**Methods**

The raw sequencing reads were first quality-checked using FastQC1 and adapter sequences were trimmed using TrimGalore (available online at https://www.bioinformatics.babraham.ac.uk/projects/trim\_galore/). The filtered reads were then mapped to the hg38 reference genome using Bowtie 2, and the genomic coordinates of the mapped reads were assigned to the reference genome annotation file using Bedtools 3. The annotation file used was based on the hg38 assembly and provided the genomic coordinates for known small RNA genes and features. The miRBase database (release v22.0) was used to identify and annotate microRNAs. The expression levels of small RNAs were quantified using R, and differential expression analysis was performed using the using DESeq2 package 4.

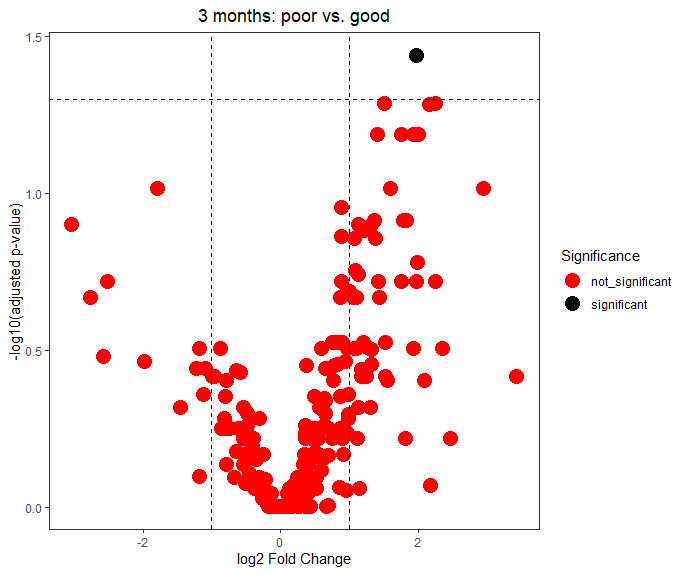
**Results**

3 months poor Vs good function

design = ~ three\_month\_status + Donor\_Age + Donor\_Gender\_M\_F

contrast\_3P <- c("three\_month\_status", "Poor", "Good")

Figure 1. Differential expression of miRNAs in individuals with poor versus good function at 3 months, highlighting the significant (P< 0.05) upregulation of a miRNA (hsa-miR-486-5p) in the poor functional status group compared to the good functional status group, after accounting for the effects of donor age and gender.



**12 month poor Vs good function**

design = ~ twelve\_month\_status + Donor\_Age + Donor\_Gender\_M\_F

contrast\_12P <- c("twelve\_month\_status", "Poor", "Good")

Figure 2. Differential expression of miRNAs in individuals with poor versus good function at 3 months, highlighting the significant (P< 0.05) downregulation of 17 miRNAs in the poor functional status group compared to the good functional status group, after accounting for the effects of donor age and gender.

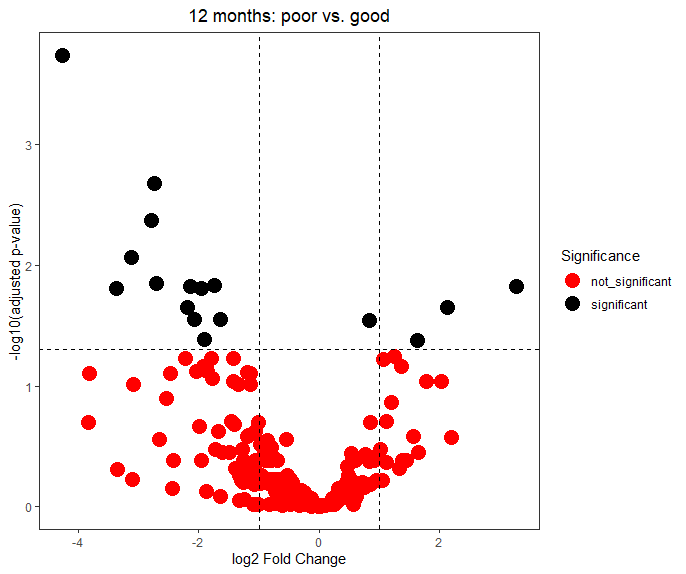
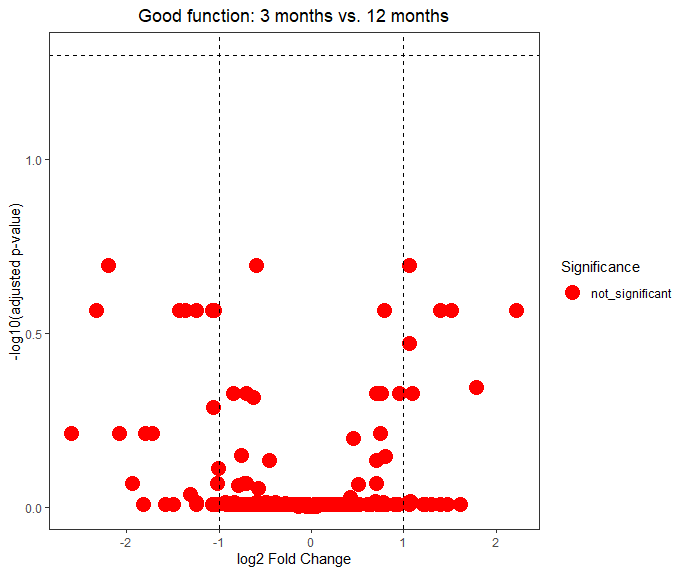


Figure 3. Differential expression of miRNAs in individuals with poor versus good function at 3 months, highlighting the significant (P< 0.05) downregulation of 17 miRNAs in the poor functional status group compared to the good functional status group, after accounting for the effects of donor age and gender.

**3 months Good vs 12 months Good**

design = ~ time\_point + Donor\_Age + Donor\_Gender\_M\_F

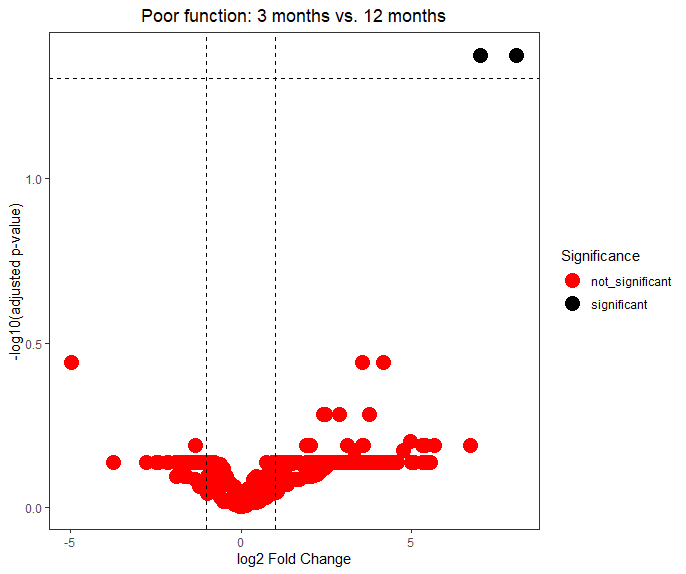
contrast <- c("time\_point", "three\_P", "twelve\_P")

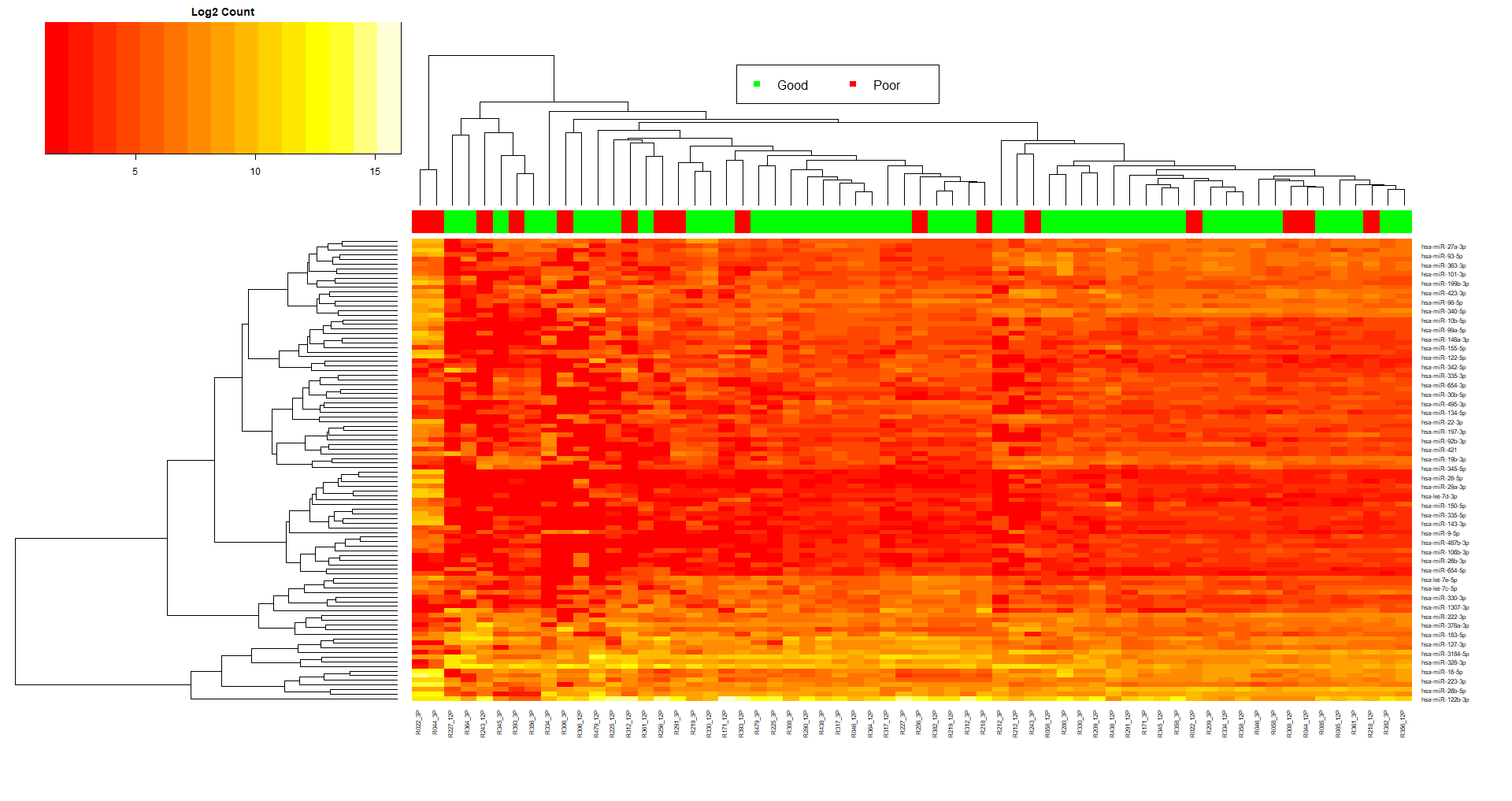


**3 months poor Vs 12 months poor**

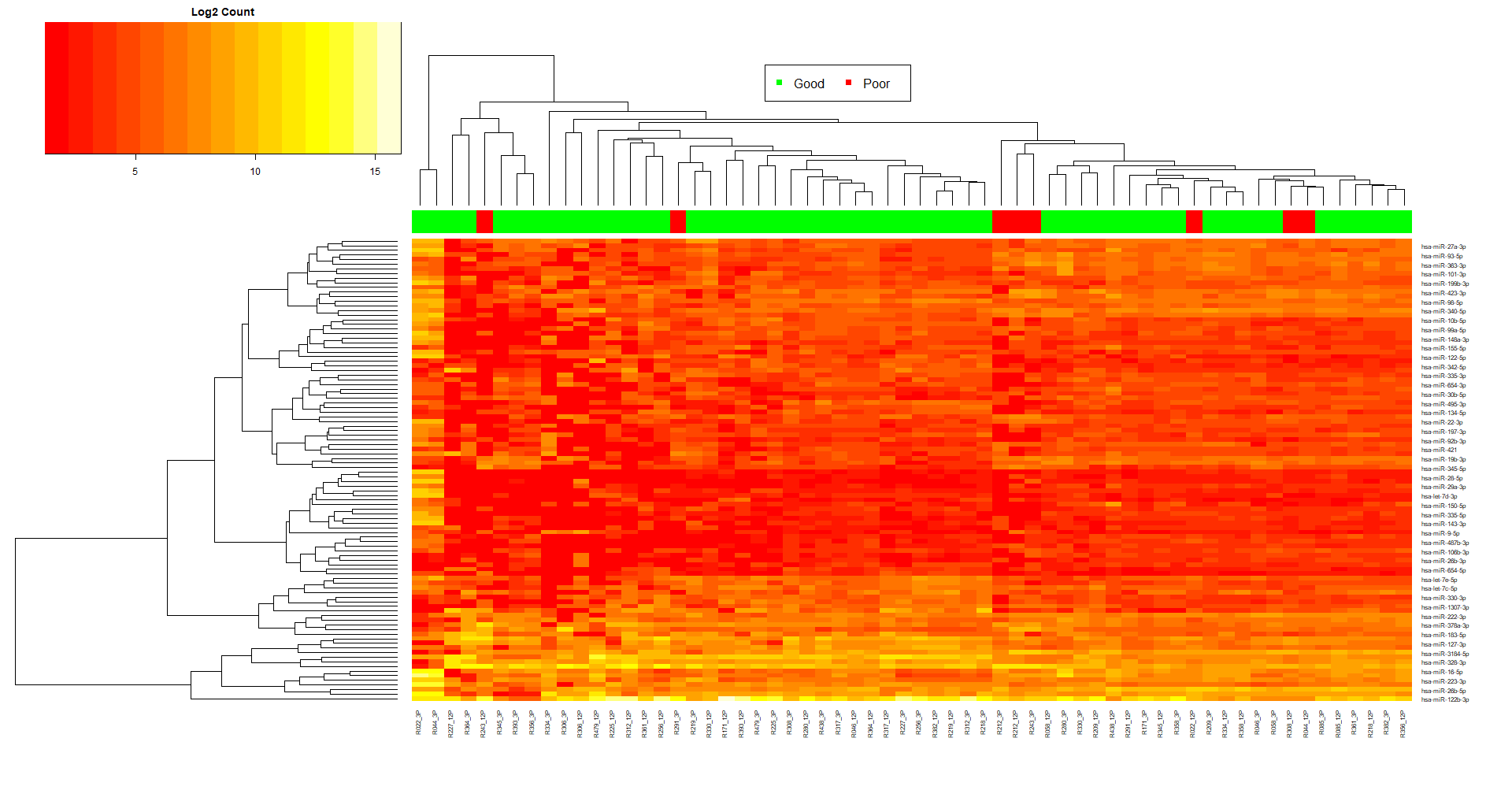
design = ~ time\_point + Donor\_Age + Donor\_Gender\_M\_F

contrast <- c("time\_point", "three\_P", "twelve\_P")



****

**Unsupervised HC: three months function**

****

**Unsupervised HC: twelve months function**

1. Andrews, S. FastQC: a quality control tool for high throughput sequence data. Vol. 2020 (2010).

2. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S.L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome biology* **10**, R25 (2009).

3. Quinlan, A.R. & Hall, I.M. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841-842 (2010).

4. Love, M.I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology* **15**(2014).