

RNA-seq Analysis

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2024-12-05

Change for each project

Prepare counts matrix

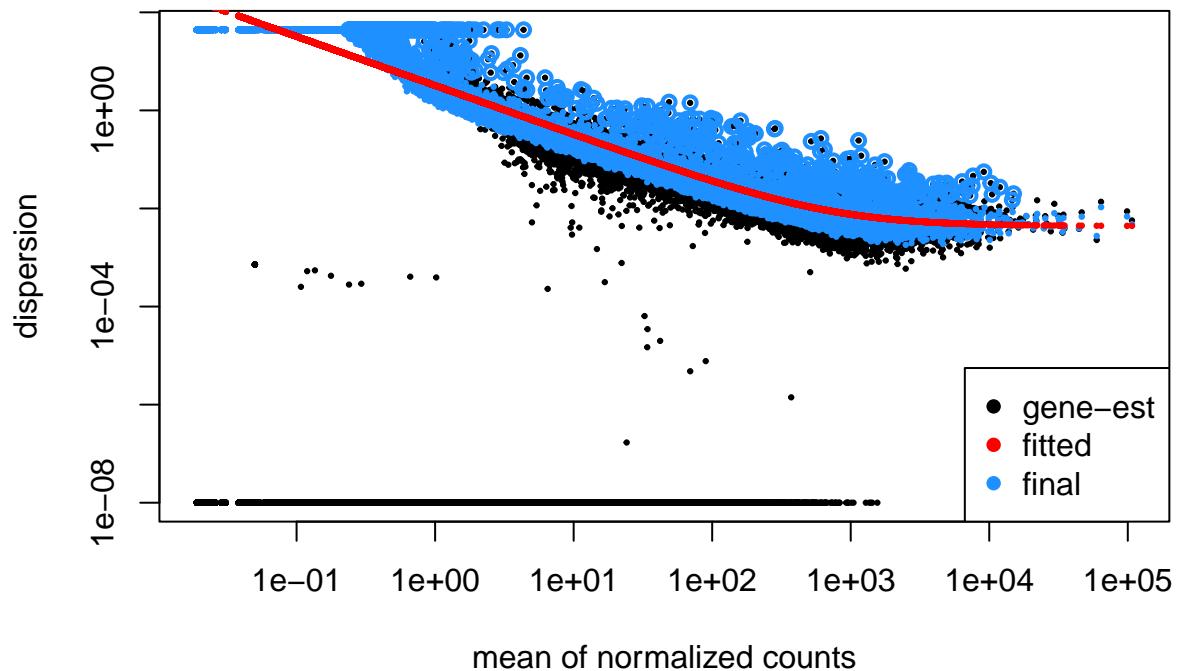
```
## [1] "Levels of gRNA factor:"  
  
## [1] "LUC"      "BMI1.1"   "BMI1.3"   "CBX2.1"   "CBX2.3"   "CBX4.1"   "CBX4.2"  
## [8] "CBX8.1"   "CBX8.2"   "EZH2.3"   "EZH2.5"   "H3F3A.2"   "H3F3A.3"  
  
## [1] "Levels of gene target factor:"  
  
## [1] "LUC"      "BMI1"     "CBX2"     "CBX4"     "CBX8"     "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] "Number of genes in data set: 64272"
```



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"

```

Volcano plots with IfcShrink

Gene expression heatmaps (all genes)

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+"
```

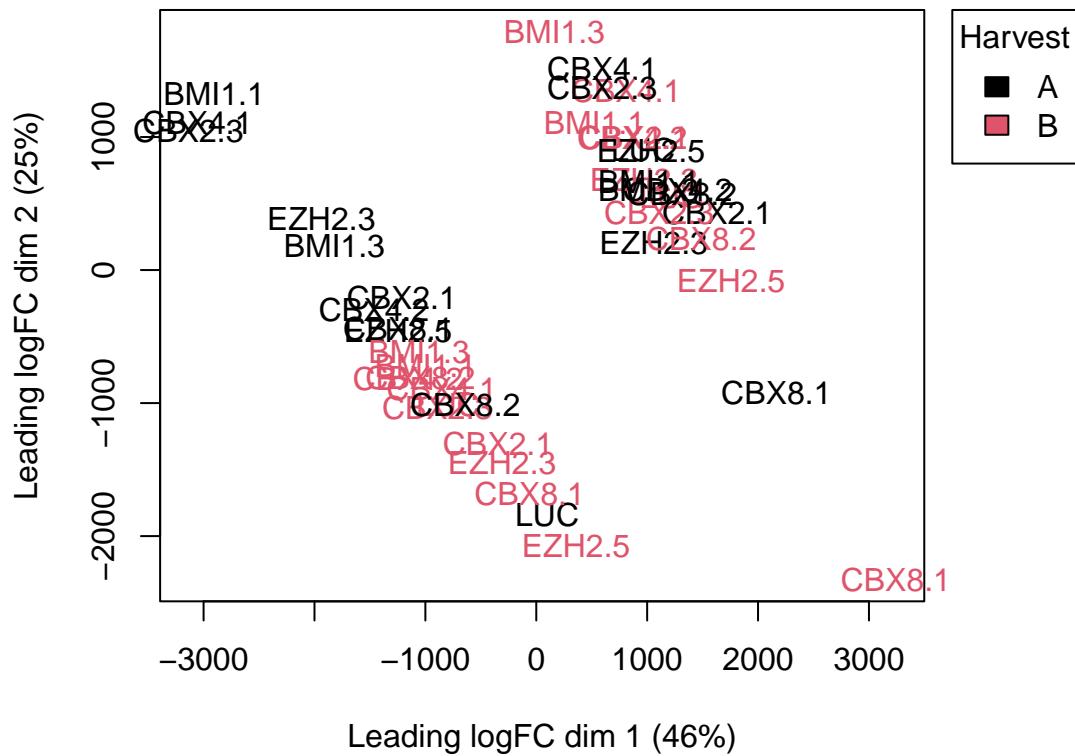
```
## [1] "Making comparison: CBX2.3 K27M+ vs LUC K27M+"
```

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

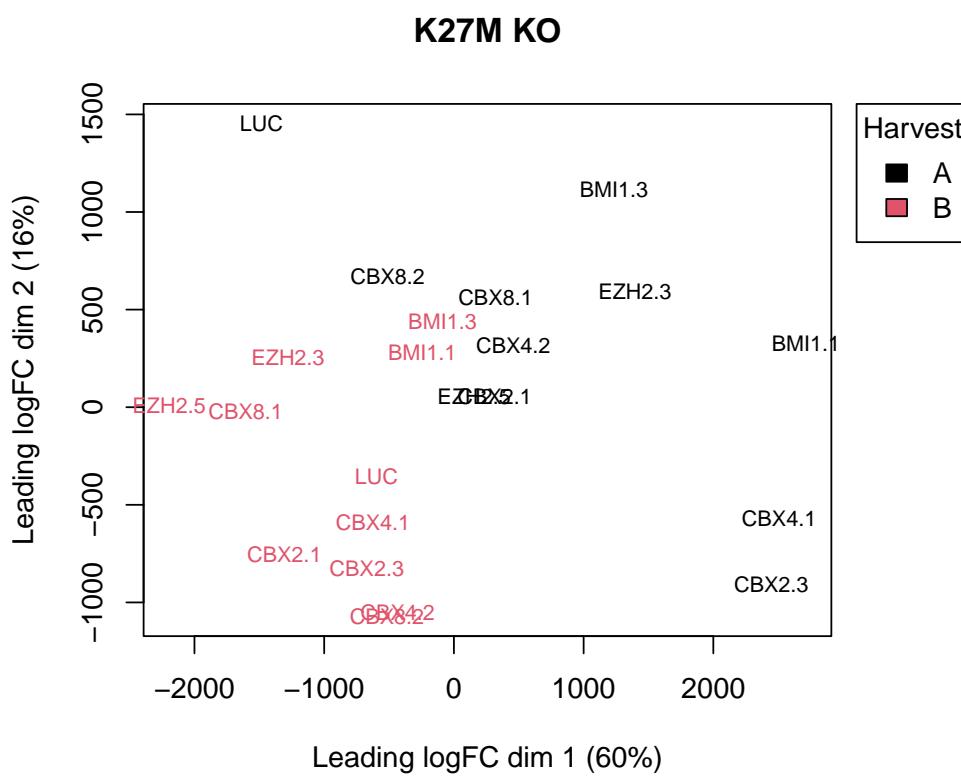
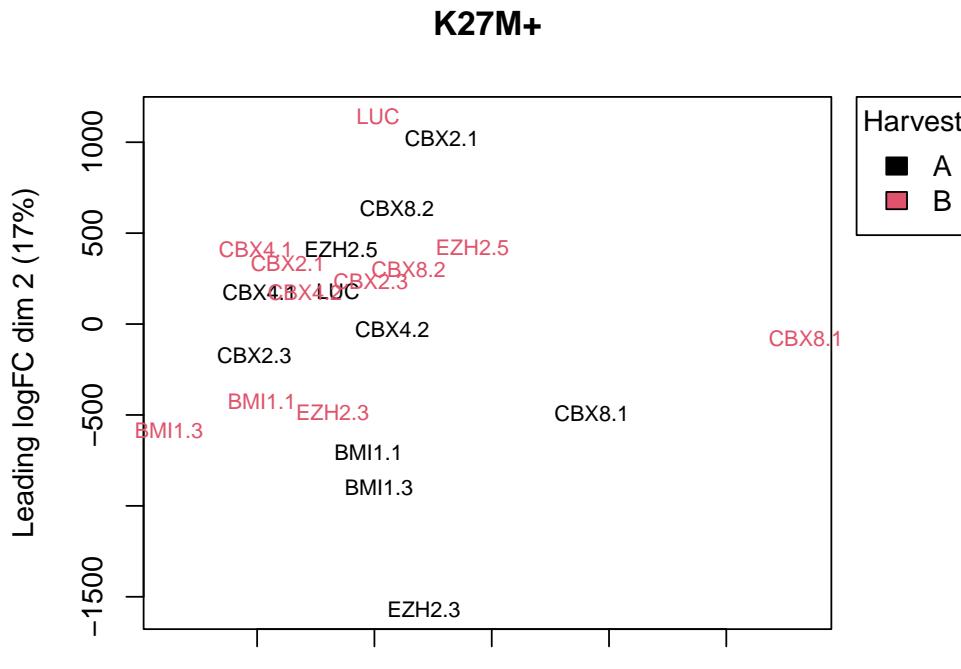
```
## [1] "Making comparison: CBX8.2 K27M+ vs LUC K27M+"
```

lfcShrink volcano plots

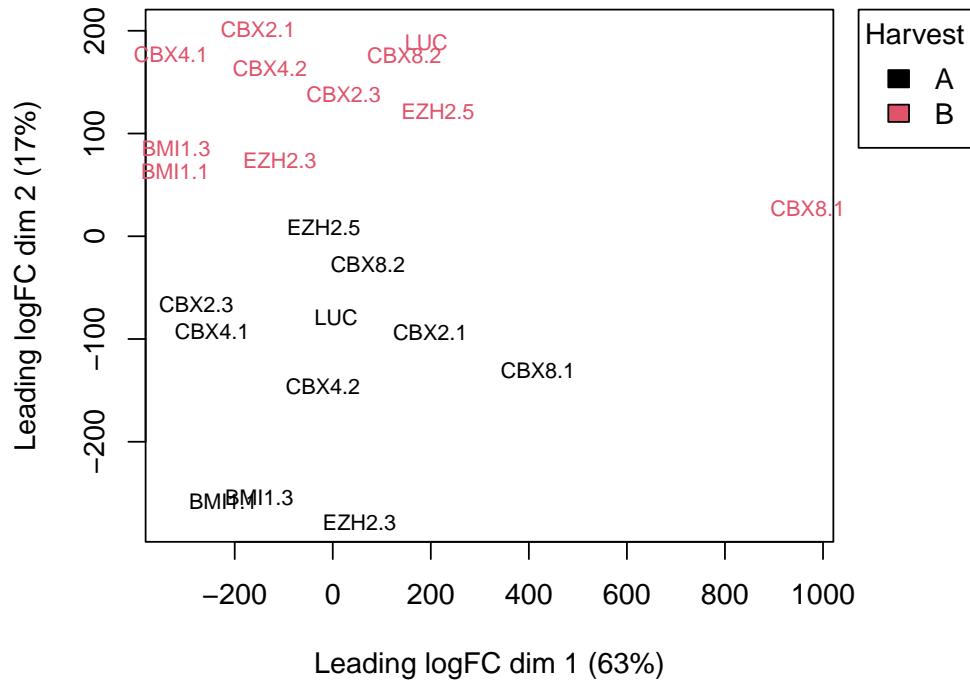
MDS plots (all genotypes)



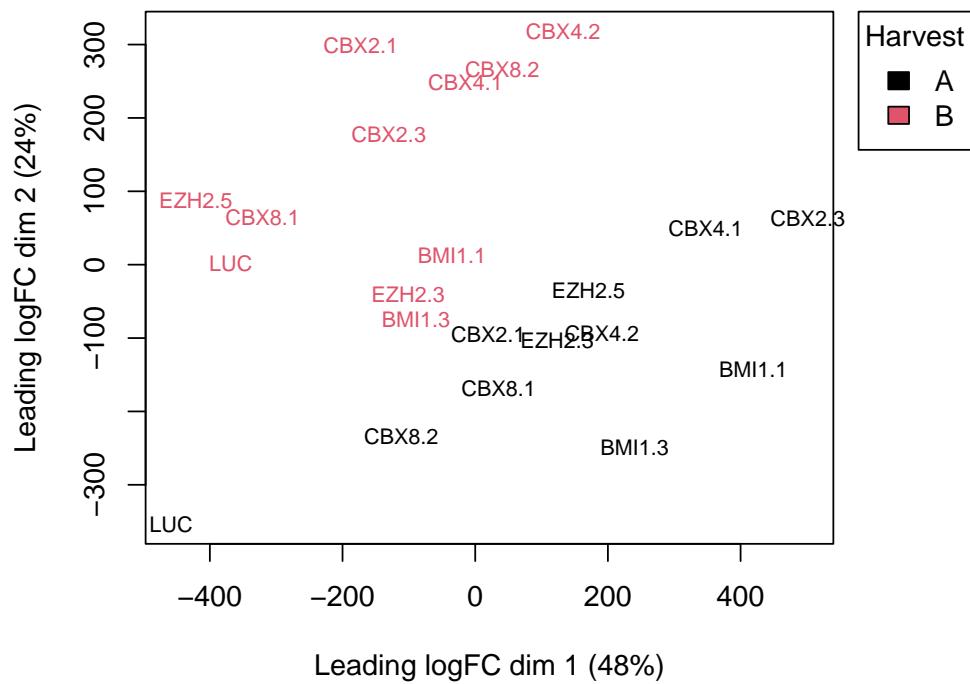
MDS plots (by genotypes)



**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+"

```

Prepare data for Q-Q plot comparing different KOs - upregulated

```

## [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] "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_KO_1_A LUC_K27M_KO_1_B LUC_K27M+_1_A LUC_K27M+_1_B
## DMRTA2      9.905174     9.773964    5.127327   4.983322
## EFHD1       8.872999     8.754798    6.652144   6.718962
## ZIC4        6.796677     6.827865    4.389217   4.329533
## PDLIM3      9.014078     8.662330    5.052239   5.048261
## FOXD1       4.644794     4.383846    4.212172   4.091266
## PCDH1       8.901578     8.301141    5.670365   5.432514
## SCUBE3      5.552530     5.413173    5.009332   5.270702
## CPNE5        8.464092     7.655380    5.738217   6.062448
## EYA4         10.251749    10.279381   6.664907   6.625677
## CDKN2A      7.669859     7.369515    8.131389   7.955023
## IGSF9B       8.117478     8.667190    6.400612   6.272158
## ITGA5        8.581764     8.354264    6.843192   6.414037
## RTN1         8.856148     8.993226    6.922934   7.219950
## ACTC1        5.010204     4.465308    3.961457   3.877563
## TLE2         3.963068     4.068613    3.308481   3.295542

##      LUC_K27M_KO_1_A LUC_K27M_KO_1_B LUC_K27M+_1_A LUC_K27M+_1_B
## Min.   :-2.3898   Min.   :-2.390   Min.   :-2.3900  Min.   :-2.3901
## 1st Qu.:-0.5243   1st Qu.:-0.519   1st Qu.:-0.5233  1st Qu.:-0.5185
## Median : 0.0000   Median : 0.000   Median : 0.0000  Median : 0.0000
## Mean   : 1.6102   Mean   : 1.611   Mean   : 1.6122  Mean   : 1.6168
## 3rd Qu.: 3.4329   3rd Qu.: 3.449   3rd Qu.: 3.4583  3rd Qu.: 3.5069
## Max.   :16.9822   Max.   :16.889   Max.   :16.7283  Max.   :16.5795

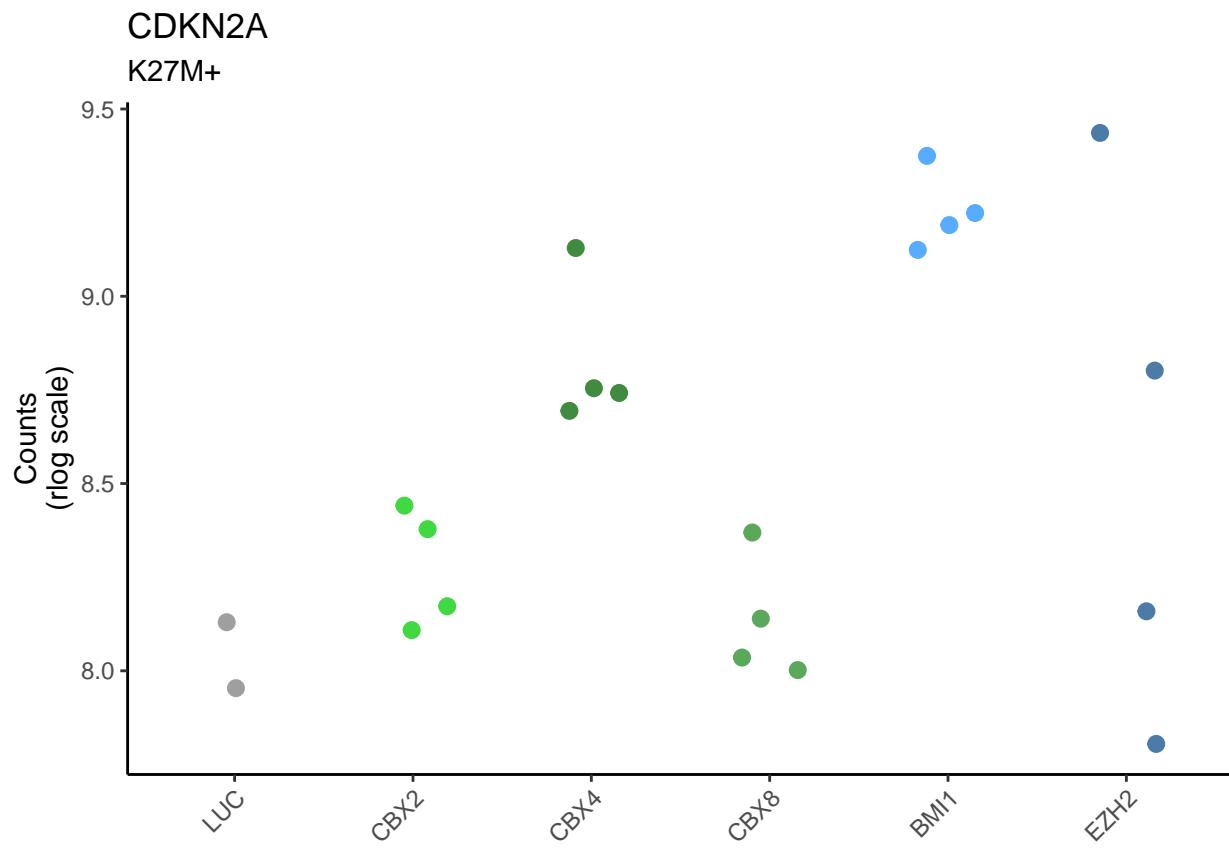
## [1] "Common Genes at Baseline (Z-score of the rlog transform)"

##      LUC_K27M_KO_1_A LUC_K27M_KO_1_B LUC_K27M+_1_A LUC_K27M+_1_B
## DMRTA2      2.1744381    2.1441555    0.9229741   0.8844507
## EFHD1       1.9038636    1.8764591    1.3233536   1.3404335
## ZIC4        1.3595757    1.3703268    0.7291643   0.7126890
## PDLIM3      1.9408460    1.8521714    0.9032577   0.9015113
## FOXD1       0.7954805    0.7283755    0.6826769   0.6500923
## PCDH1       1.9113551    1.7573006    1.0655625   1.0024614
## SCUBE3      1.0334346    0.9987407    0.8919915   0.9599504
## CPNE5        1.7966725    1.5876837    1.0833787   1.1679559
## EYA4         2.2652896    2.2769095    1.3267047   1.3159259
## CDKN2A      1.5884719    1.5125978    1.7117670   1.6651682
## IGSF9B       1.7058109    1.8534479    1.2573074   1.2230504
## ITGA5        1.8275191    1.7712540    1.3735180   1.2603244
## RTN1         1.8994461    1.9390852    1.3944564   1.4720518
## ACTC1        0.8912691    0.7497724    0.6168452   0.5939487
## TLE2         0.6167725    0.6455757    0.4453897   0.4410417

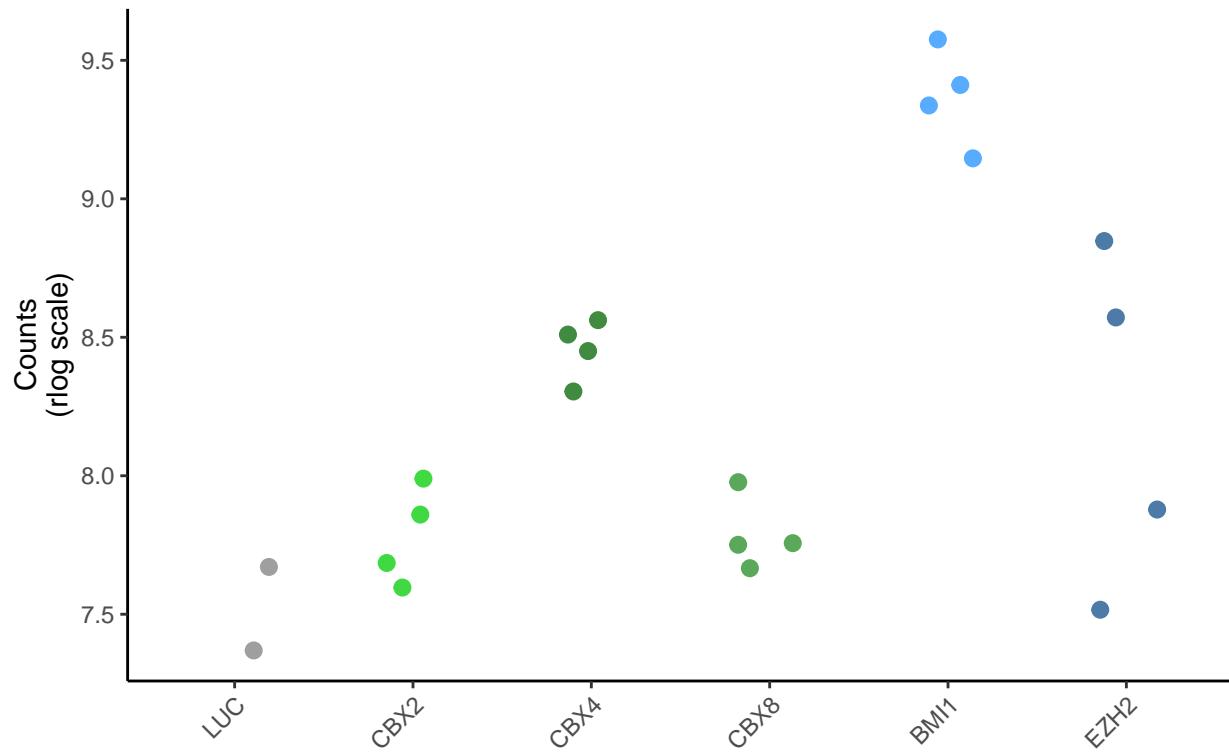
##      LUC_K27M_KO_1_A LUC_K27M_KO_1_B LUC_K27M+_1_A LUC_K27M+_1_B
## Min.   :-1.0486   Min.   :-1.0509   Min.   :-1.0509  Min.   :-1.0527
## 1st Qu.:-0.5595   1st Qu.:-0.5594   1st Qu.:-0.5608  1st Qu.:-0.5610
## Median :-0.4221   Median :-0.4231   Median :-0.4233  Median :-0.4248

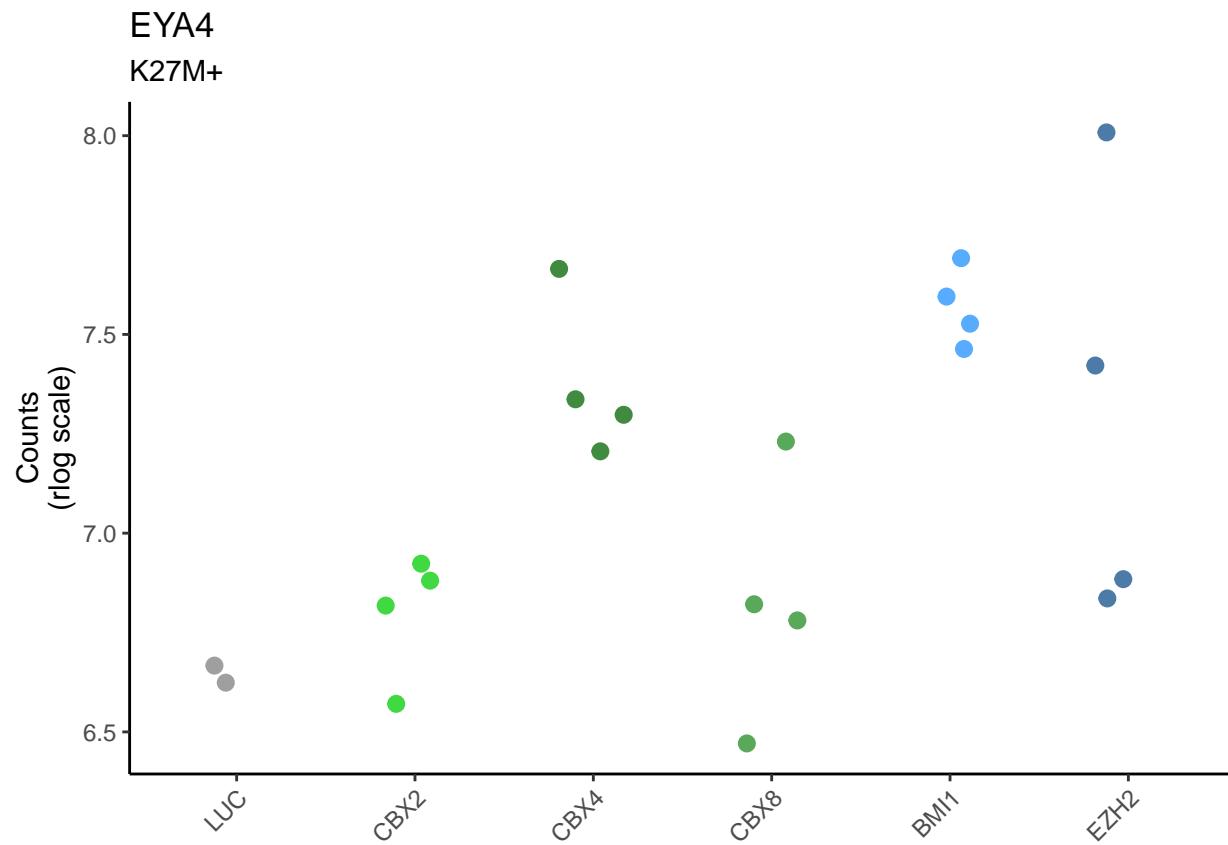
```

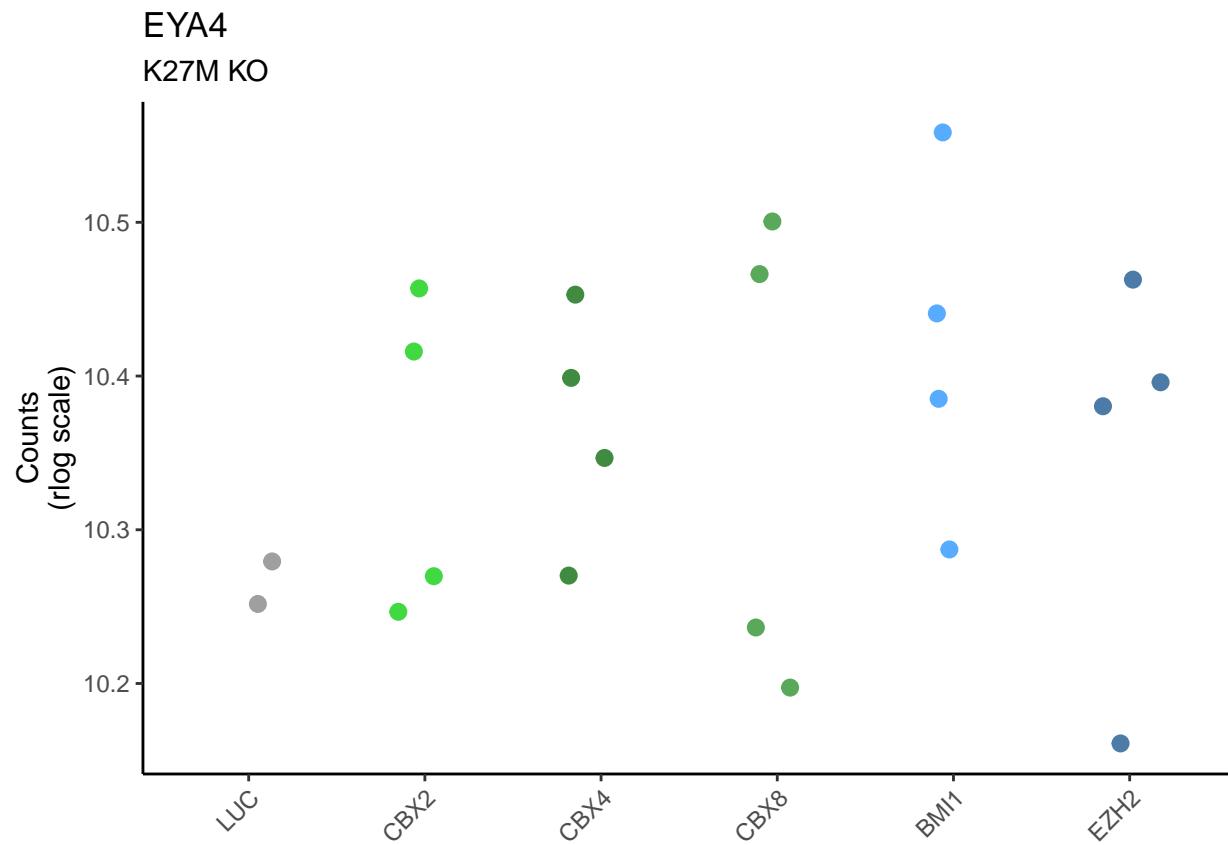
```
##  Mean   : 0.0000  Mean   : 0.0000  Mean   : 0.0000  Mean   : 0.0000  
## 3rd Qu.: 0.4778  3rd Qu.: 0.4827  3rd Qu.: 0.4847  3rd Qu.: 0.4966  
## Max.   : 4.0296  Max.   : 4.0130  Max.   : 3.9691  Max.   : 3.9310
```

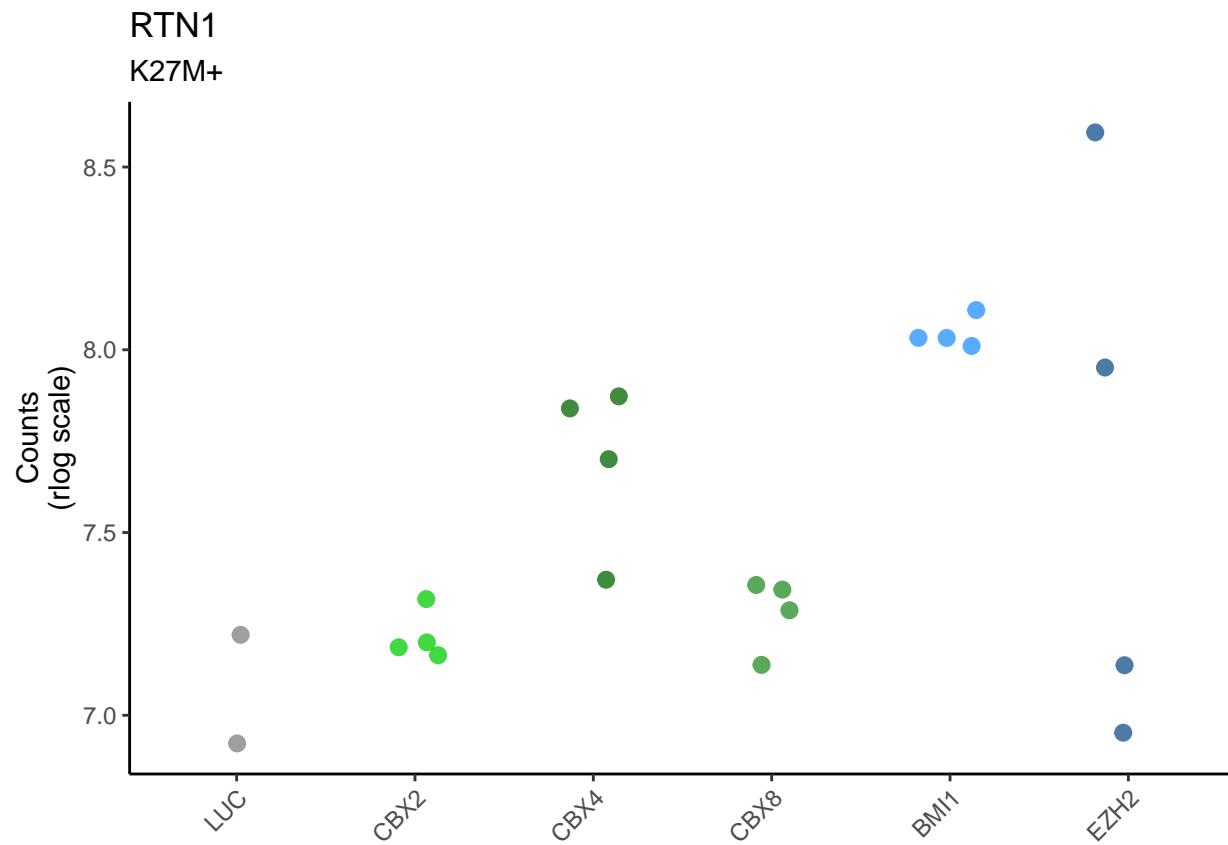


CDKN2A
K27M KO

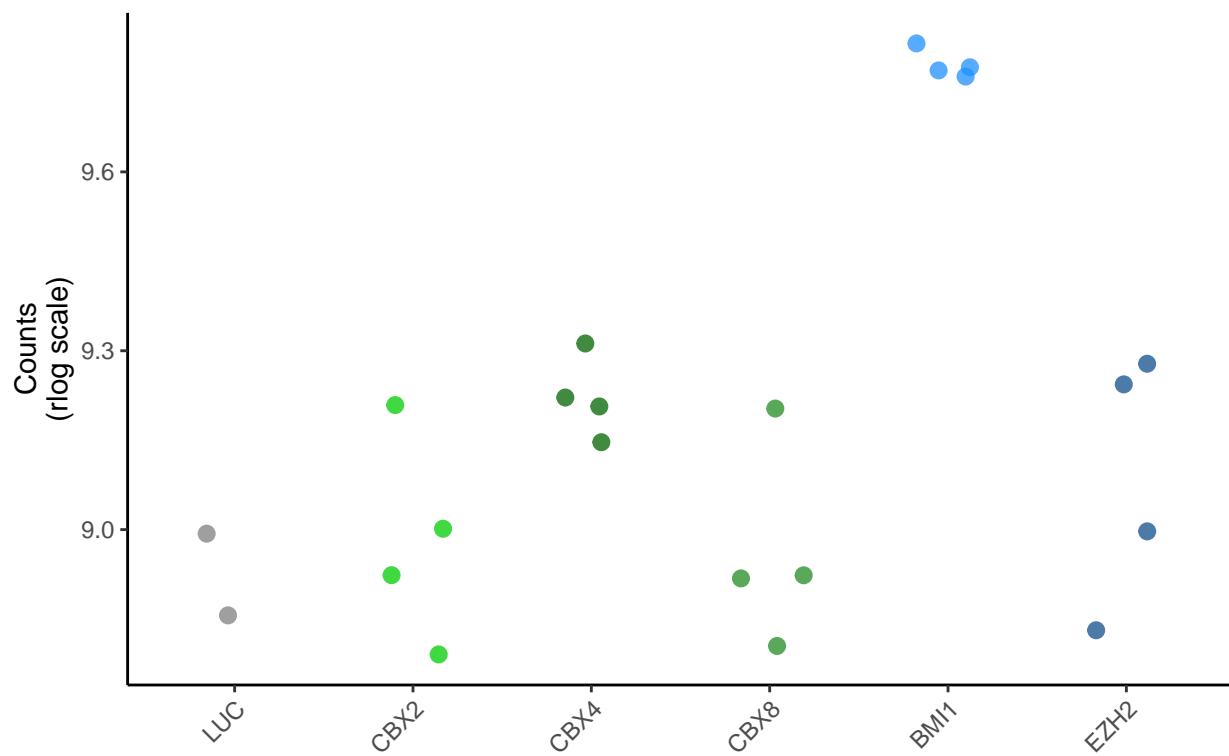


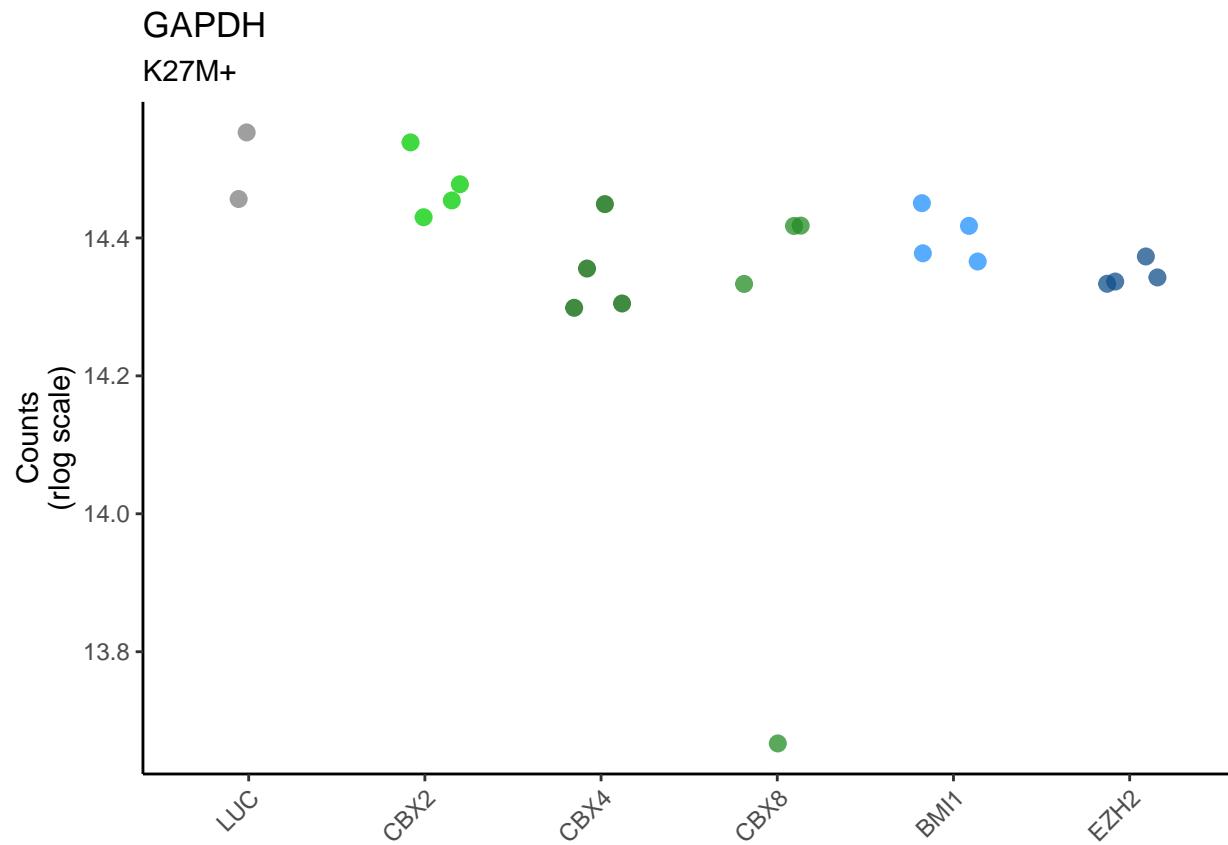


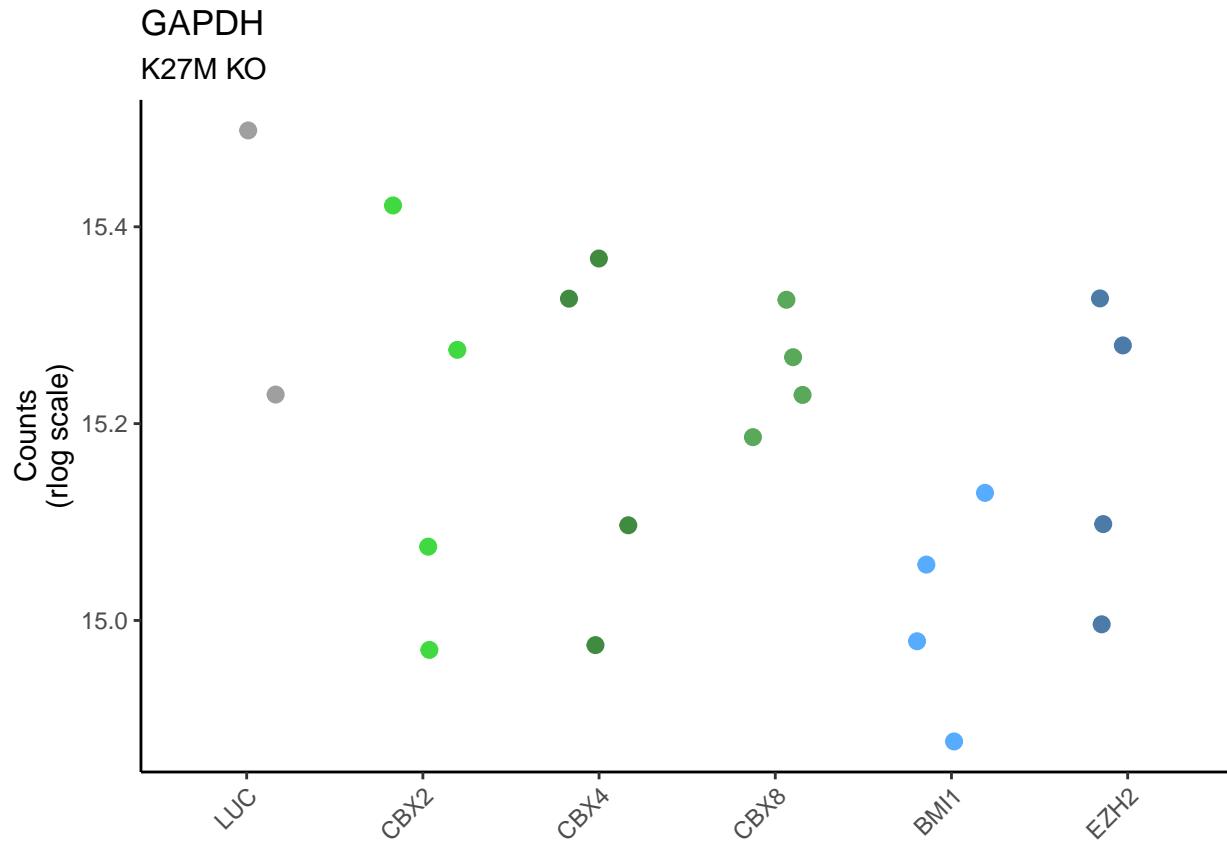




RTN1
K27M KO







MA Plots (K27M+)

```
## [1] "Making comparison: CBX4.1_K27M+_1_A" "Making comparison: CBX4.1_K27M+_1_B"
## [3] "Making comparison: CBX2.1_K27M+_1_A" "Making comparison: CBX2.1_K27M+_1_B"
## [1] "Contrasting: condition"      "Contrasting: CBX4.1_K27M+"
## [3] "Contrasting: CBX2.1_K27M+"

## [1] "Making comparison: CBX4.2_K27M+_1_A" "Making comparison: CBX4.2_K27M+_1_B"
## [3] "Making comparison: CBX2.1_K27M+_1_A" "Making comparison: CBX2.1_K27M+_1_B"
## [1] "Contrasting: condition"      "Contrasting: CBX4.2_K27M+"
## [3] "Contrasting: CBX2.1_K27M+"

## [1] "Making comparison: CBX4.1_K27M+_1_A" "Making comparison: CBX4.1_K27M+_1_B"
## [3] "Making comparison: CBX2.3_K27M+_1_A" "Making comparison: CBX2.3_K27M+_1_B"
## [1] "Contrasting: condition"      "Contrasting: CBX4.1_K27M+"
## [3] "Contrasting: CBX2.3_K27M+"

## [1] "Making comparison: CBX4.2_K27M+_1_A" "Making comparison: CBX4.2_K27M+_1_B"
## [3] "Making comparison: CBX2.3_K27M+_1_A" "Making comparison: CBX2.3_K27M+_1_B"
## [1] "Contrasting: condition"      "Contrasting: CBX4.2_K27M+"
## [3] "Contrasting: CBX2.3_K27M+"

## [1] "Making comparison: CBX4.1_K27M+_1_A" "Making comparison: CBX4.1_K27M+_1_B"
## [3] "Making comparison: CBX8.1_K27M+_1_A" "Making comparison: CBX8.1_K27M+_1_B"
```



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## [1] "Making comparison: CBX4.2_K27M+_1_A" "Making comparison: CBX4.2_K27M+_1_B"
## [3] "Making comparison: BMI1.3_K27M+_1_A" "Making comparison: BMI1.3_K27M+_1_B"
## [1] "Contrasting: condition"      "Contrasting: CBX4.2_K27M+"
## [3] "Contrasting: BMI1.3_K27M+"

## [1] "Making comparison: CBX4.1_K27M+_1_A"
## [2] "Making comparison: CBX4.1_K27M+_1_B"
## [3] "Making comparison: CBX4.1_K27M_KO_1_A"
## [4] "Making comparison: CBX4.1_K27M_KO_1_B"
## [1] "Contrasting: condition"      "Contrasting: CBX4.1_K27M+"
## [3] "Contrasting: CBX4.1_K27M_KO"

## [1] "Making comparison: CBX4.2_K27M+_1_A"
## [2] "Making comparison: CBX4.2_K27M+_1_B"
## [3] "Making comparison: CBX4.1_K27M_KO_1_A"
## [4] "Making comparison: CBX4.1_K27M_KO_1_B"
## [1] "Contrasting: condition"      "Contrasting: CBX4.2_K27M+"
## [3] "Contrasting: CBX4.1_K27M_KO"

## [1] "Making comparison: CBX4.1_K27M+_1_A"
## [2] "Making comparison: CBX4.1_K27M+_1_B"
## [3] "Making comparison: CBX4.2_K27M_KO_1_A"
## [4] "Making comparison: CBX4.2_K27M_KO_1_B"
## [1] "Contrasting: condition"      "Contrasting: CBX4.1_K27M+"
## [3] "Contrasting: CBX4.2_K27M_KO"

## [1] "Making comparison: CBX4.2_K27M+_1_A"
## [2] "Making comparison: CBX4.2_K27M+_1_B"
## [3] "Making comparison: CBX4.2_K27M_KO_1_A"
## [4] "Making comparison: CBX4.2_K27M_KO_1_B"
## [1] "Contrasting: condition"      "Contrasting: CBX4.2_K27M+"
## [3] "Contrasting: CBX4.2_K27M_KO"

```