

# RNA-seq Analysis

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Change for each project

Prepare counts matrix

```
## [1] "Levels of gRNA factor:"  
  
## [1] "LUC"      "BMI1.1"   "BMI1.3"   "CBX2.1"   "CBX2.3"   "CBX4.1"   "CBX4.2"  
## [8] "CBX8.1"   "CBX8.2"   "EZH2.3"   "EZH2.5"   "H3F3A.2"   "H3F3A.3"  
  
## [1] "Levels of gene target factor:"  
  
## [1] "LUC"      "BMI1"     "CBX2"     "CBX4"     "CBX8"     "EZH2"     "H3F3A"  
  
## [1] "Levels of genotype factor:"  
  
## [1] "K27M+"    "K27M_KO"  
  
## [1] "Levels of condition factor:"  
  
## [1] "LUC_K27M+"      "BMI1.1_K27M_KO"  "BMI1.1_K27M+"  "BMI1.3_K27M_KO"  
## [5] "BMI1.3_K27M+"  "CBX2.1_K27M_KO"  "CBX2.1_K27M+"  "CBX2.3_K27M_KO"  
## [9] "CBX2.3_K27M+"  "CBX4.1_K27M_KO"  "CBX4.1_K27M+"  "CBX4.2_K27M_KO"  
## [13] "CBX4.2_K27M+"  "CBX8.1_K27M_KO"  "CBX8.1_K27M+"  "CBX8.2_K27M_KO"  
## [17] "CBX8.2_K27M+"  "EZH2.3_K27M_KO"  "EZH2.3_K27M+"  "EZH2.5_K27M_KO"  
## [21] "EZH2.5_K27M+"  "H3F3A.2_K27M_KO"  "H3F3A.2_K27M+"  "H3F3A.3_K27M_KO"  
## [25] "H3F3A.3_K27M+" "LUC_K27M_KO"  
  
## [1] "Levels of treatment factor:"  
  
## [1] "LUC_K27M+"      "BMI1_K27M_KO"  "BMI1_K27M+"  "CBX2_K27M_KO"  
## [5] "CBX2_K27M+"    "CBX4_K27M_KO"  "CBX4_K27M+"  "CBX8_K27M_KO"  
## [9] "CBX8_K27M+"    "EZH2_K27M_KO"  "EZH2_K27M+"  "H3F3A_K27M_KO"  
## [13] "H3F3A_K27M+"  "LUC_K27M_KO"  
  
## [1] "Levels of group factor:"  
  
## [1] "LUC_K27M+_A"    "BMI1_K27M_KO_A"  "BMI1_K27M_KO_B"  "BMI1_K27M+_A"  
## [5] "BMI1_K27M+_B"    "CBX2_K27M_KO_A"  "CBX2_K27M_KO_B"  "CBX2_K27M+_A"  
## [9] "CBX2_K27M+_B"    "CBX4_K27M_KO_A"  "CBX4_K27M_KO_B"  "CBX4_K27M+_A"  
## [13] "CBX4_K27M+_B"    "CBX8_K27M_KO_A"  "CBX8_K27M_KO_B"  "CBX8_K27M+_A"  
## [17] "CBX8_K27M+_B"    "EZH2_K27M_KO_A"  "EZH2_K27M_KO_B"  "EZH2_K27M+_A"  
## [21] "EZH2_K27M+_B"    "H3F3A_K27M_KO_A"  "H3F3A_K27M_KO_B"  "H3F3A_K27M+_A"  
## [25] "H3F3A_K27M+_B"  "LUC_K27M_KO_A"  "LUC_K27M_KO_B"  "LUC_K27M+_B"
```

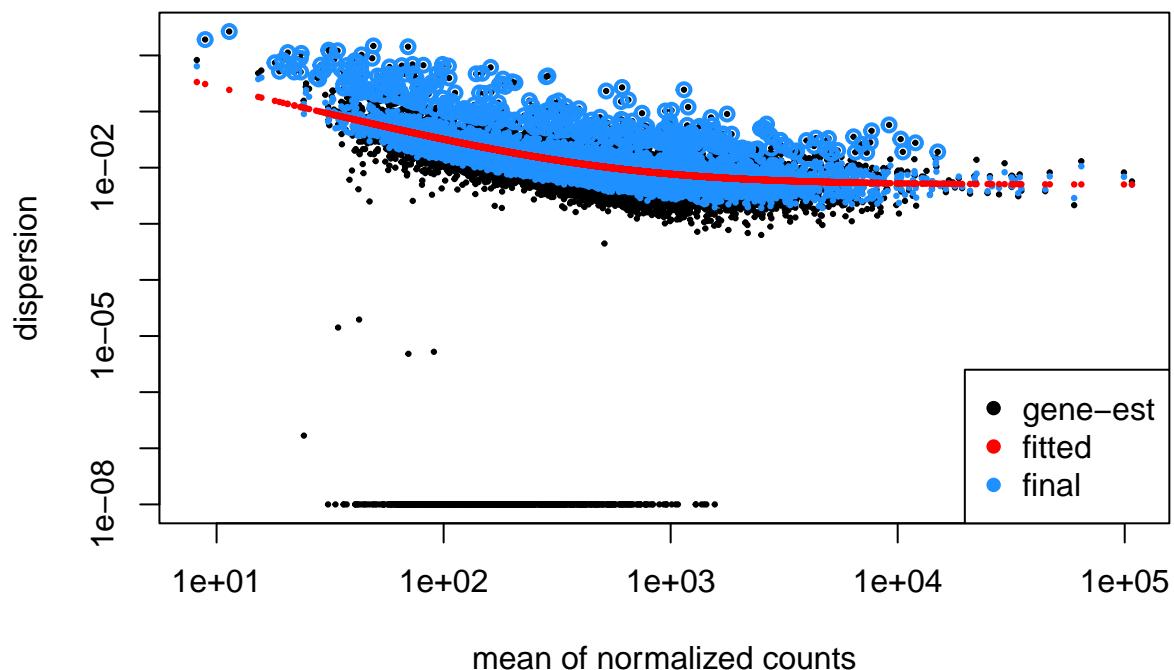
QC

```
## pdf  
## 2
```

Create DESeqDataSet and fit model

```
## [1] 64272
```

```
## [1] 12571
```



Statistical test (Wald's test)

Volcano plots

```
## [1] "condition"    "LUC_K27M_KO"  "LUC_K27M+"  
  
## [1] "condition"    "CBX4.1_K27M_KO" "LUC_K27M+"  
  
## [1] "condition"    "CBX4.2_K27M_KO" "LUC_K27M+"  
  
## [1] "condition"    "CBX2.1_K27M_KO" "LUC_K27M+"
```

```

## [1] "condition"      "CBX2.3_K27M_KO" "LUC_K27M+"

## [1] "condition"      "EZH2.3_K27M_KO" "LUC_K27M+"

## [1] "condition"      "EZH2.5_K27M_KO" "LUC_K27M+"

## [1] "condition"      "CBX8.1_K27M_KO" "LUC_K27M+"

## [1] "condition"      "CBX8.2_K27M_KO" "LUC_K27M+"

## [1] "condition"      "BMI1.1_K27M_KO" "LUC_K27M+"

## [1] "condition"      "BMI1.3_K27M_KO" "LUC_K27M+"

## [1] "condition"      "CBX4.1_K27M+"  "LUC_K27M+"

## [1] "condition"      "CBX4.2_K27M+"  "LUC_K27M+"

## [1] "condition"      "CBX2.1_K27M+"  "LUC_K27M+"

## [1] "condition"      "CBX2.3_K27M+"  "LUC_K27M+"

## [1] "condition"      "EZH2.3_K27M+"  "LUC_K27M+"

## [1] "condition"      "EZH2.5_K27M+"  "LUC_K27M+"

## [1] "condition"      "CBX8.1_K27M+"  "LUC_K27M+"

## [1] "condition"      "CBX8.2_K27M+"  "LUC_K27M+"

## [1] "condition"      "BMI1.1_K27M+"  "LUC_K27M+"

## [1] "condition"      "BMI1.3_K27M+"  "LUC_K27M+"

## [1] "condition"      "CBX4.1_K27M_KO" "LUC_K27M_KO"

## [1] "condition"      "CBX4.2_K27M_KO" "LUC_K27M_KO"

## [1] "condition"      "CBX2.1_K27M_KO" "LUC_K27M_KO"

## [1] "condition"      "CBX2.3_K27M_KO" "LUC_K27M_KO"

## [1] "condition"      "EZH2.3_K27M_KO" "LUC_K27M_KO"

## [1] "condition"      "EZH2.5_K27M_KO" "LUC_K27M_KO"

## [1] "condition"      "CBX8.1_K27M_KO" "LUC_K27M_KO"

```

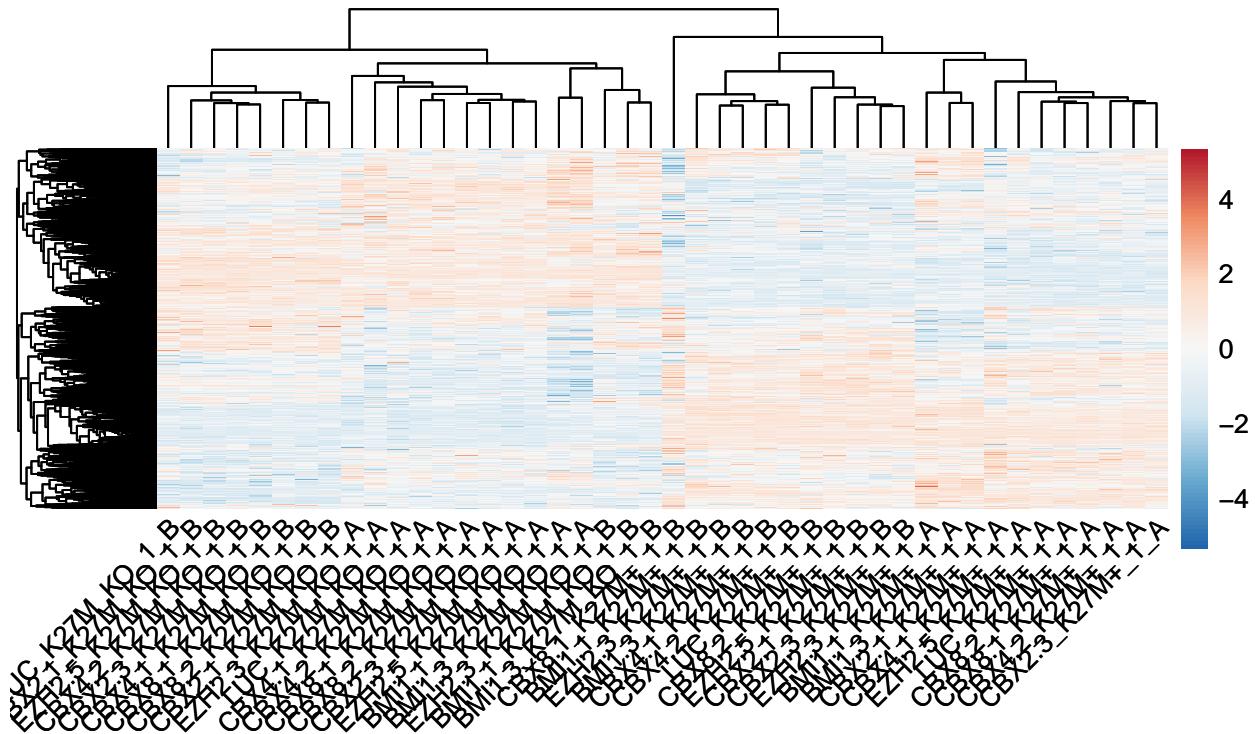
```
## [1] "condition"      "CBX8.2_K27M_KO" "LUC_K27M_KO"  
  
## [1] "condition"      "BMI1.1_K27M_KO" "LUC_K27M_KO"  
  
## [1] "condition"      "BMI1.3_K27M_KO" "LUC_K27M_KO"  
  
## [1] "condition"      "LUC_K27M+"    "LUC_K27M_KO"  
  
## [1] "condition"      "CBX4.1_K27M+"  "LUC_K27M_KO"  
  
## [1] "condition"      "CBX4.2_K27M+"  "LUC_K27M_KO"  
  
## [1] "condition"      "CBX2.1_K27M+"  "LUC_K27M_KO"  
  
## [1] "condition"      "CBX2.3_K27M+"  "LUC_K27M_KO"  
  
## [1] "condition"      "EZH2.3_K27M+"  "LUC_K27M_KO"  
  
## [1] "condition"      "EZH2.5_K27M+"  "LUC_K27M_KO"  
  
## [1] "condition"      "CBX8.1_K27M+"  "LUC_K27M_KO"  
  
## [1] "condition"      "CBX8.2_K27M+"  "LUC_K27M_KO"  
  
## [1] "condition"      "BMI1.1_K27M+"  "LUC_K27M_KO"  
  
## [1] "condition"      "BMI1.3_K27M+"  "LUC_K27M_KO"
```

## Gene expression heatmaps (all genes)

```
## pdf  
## 2
```

## Differentially expressed genes

N = 12571



## Q-Q plots

```
## [1] "Making comparison: CBX4.1_K27M+_vs_K27M_KO"  
  
## [1] "Making comparison: CBX4.2_K27M+_vs_K27M_KO"  
  
## [1] "Making comparison: CBX2.1_K27M+_vs_K27M_KO"  
  
## [1] "Making comparison: CBX2.3_K27M+_vs_K27M_KO"  
  
## [1] "Making comparison: EZH2.3_K27M+_vs_K27M_KO"  
  
## [1] "Making comparison: EZH2.5_K27M+_vs_K27M_KO"  
  
## [1] "Making comparison: CBX8.1_K27M+_vs_K27M_KO"  
  
## [1] "Making comparison: CBX8.2_K27M+_vs_K27M_KO"  
  
## [1] "Making comparison: BMI1.1_K27M+_vs_K27M_KO"  
  
## [1] "Making comparison: BMI1.3_K27M+_vs_K27M_KO"
```

### Scaled volcano plots

```
## [1] "Making comparison: CBX4.1 K27M+ vs LUC K27M+"

## [1] "Making comparison: CBX4.2 K27M+ vs LUC K27M+"

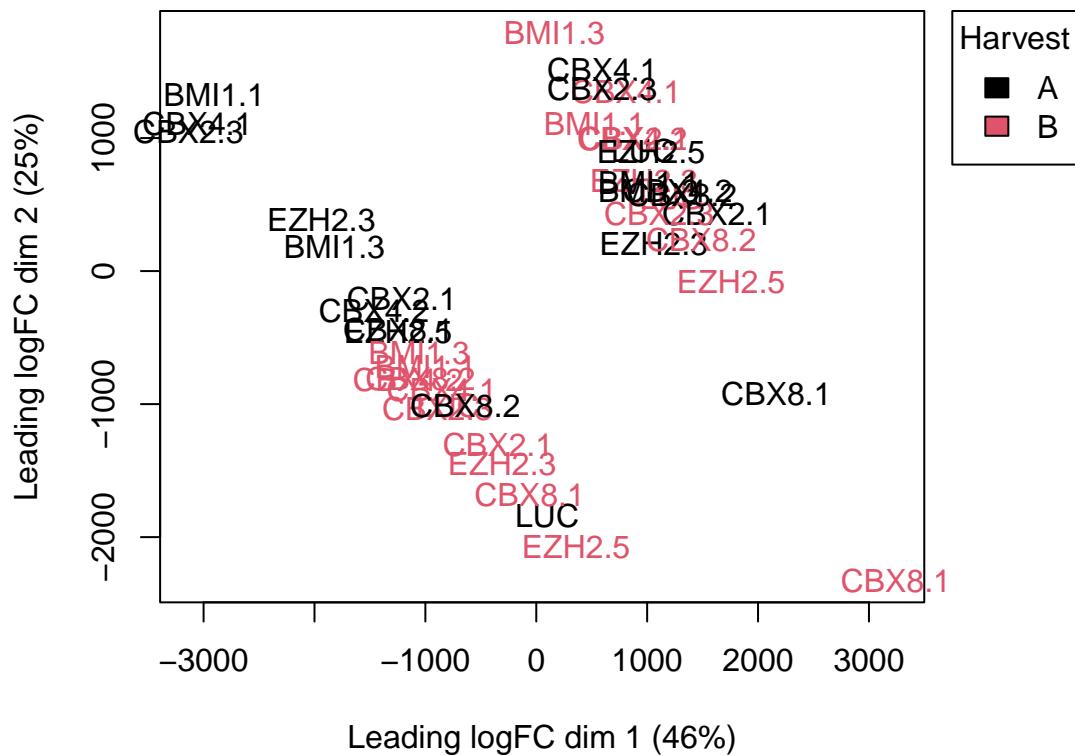
## [1] "Making comparison: CBX2.1 K27M+ vs LUC K27M+"

## [1] "Making comparison: CBX2.3 K27M+ vs LUC K27M+"

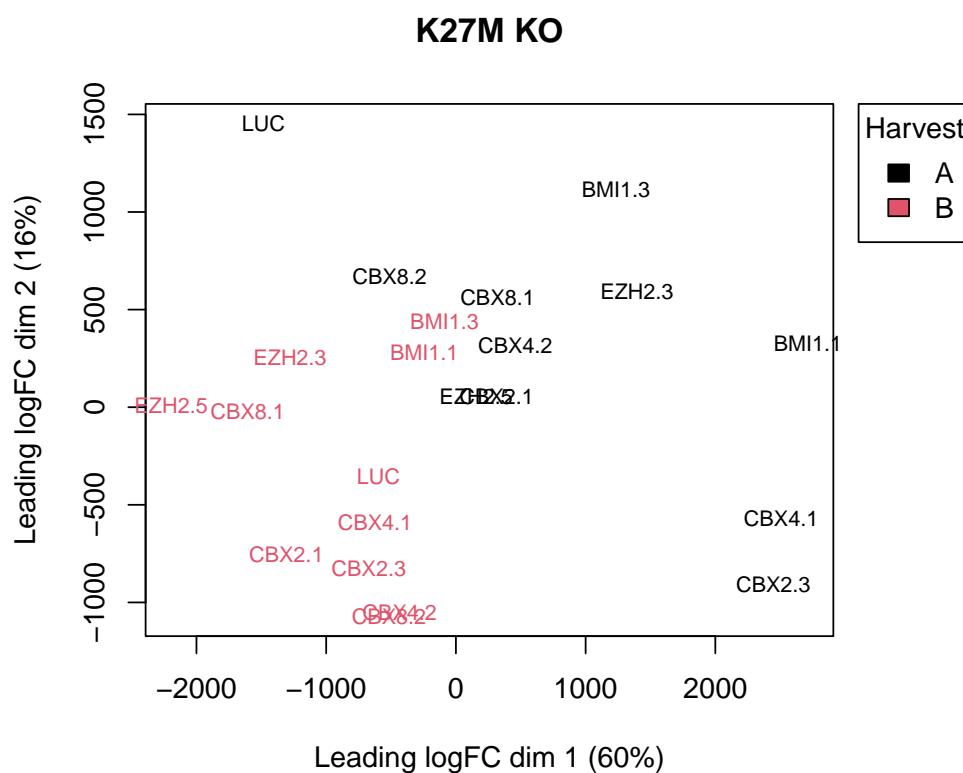
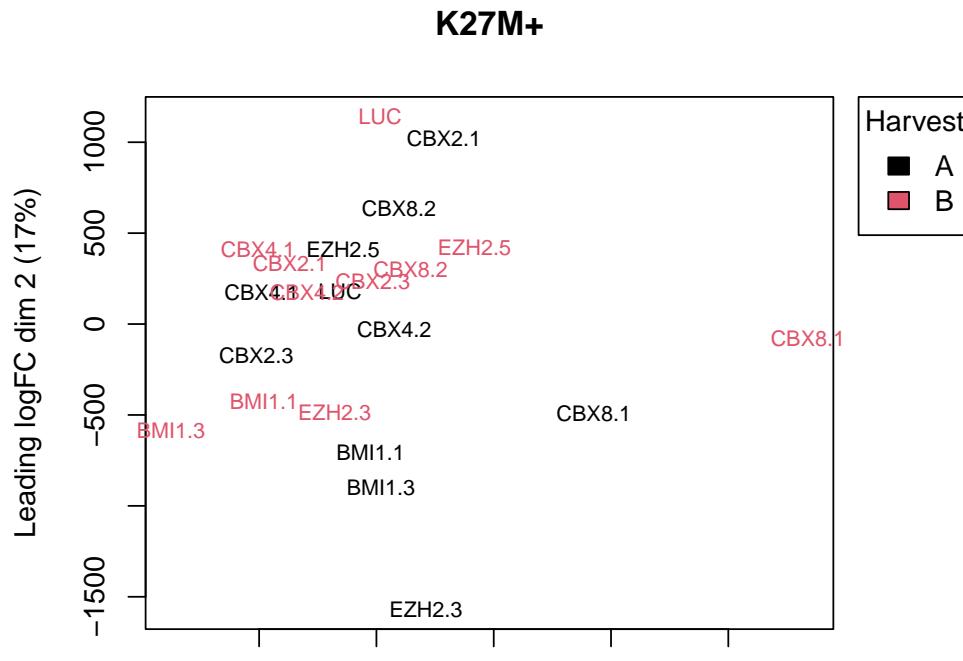
## [1] "Making comparison: CBX8.1 K27M+ vs LUC K27M+"

## [1] "Making comparison: CBX8.2 K27M+ vs LUC K27M+"
```

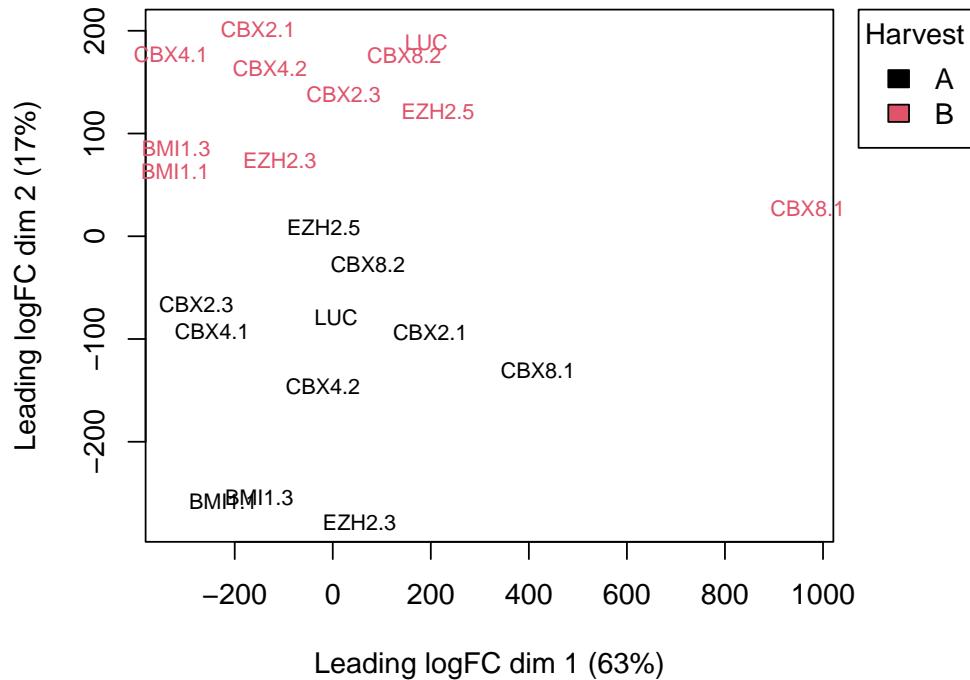
### MDS plots (all genotypes)



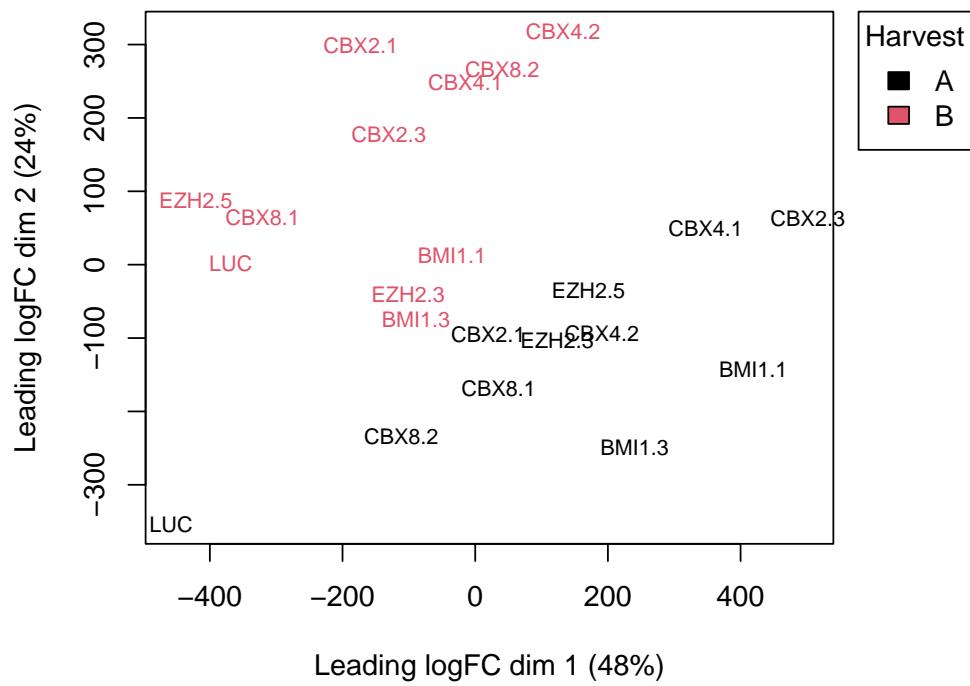
MDS plots (by genotypes)



**PcB targets  
K27M+**



**PcB targets  
K27M KO**



```

## [1] "Making comparison: EZH2.3_K27M+_vs_CBX4.1_K27M+"

## [1] "Making comparison: EZH2.3_K27M+_vs_CBX4.2_K27M+"

## [1] "Making comparison: EZH2.5_K27M+_vs_CBX4.1_K27M+"

## [1] "Making comparison: EZH2.5_K27M+_vs_CBX4.2_K27M+"

## [1] "Making comparison: BMI1.1_K27M+_vs_CBX4.1_K27M+"

## [1] "Making comparison: BMI1.1_K27M+_vs_CBX4.2_K27M+"

## [1] "Making comparison: BMI1.3_K27M+_vs_CBX4.1_K27M+"

## [1] "Making comparison: BMI1.3_K27M+_vs_CBX4.2_K27M+"

## [1] "Making comparison: CBX2.1_K27M+_vs_CBX4.1_K27M+"

## [1] "Making comparison: CBX2.1_K27M+_vs_CBX4.2_K27M+"

## [1] "Making comparison: CBX2.3_K27M+_vs_CBX4.1_K27M+"

## [1] "Making comparison: CBX2.3_K27M+_vs_CBX4.2_K27M+"

## [1] "Making comparison: CBX8.1_K27M+_vs_CBX4.1_K27M+"

## [1] "Making comparison: CBX8.1_K27M+_vs_CBX4.2_K27M+"

## [1] "Making comparison: CBX8.2_K27M+_vs_CBX4.1_K27M+"

## [1] "Making comparison: CBX8.2_K27M+_vs_CBX4.2_K27M+"

## [1] "Common Genes at Baseline (Regularized log-transformed counts)"

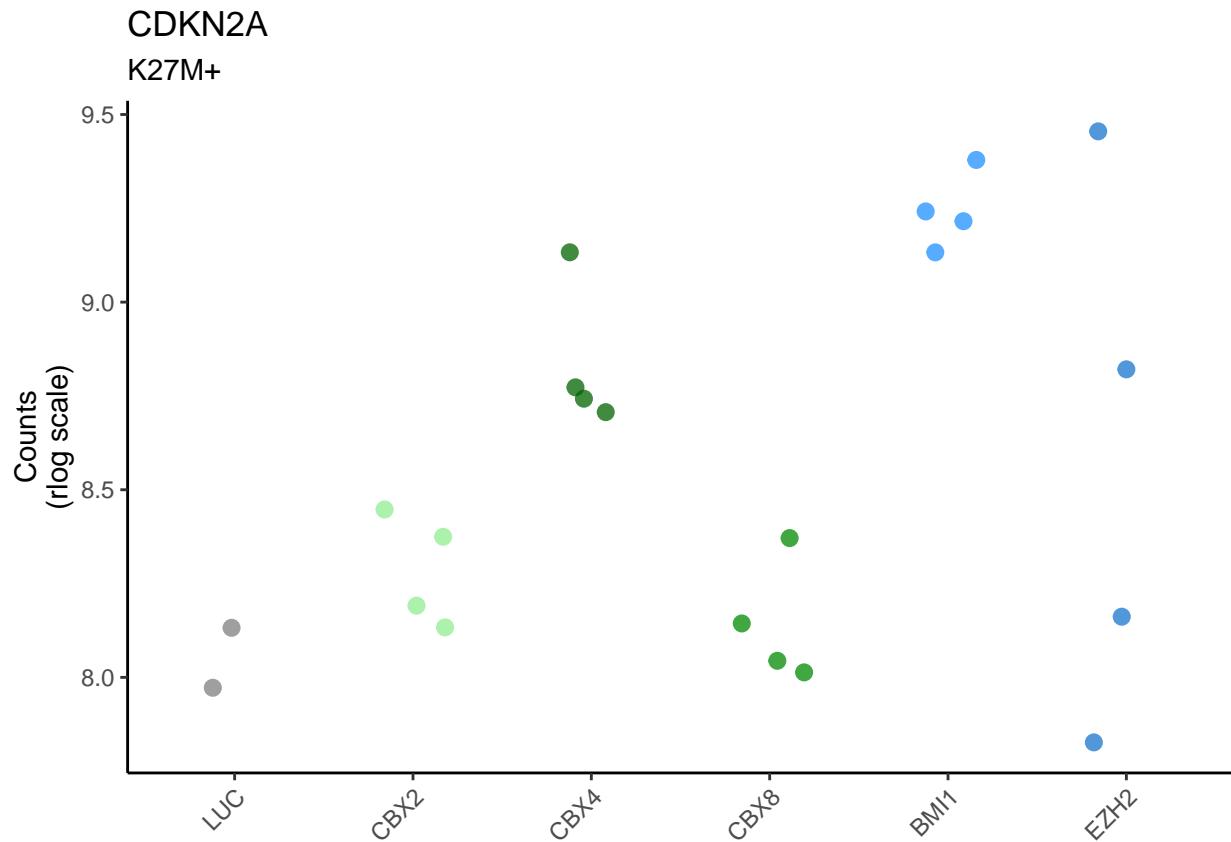
##          LUC_K27M_K0_1_A  LUC_K27M_K0_1_B  LUC_K27M+_1_A  LUC_K27M+_1_B
## DMRTA2        9.874697      9.758507     5.247739     5.119990
## PDLIM3        8.992170      8.656218     5.158113     5.157734
## PCDH1        8.880890      8.297928     5.734141     5.518013
## EYA4         10.223383     10.264720     6.719321     6.692411
## CDKN2A        7.673145      7.389676     8.132096     7.972329
## RTN1         8.840179      8.988995     6.951826     7.252024
## ACTC1        5.013047      4.484721     4.000596     3.922409

##  LUC_K27M_K0_1_A  LUC_K27M_K0_1_B  LUC_K27M+_1_A  LUC_K27M+_1_B
## Min.    : 1.886   Min.    : 1.830   Min.    : 1.866   Min.    : 1.790
## 1st Qu.: 7.203   1st Qu.: 7.243   1st Qu.: 7.213   1st Qu.: 7.302
## Median  : 8.422   Median  : 8.429   Median  : 8.409   Median  : 8.420
## Mean    : 8.447   Mean    : 8.444   Mean    : 8.445   Mean    : 8.459
## 3rd Qu.: 9.572   3rd Qu.: 9.551   3rd Qu.: 9.538   3rd Qu.: 9.507
## Max.    :16.965   Max.    :16.888   Max.    :16.722   Max.    :16.591

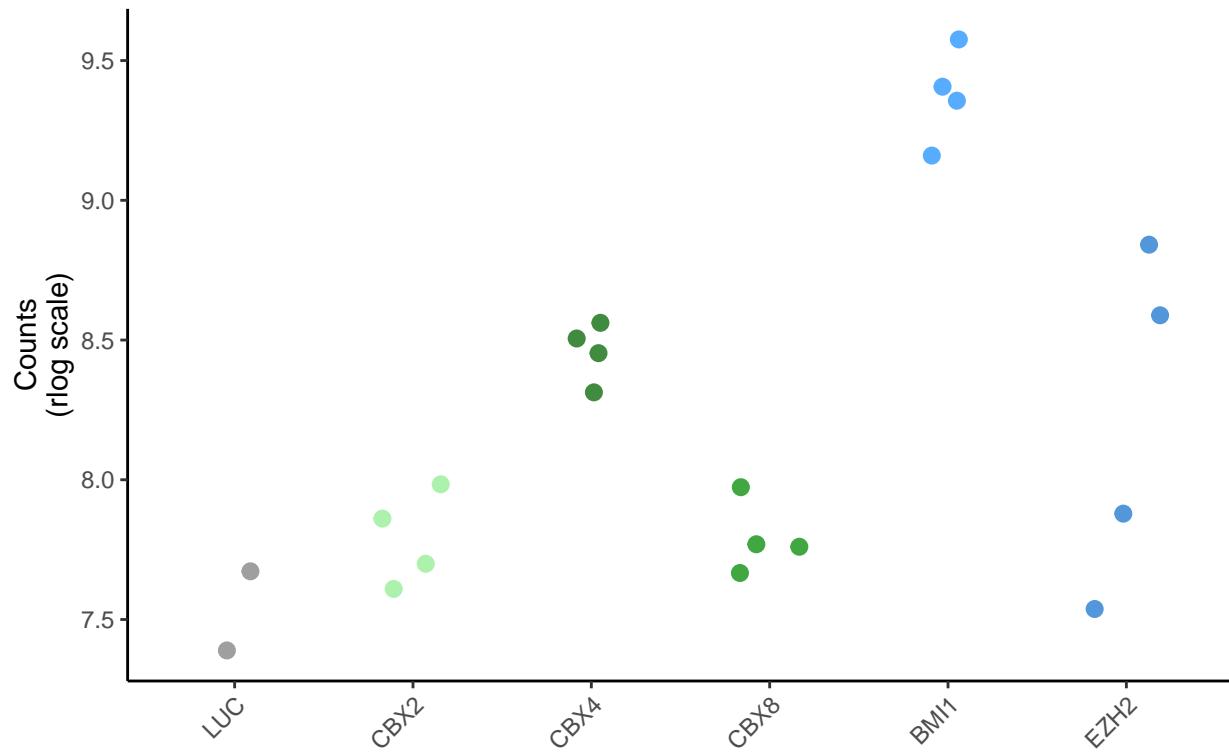
```

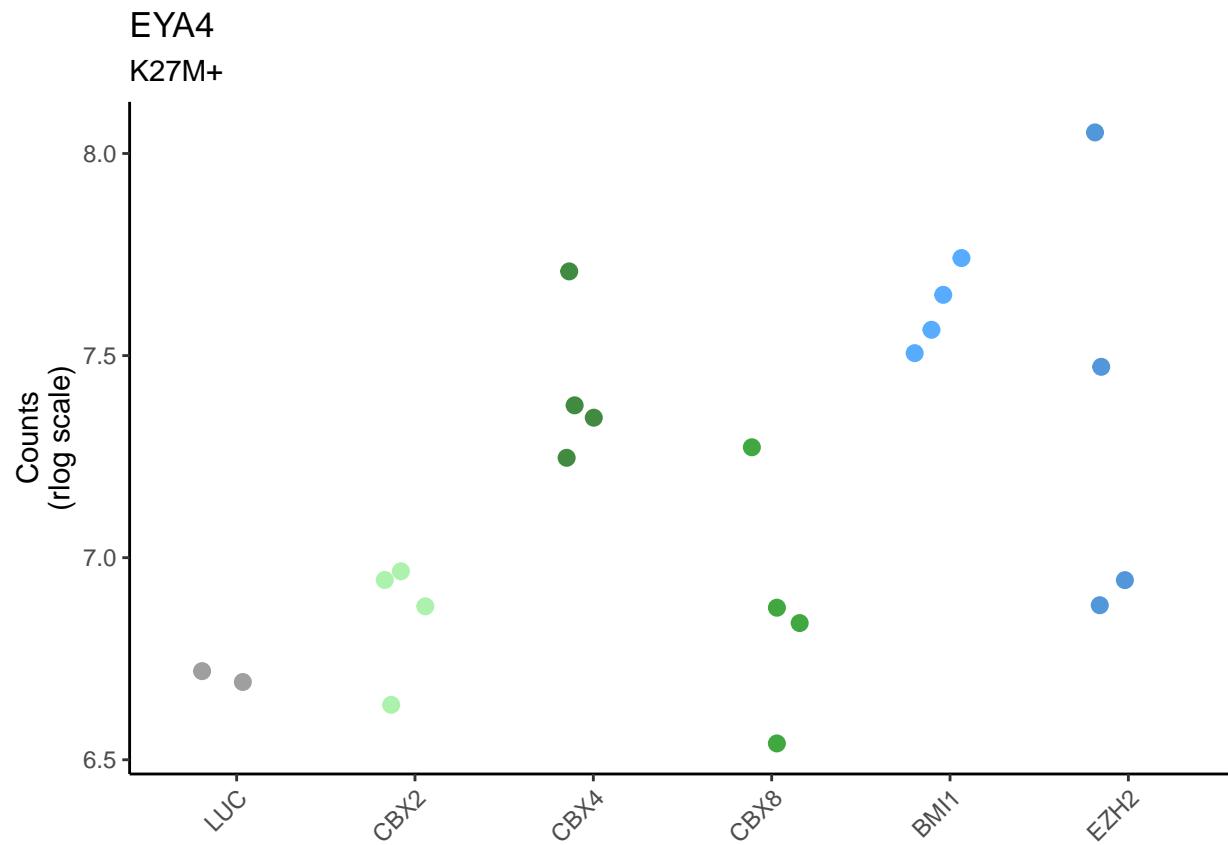
```
## [1] "Common Genes at Baseline (Z-score of the rlog transform)"
```

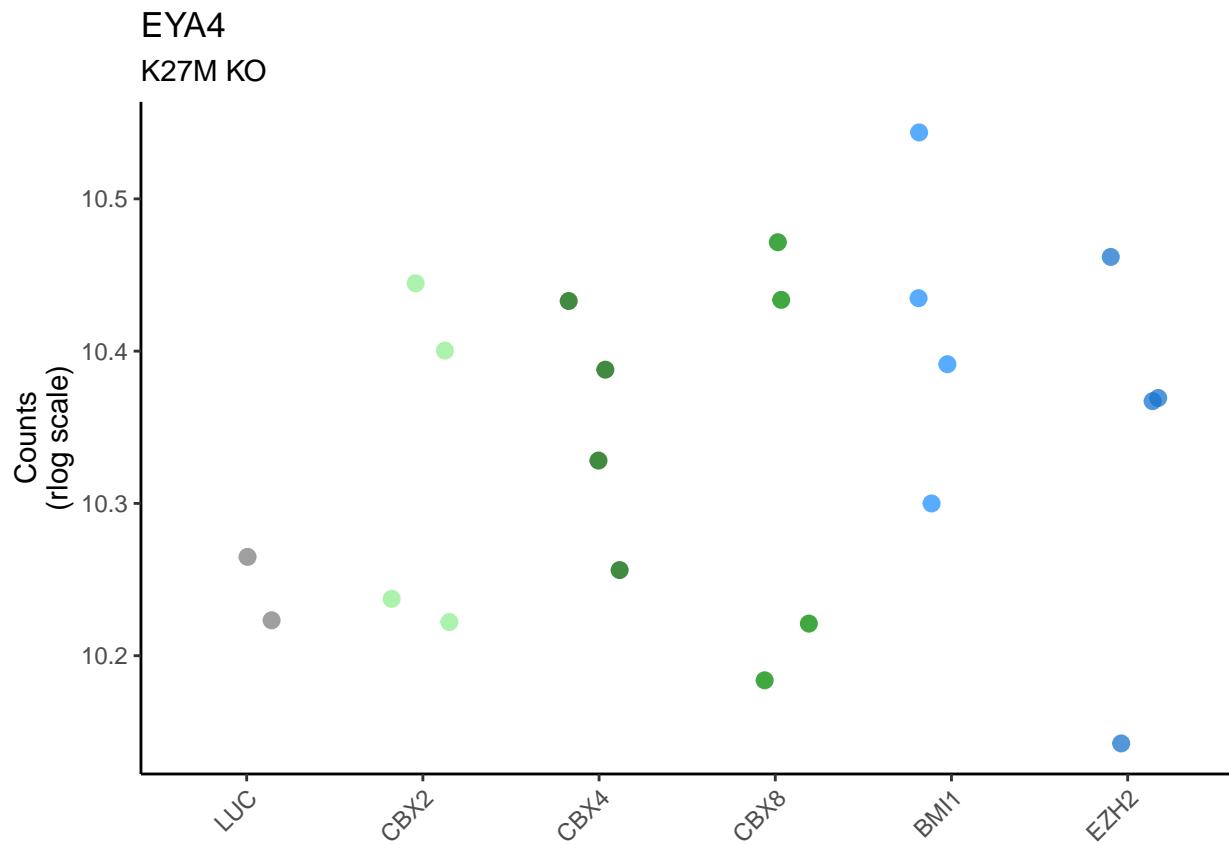
```
##          LUC_K27M_K0_1_A  LUC_K27M_K0_1_B  LUC_K27M+_1_A  LUC_K27M+_1_B
## DMRTA2      0.8181314     0.76784142   -1.8920072    -2.0355356
## PDLIM3      0.3123497     0.12397093   -1.9450411    -2.0125246
## PCDH1       0.2485743    -0.08531372   -1.6041918    -1.7928800
## EYA4        1.0179654     1.06353080   -1.0212379    -1.0769045
## CDKN2A     -0.4435920    -0.61584233   -0.1852662    -0.2965996
## RTN1        0.2252424     0.31835312   -0.8836593    -0.7357355
## ACTC1     -1.9681109    -2.31268759   -2.6299707    -2.7656441
##  LUC_K27M_K0_1_A  LUC_K27M_K0_1_B  LUC_K27M+_1_A  LUC_K27M+_1_B
## Min.   :-3.76005   Min.   :-3.863604  Min.   :-3.8932   Min.   :-4.06581
## 1st Qu.:-0.71279   1st Qu.:-0.701338  1st Qu.:-0.7289   1st Qu.:-0.70522
## Median : -0.01435   Median : -0.008853  Median : -0.0213   Median : -0.02375
## Mean    : 0.000000  Mean    : 0.000000  Mean    : 0.00000  Mean    : 0.00000
## 3rd Qu. : 0.644411  3rd Qu. : 0.646598  3rd Qu. : 0.64666  3rd Qu. : 0.63884
## Max.    : 4.88190   Max.    : 4.932158  Max.    : 4.8974   Max.    : 4.95798
```

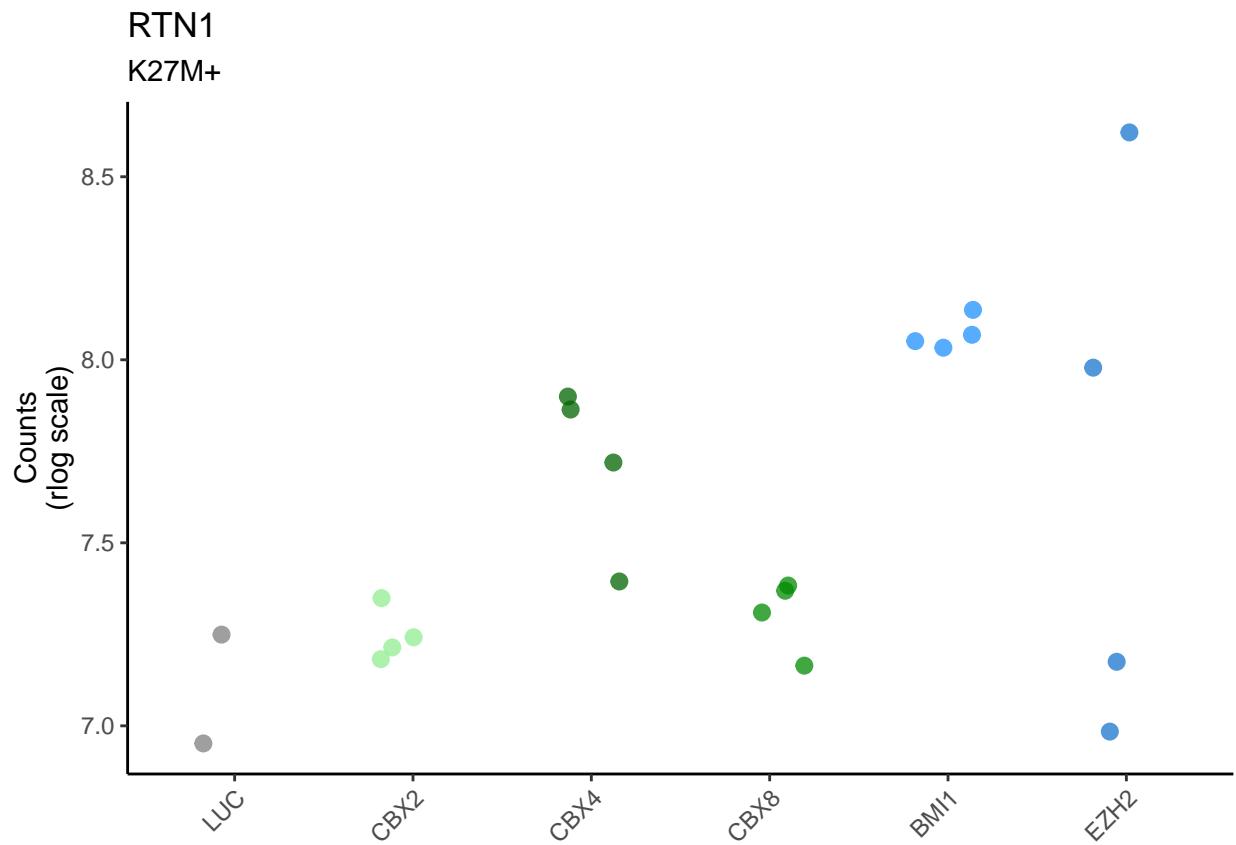


CDKN2A  
K27M KO

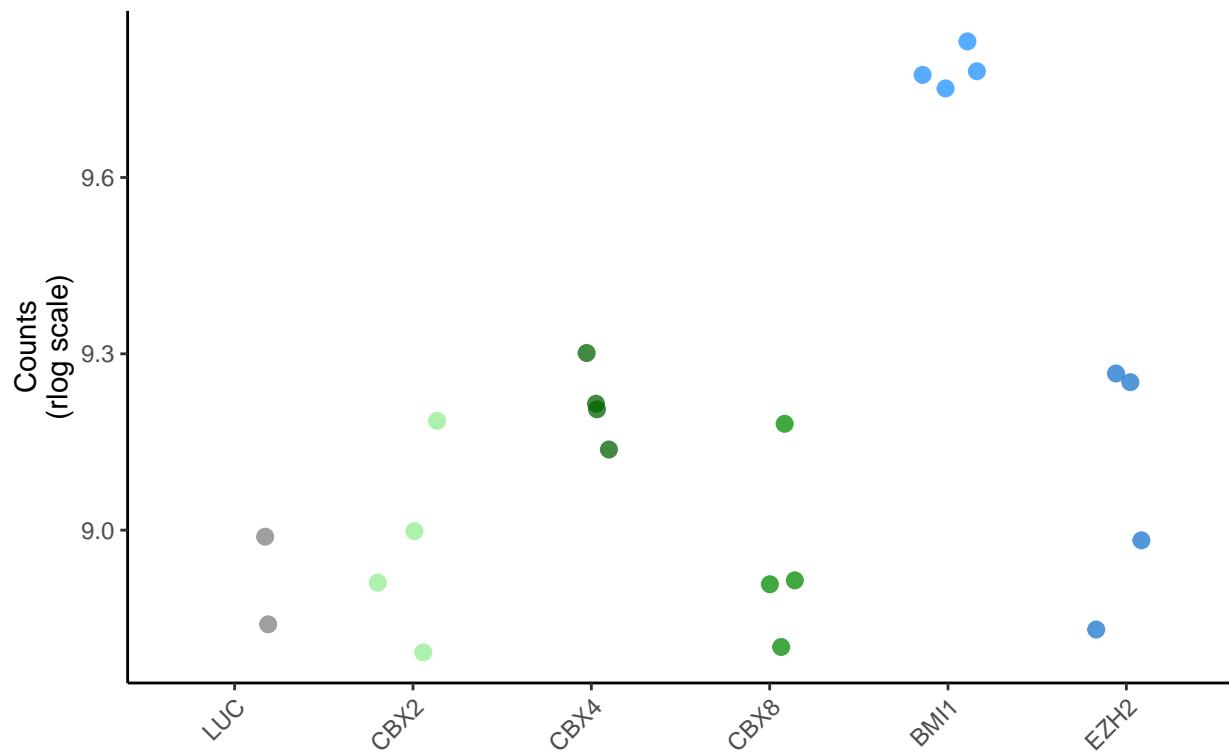


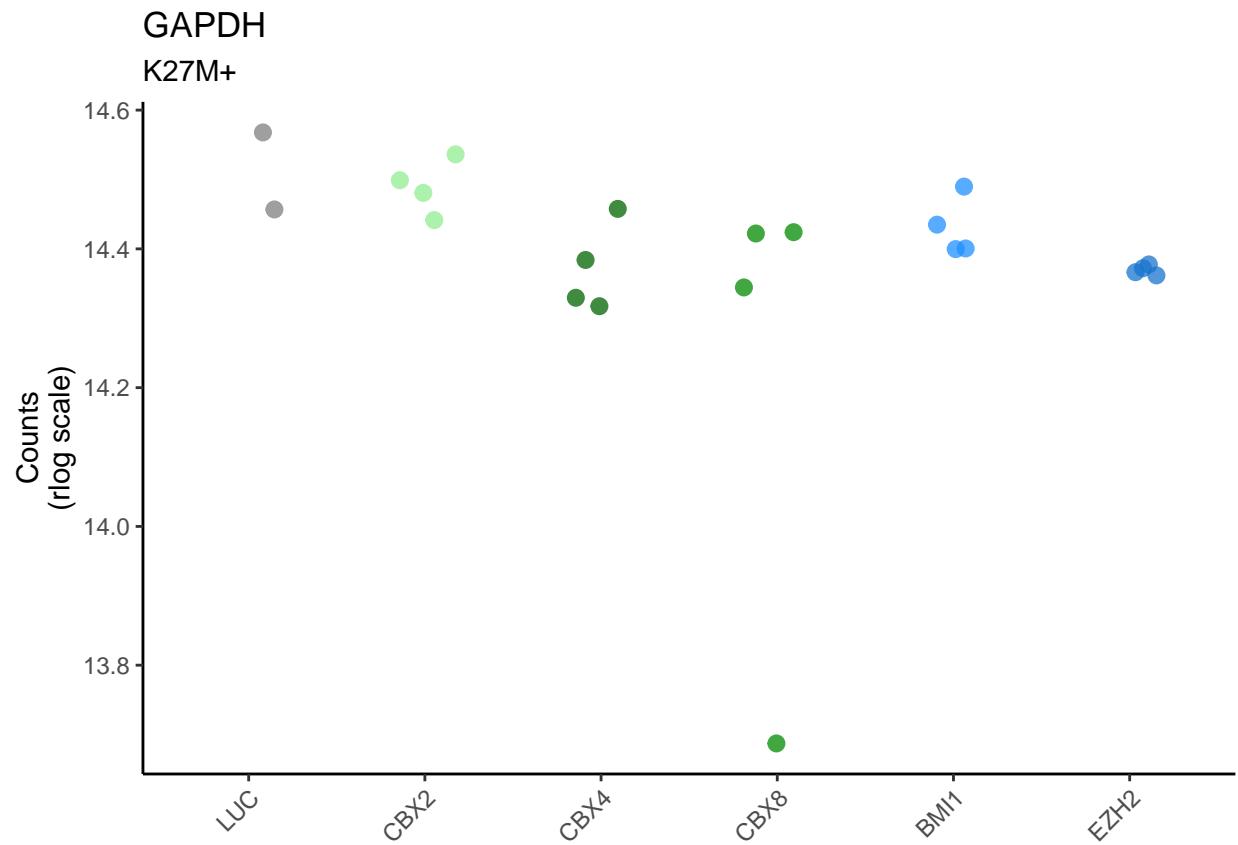


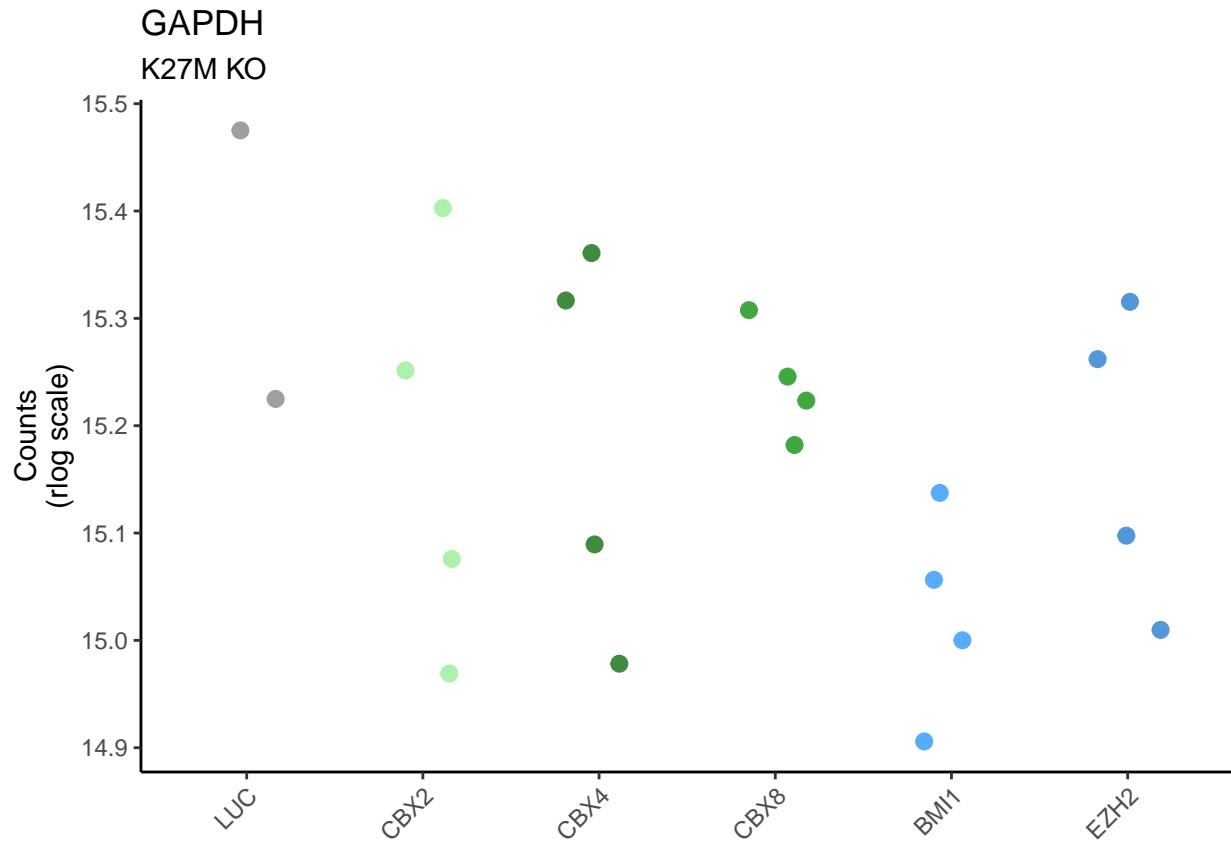




**RTN1**  
**K27M KO**







### MA Plots (K27M+)

```

## [1] "condition"      "CBX4.1_K27M+"  "CBX2.1_K27M+"
## [1] "condition"      "CBX4.2_K27M+"  "CBX2.1_K27M+"
## [1] "condition"      "CBX4.1_K27M+"  "CBX2.3_K27M+"
## [1] "condition"      "CBX4.2_K27M+"  "CBX2.3_K27M+"
## [1] "condition"      "CBX4.1_K27M+"  "CBX8.1_K27M+"
## [1] "condition"      "CBX4.2_K27M+"  "CBX8.1_K27M+"
## [1] "condition"      "CBX4.1_K27M+"  "CBX8.2_K27M+"
## [1] "condition"      "CBX4.2_K27M+"  "CBX8.2_K27M+"
## [1] "condition"      "CBX4.1_K27M+"  "EZH2.3_K27M+"
## [1] "condition"      "CBX4.2_K27M+"  "EZH2.3_K27M+"
## [1] "condition"      "CBX4.1_K27M+"  "EZH2.5_K27M+"

```

```
## [1] "condition"      "CBX4.2_K27M+"  "EZH2.5_K27M+"  
  
## [1] "condition"      "CBX4.1_K27M+"  "BMI1.1_K27M+"  
  
## [1] "condition"      "CBX4.2_K27M+"  "BMI1.1_K27M+"  
  
## [1] "condition"      "CBX4.1_K27M+"  "BMI1.3_K27M+"  
  
## [1] "condition"      "CBX4.2_K27M+"  "BMI1.3_K27M+"  
  
## [1] "condition"      "CBX4.1_K27M+"  "CBX4.1_K27M_KO"  
  
## [1] "condition"      "CBX4.2_K27M+"  "CBX4.1_K27M_KO"  
  
## [1] "condition"      "CBX4.1_K27M+"  "CBX4.2_K27M_KO"  
  
## [1] "condition"      "CBX4.2_K27M+"  "CBX4.2_K27M_KO"
```