
Most of the samples have less than 10% ribosomal content



Pre QC density scatter plot

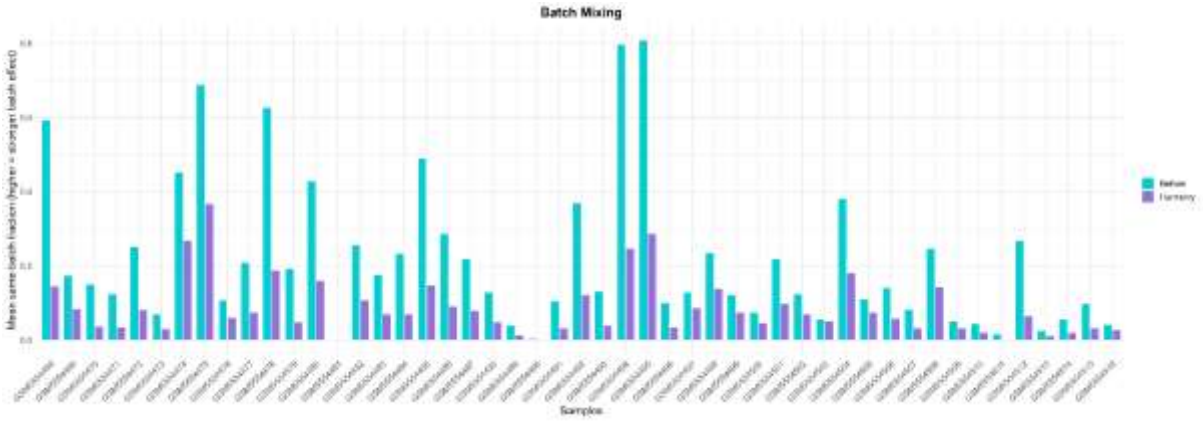


Post QC density scatter plot

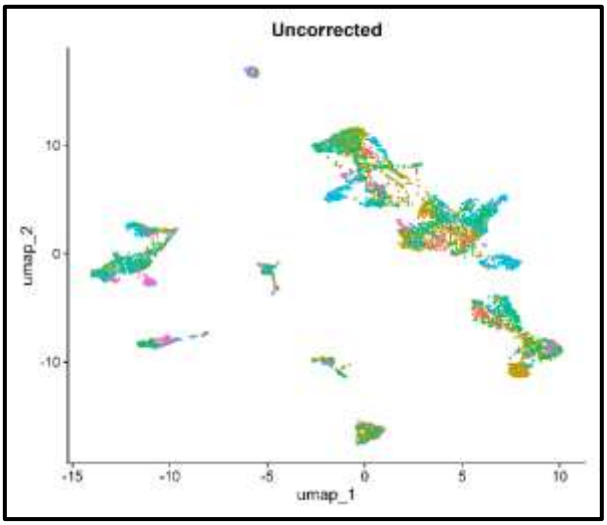


Elbow plot

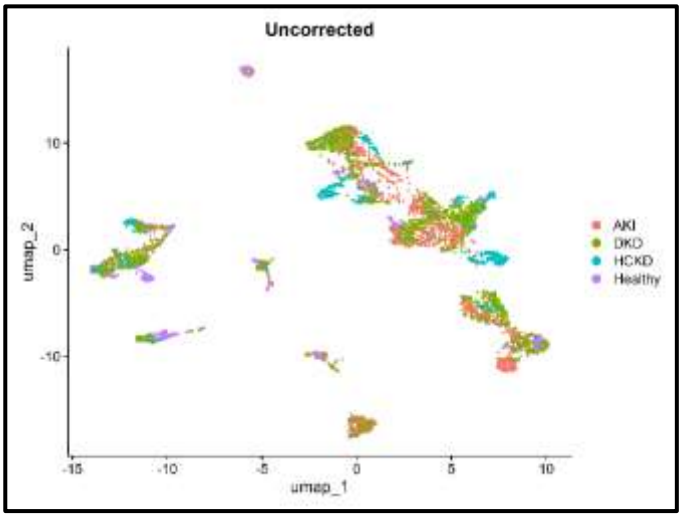
BATCH CORRECTION



Batch correction plot



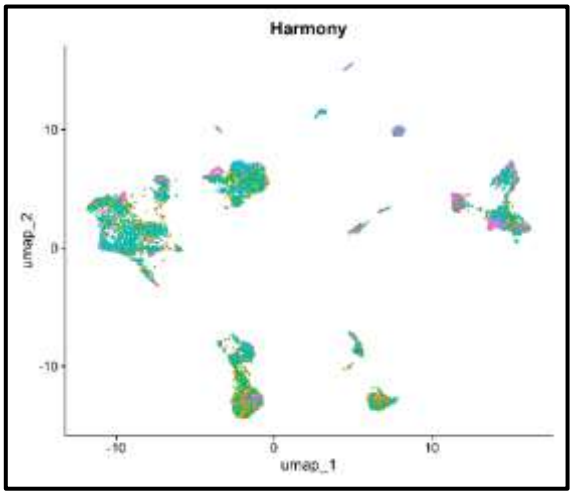
Raw PCA sample



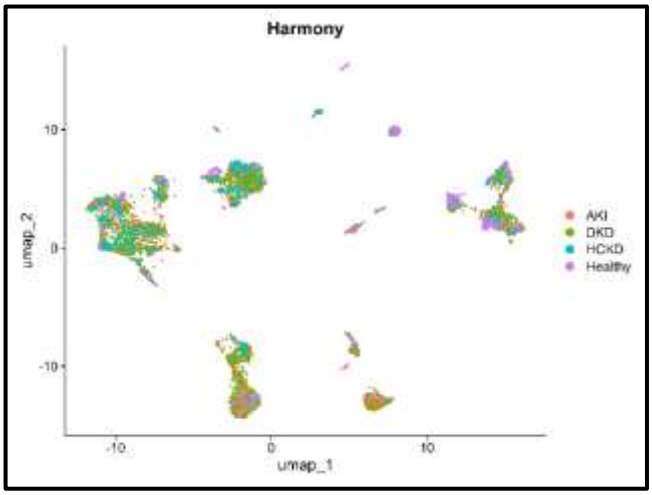
Raw PCA condition

Before batch correction, each sample forms its own cluster. It is not based on any biological conditions or grouping of replicates together. Samples are scattered across multiple clusters. There is no clear separation. The data is dominated by batch not by any condition. Hence, we can say that if we proceed without any corrections, it might give us false results.

Harmony integration



Harmony sample



Harmony condition

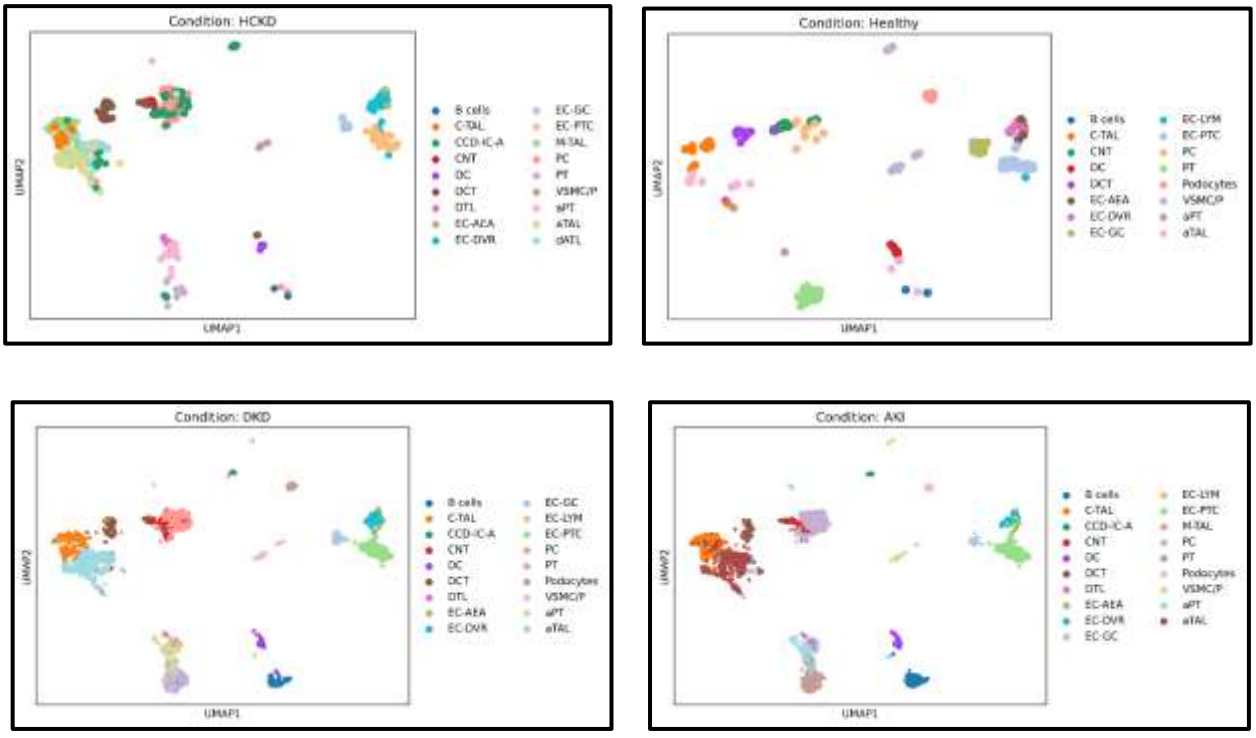
After we use Harmony, UMAP's are regenerated, the clusters are formed now based on the conditions. This is further confirmed by UMAP generation based on conditions. Bach effects are successfully removed.

CELL TYPE ANNOTATION PLOT (CELLTYPIST)

Celltypist is chosen here for annotation as it can annotate thousands of cells in a few seconds. It uses cross tissue immune and epithelial database.

Top predicted cell types are:

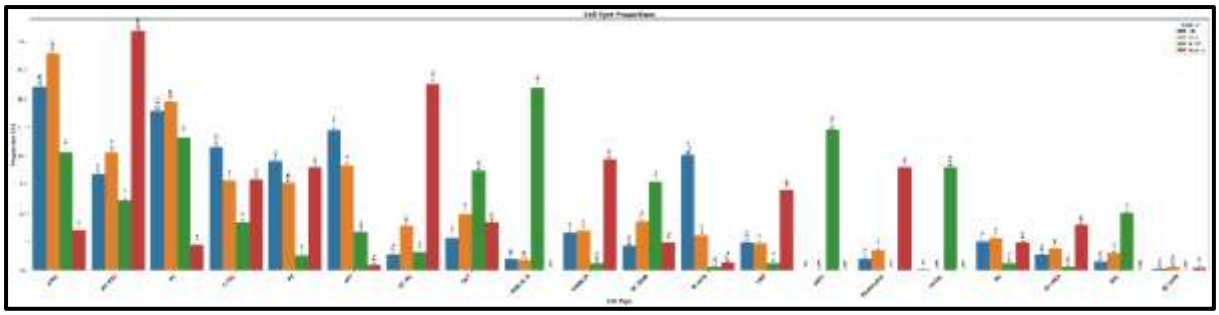
- *aTAL*
- *PC*
- EC-PTC
- *aPT*
- C-TAL



UMAP by condition

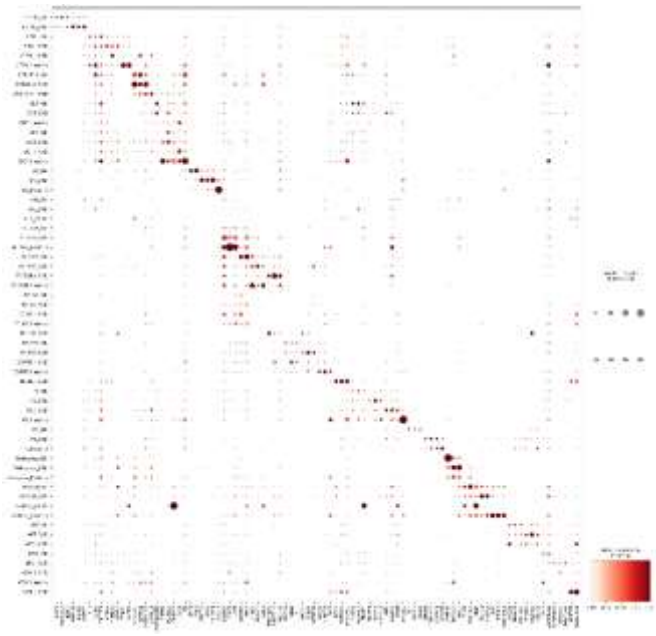
This visualization shows how different cell types appear across different conditions. AKI condition map shows highest variety of immune cells like B cells, plasma cells, macrophages. Thus, confirming acute inflammatory condition.

DKD condition map shows fibroblasts and myoblast cell populations. While healthy cells show epithelial and endothelial cells.



Cell proportions barplot

These plots show that AKI condition cells showed highest proportion of immune cells. This represents acute inflammation and tubular damage.



Cluster markergenes

DIFFERENTIAL EXPRESSION & PATHWAY ANALYSIS

Proceeded with the differential expression analysis of DKD v/s healthy to identify therapeutic target in disease condition.