

check **Ammarnas brain sex-biased gene expression issue** (exceptional more number of female-biased genes, formed a distinct second clouds in volcano plot)

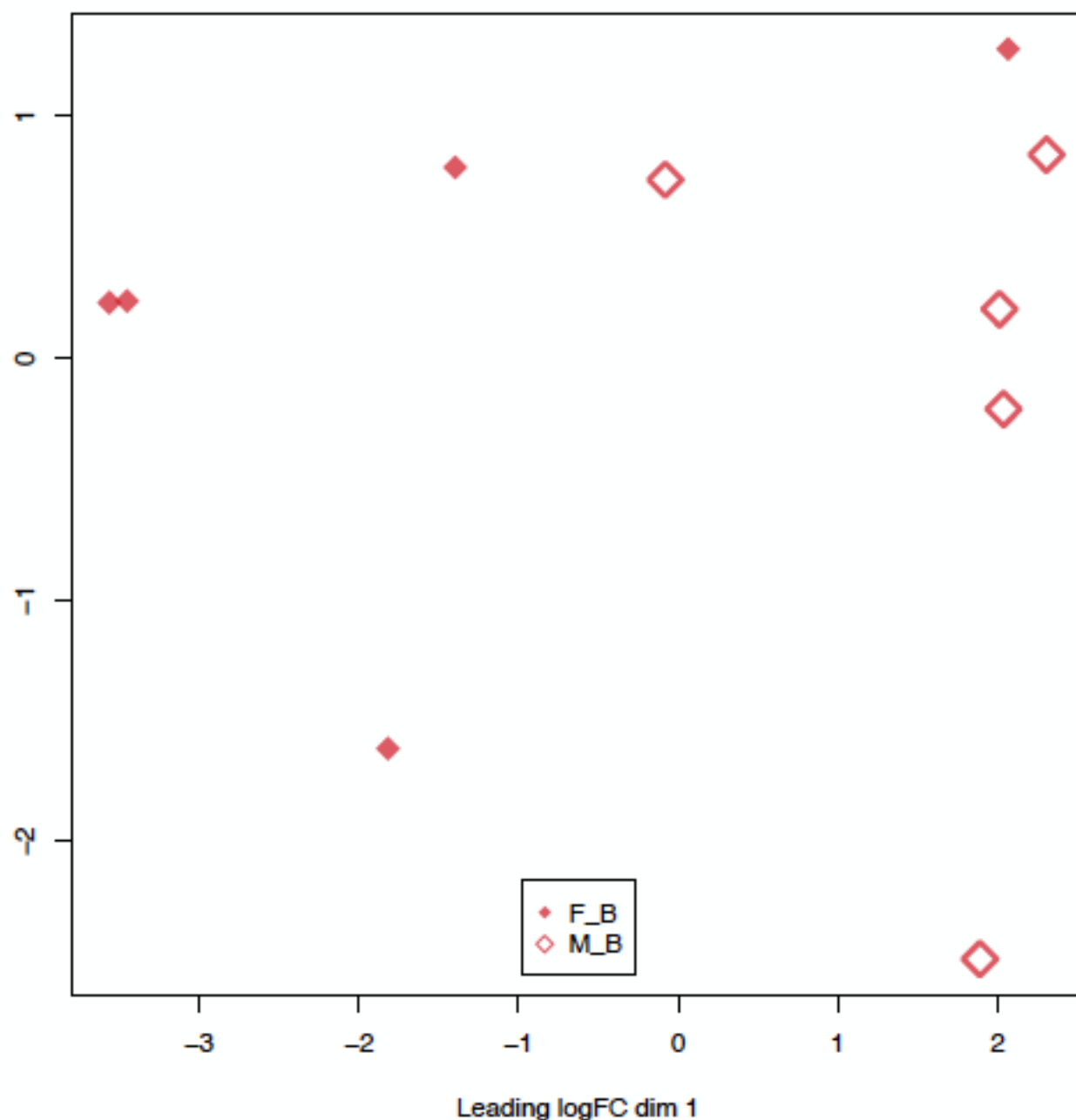
### 1. re-run analysis

the results remain the same as before (for number of SB genes and the second clouds)

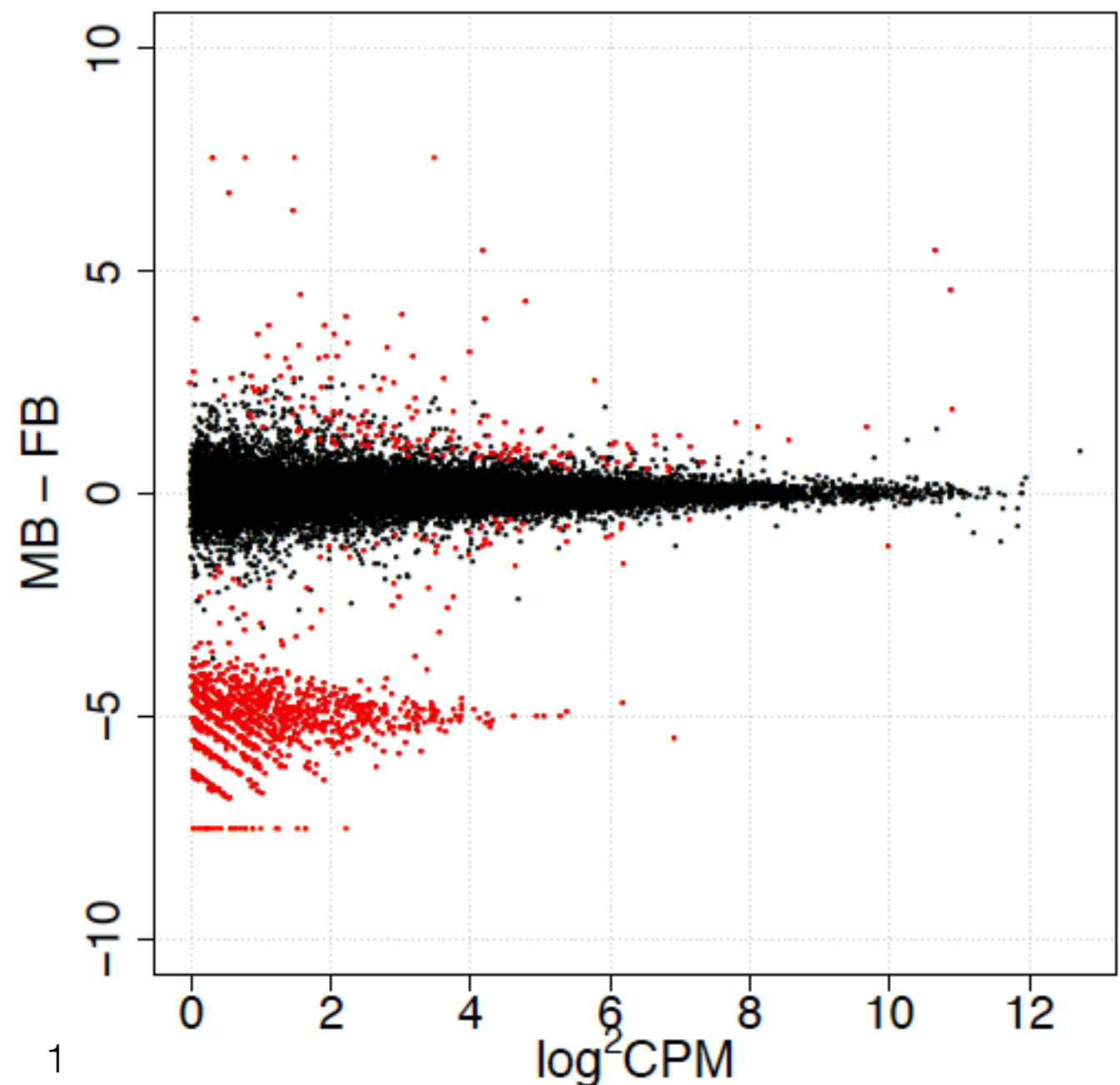
FB:1072 (FDR<0.05)

MB:166

Ammarnas MDS plot

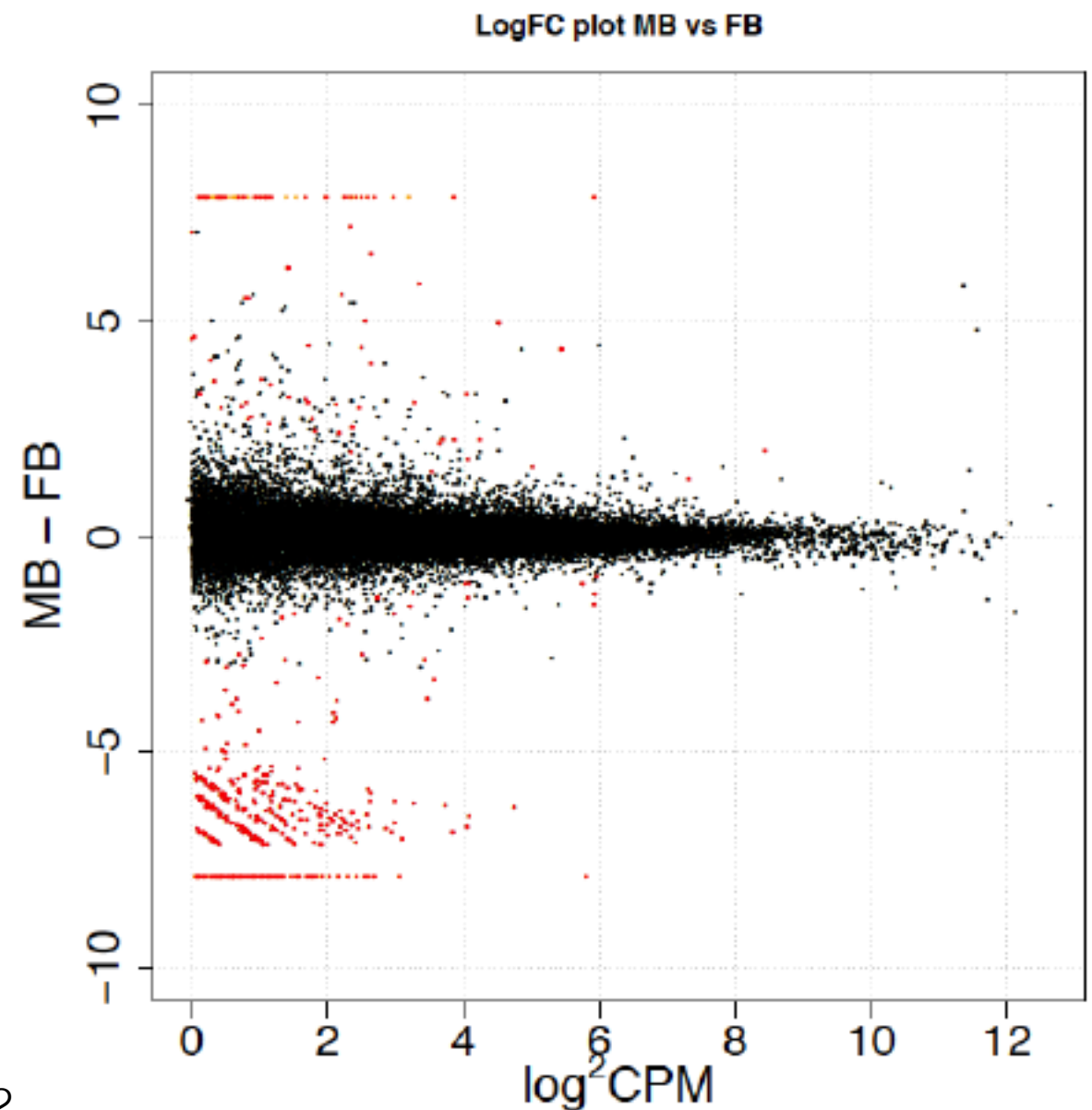
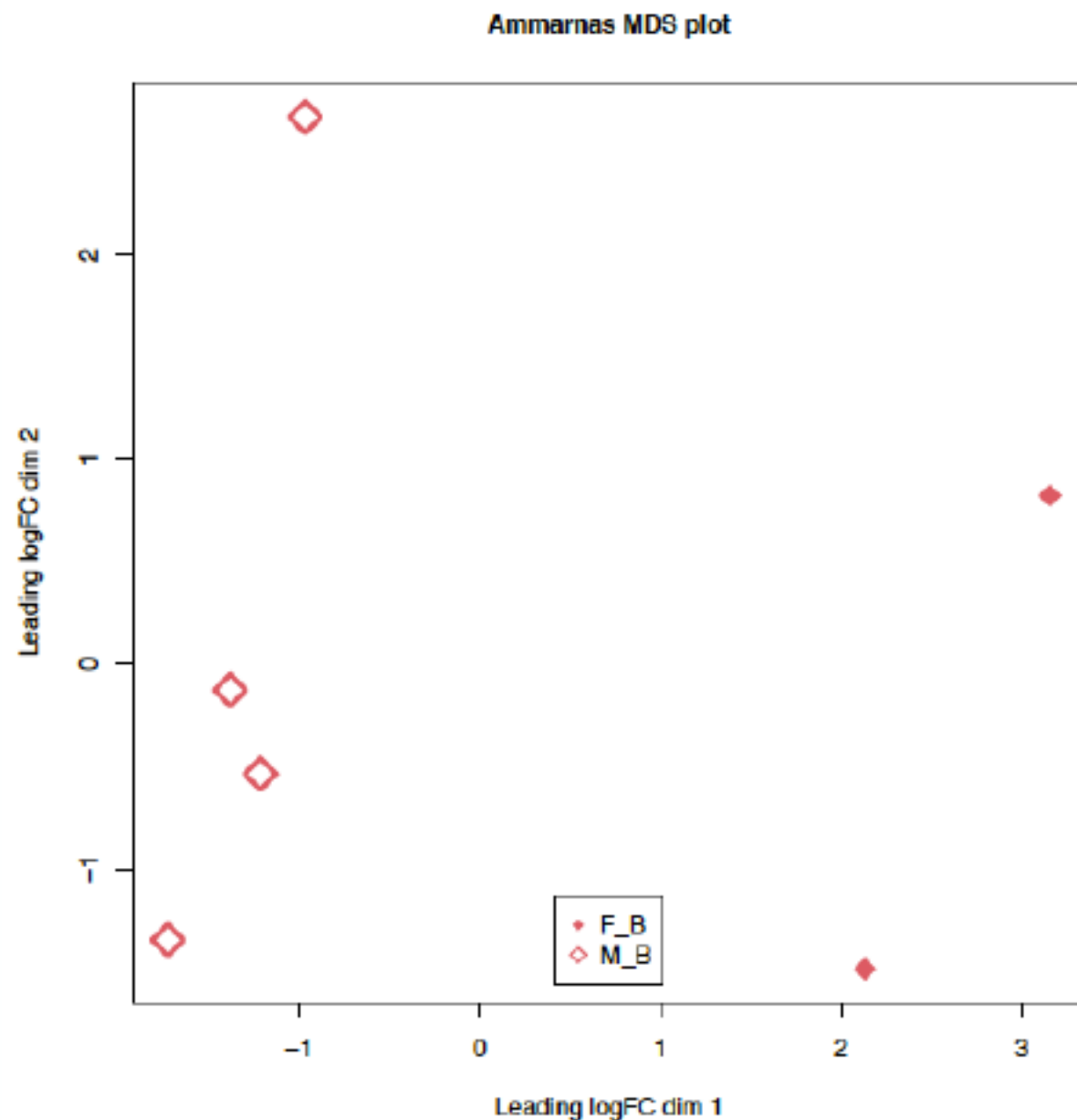


LogFC plot MB vs FB



check Ammarnas brain sex-biased gene expression issue (exceptional more number of female-biased genes, formed a distinct second clouds in volcano plot)

2. remove seem-to-be problematic samples, (remain 2 females and 4 males)  
the extra female-biased gene cloud remains.



### 3. GO term analysis:

suggests no weird/possible contaminated-tissue specific genes.

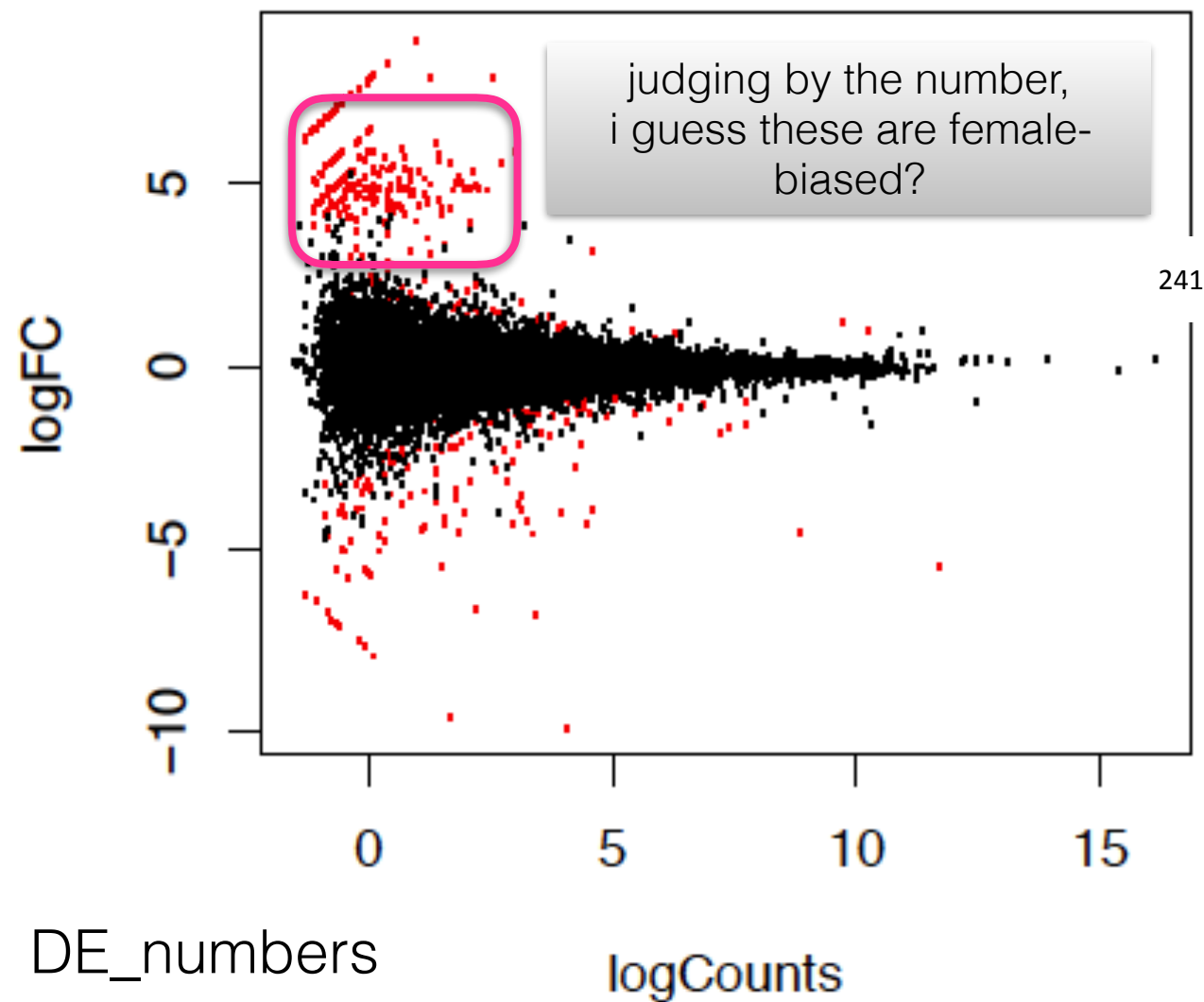
GO category	GO	Term	Annotated	Significant	Expected	topGO (Fisher)	classic (Fisher)
BP	GO:0019752	carboxylic acid metabolic process	13	3	0.49	0.008	0.008
BP	GO:0043043	peptide biosynthetic process	19	3	0.72	0.030	0.025

#### 4. Using transcriptome from Amm, Tve, Kijp 3 pops, mapping the reads

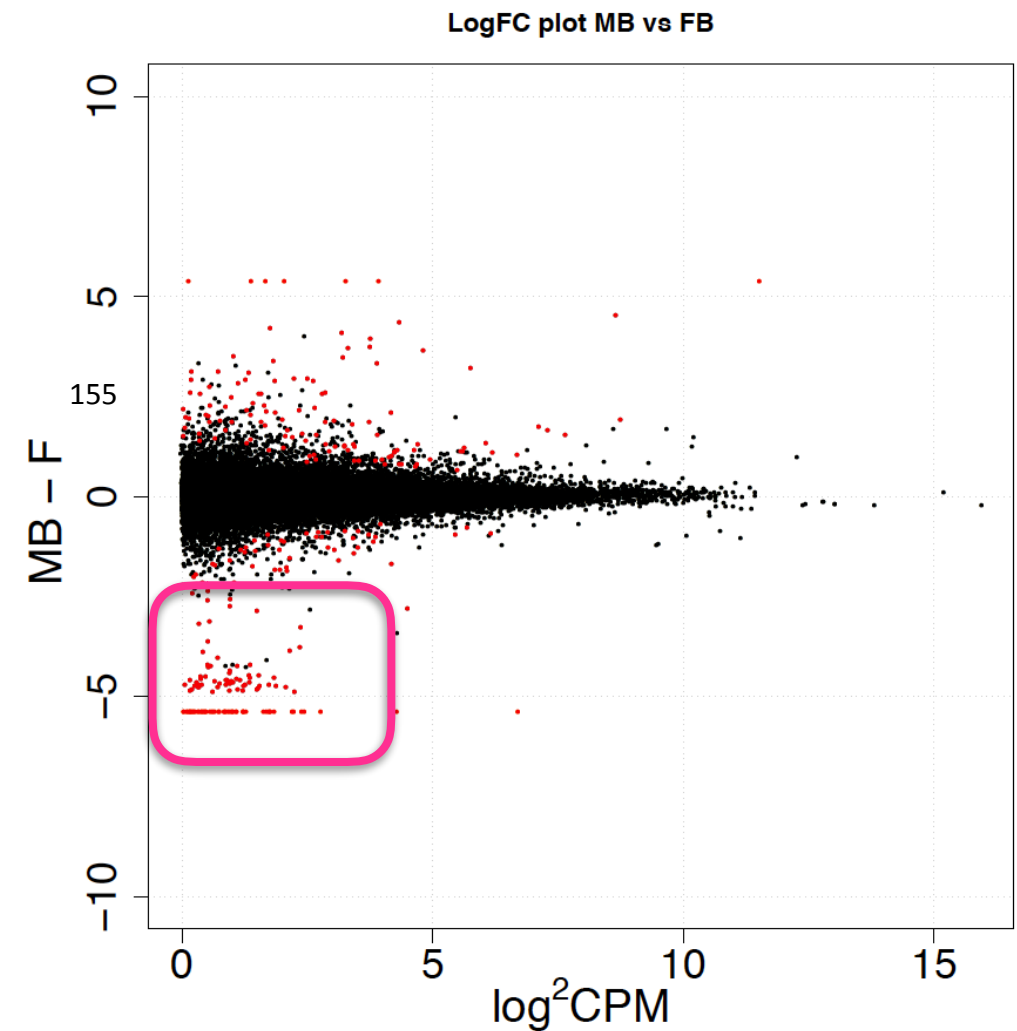
Criteria:

1.  
`dgl <- dgl[aveLogCPM(dgl) > 0,] # filter by average reads`

#### MA plot



DE\_numbers  
Total\_DE 396  
Male-bias 155  
Female-bias 241



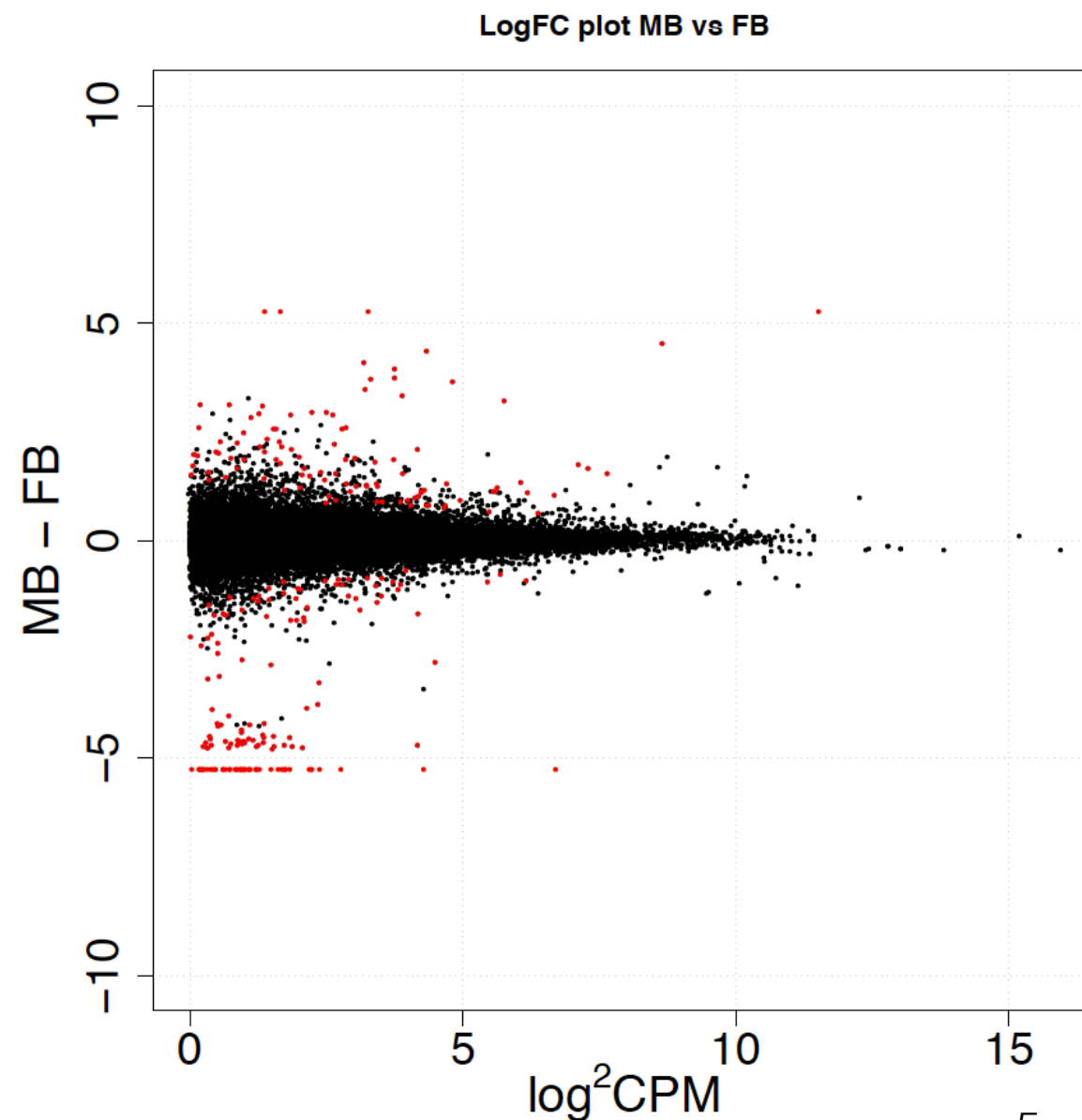
DE\_numbers  
Total\_DE 324  
Male-bias 142  
Female-bias 182

#### 4. Using transcriptome from Amm, Tve, Kijp 3 pops, mapping the reads

Criteria:

2.

```
dgl <- dgl[aveLogCPM(dgl) > 0,] # filter by average reads  
dgl <- dgl[rowSums(cpm(dgl)>1) >= 3,] #filter by minimum expression in at least half of sample size  
per sex
```



DE\_numbers

Total\_DE 287

Male-bias 121

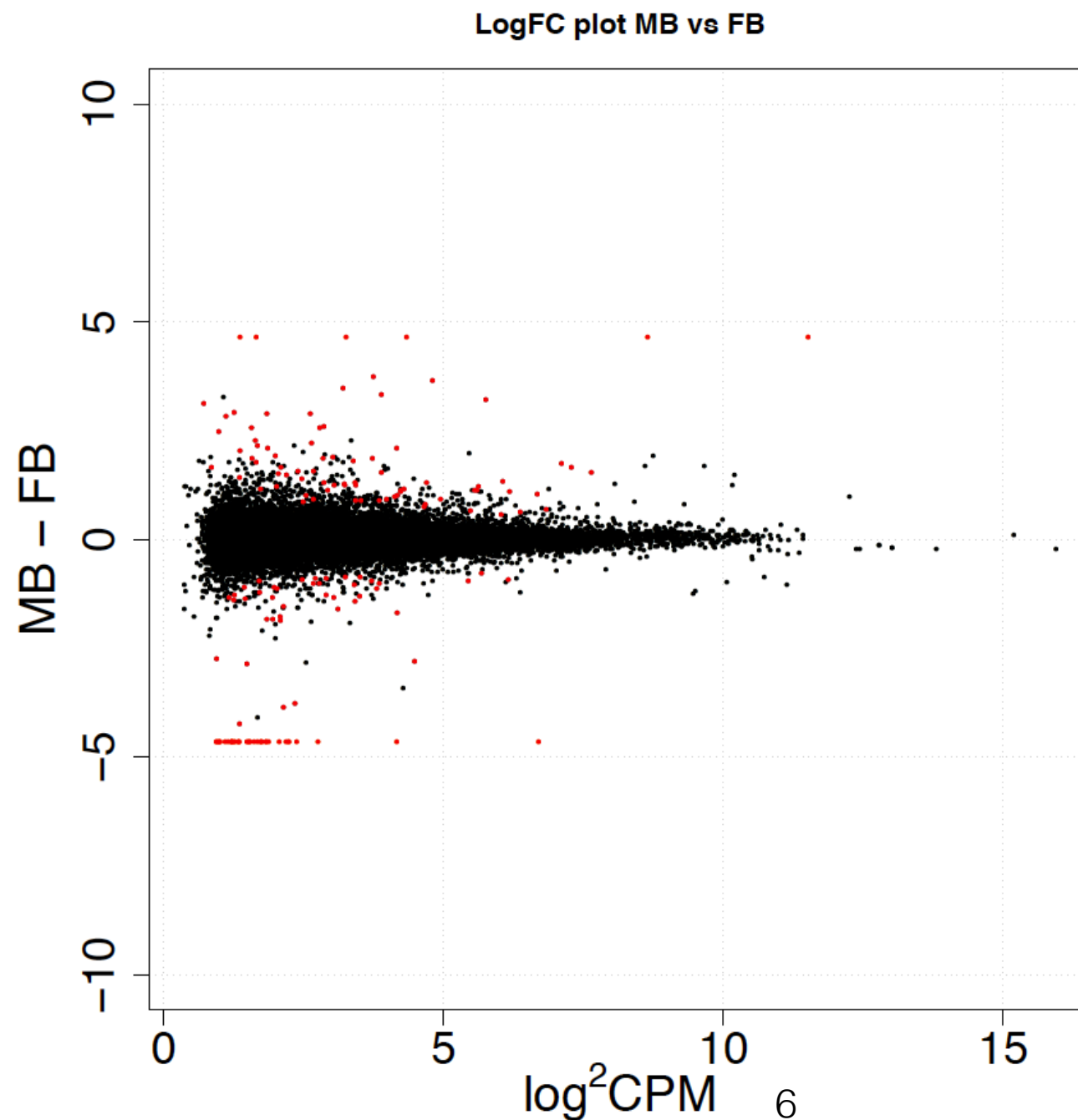
Female-bias 166

4. Using transcriptome from Amm, Tve, Kijp 3 pops, mapping the reads

Criteria:

3.

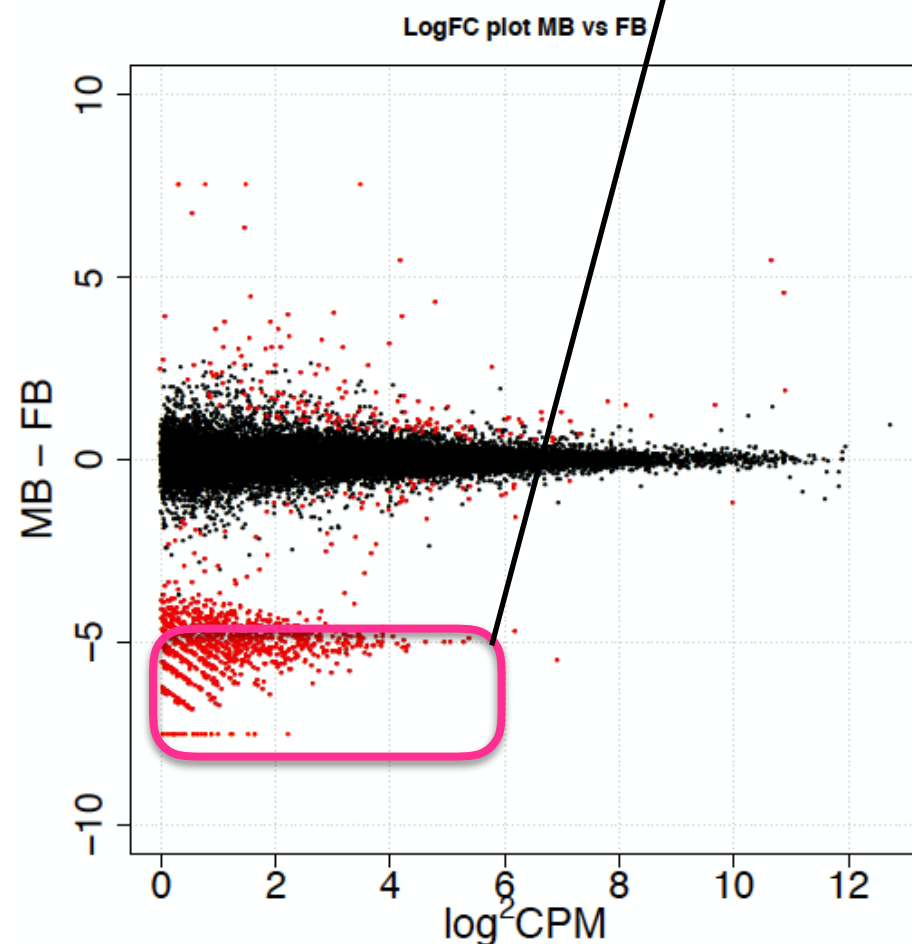
```
dgl <- dgl[aveLogCPM(dgl) > 0,] # filter by average reads  
dgl <- dgl[rowSums(cpm(dgl)>2) >= 4,] #filter by minimum expression in at least half of sample size  
per sex
```



DE\_numbers  
Total\_DE 158  
Male-bias 80  
Female-bias 78

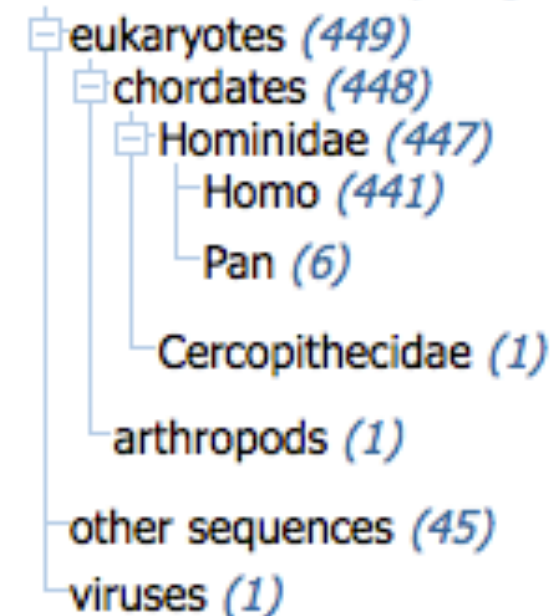
Amm transcriptome: subtract genes highly expressed in females ( $\log_2 \geq 5$ )  
 reflecting the transcripts from the second clouds —>  
 Blast 519 highly expressed in Amm female brain, 444 out of 495 (90%) blast  
 results are human sapiens.

```
dgl <- dgl[aveLogCPM(dgl) > 0,] # filter by average reads
dgl <- dgl[rowSums(cpm(dgl)>1) >= 3,] #filter by minimum expression in at least half of sample size per sex
```



### Results by taxon

#### Taxonomic Groups [\[List\]](#)



Would it be somewhat contamination by human sample?