Replication: Thomas et al., LAA/RAA RNAseq AF and ctrls (GSE128188)

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1 Load data

Define genes of interest:

```
## [1] "eQTL genes:"
```

```
## [1] "NKX2-5" "TNNT2"
```

```
## [1] "pQTL genes:"
```

```
## [1] "CYB5R3" "NDUFB3" "HIBADH" "NDUFA9" "DLAT"
```

```
## [1] "NKX2-5 targets:"
```

```
##  [1] "PPIF"   "MYL4"   "CKM"    "MYL7"   "PGAM2"  "TNNC1"  "CYC1"   "ETFB"   "PRDX5"
## [10] "AK1"    "ALDOA"  "TCAP"   "TOM1L2"
```

```
## [1] "Genes in fibrosis score: "
```

```
##  [1] "ELN"      "FGF10"    "JAG1"     "KIAA1199" "CPXM2"    "FOSB"     "FCRL2"
##  [8] "SCN7A"    "NOV"      "ARHGAP20" "CILP"     "FRAS1"    "DCDC2"    "NRG1"
## [15] "CLEC3B"   "AFAP1L2"  "COL14A1"  "ITGBL1"
```

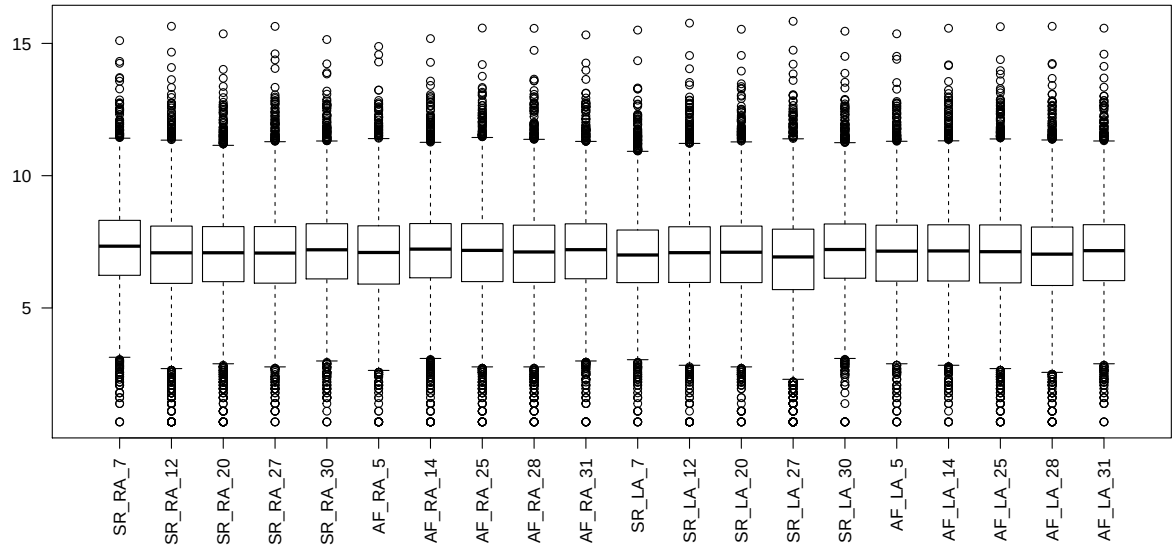
1.1 Gene length annotations

2 Replication: Left atrium AF vs. healthy

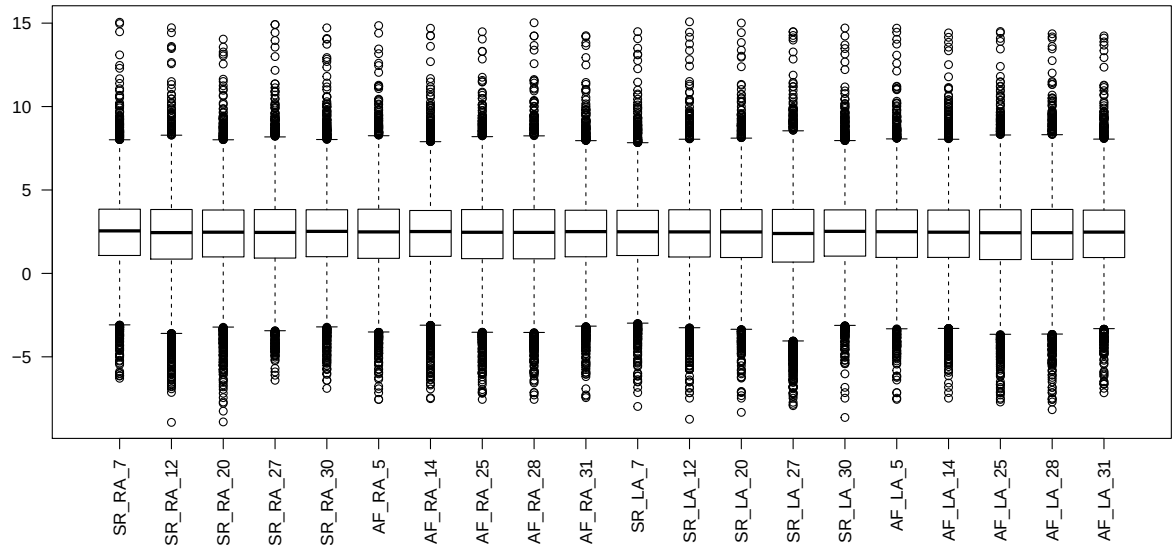
2.1 Load counts and correct for library size

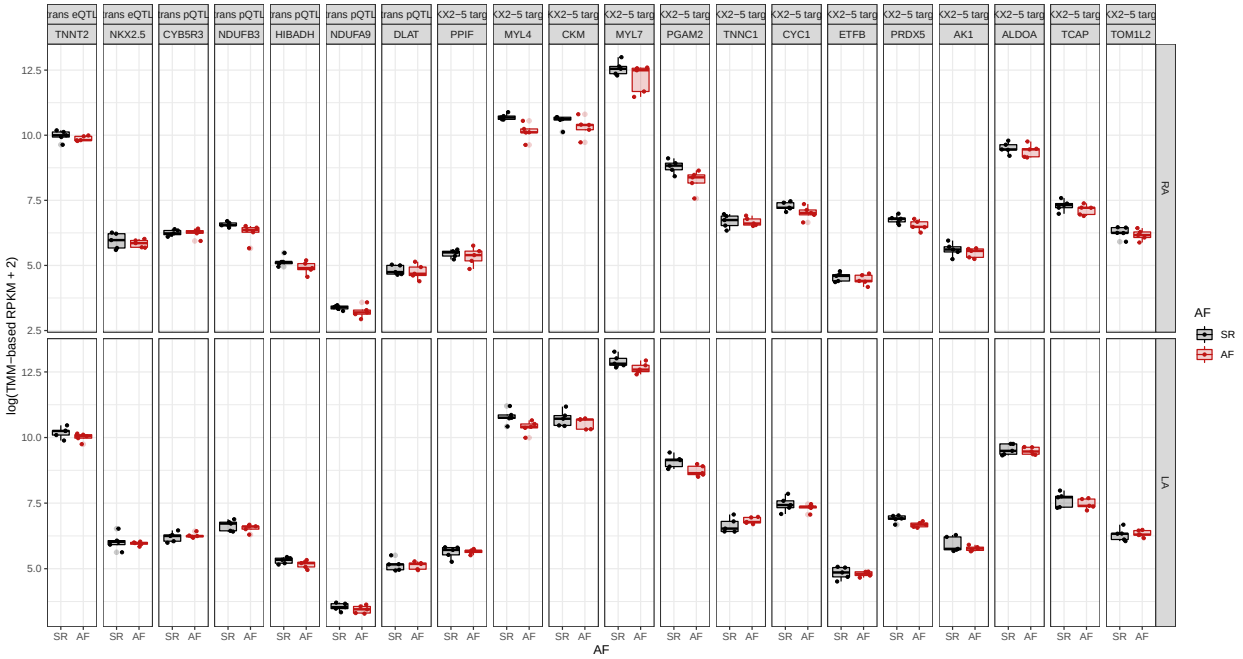
```
## [1] "All trans QTL genes and NKX2-5 targets measured."
```

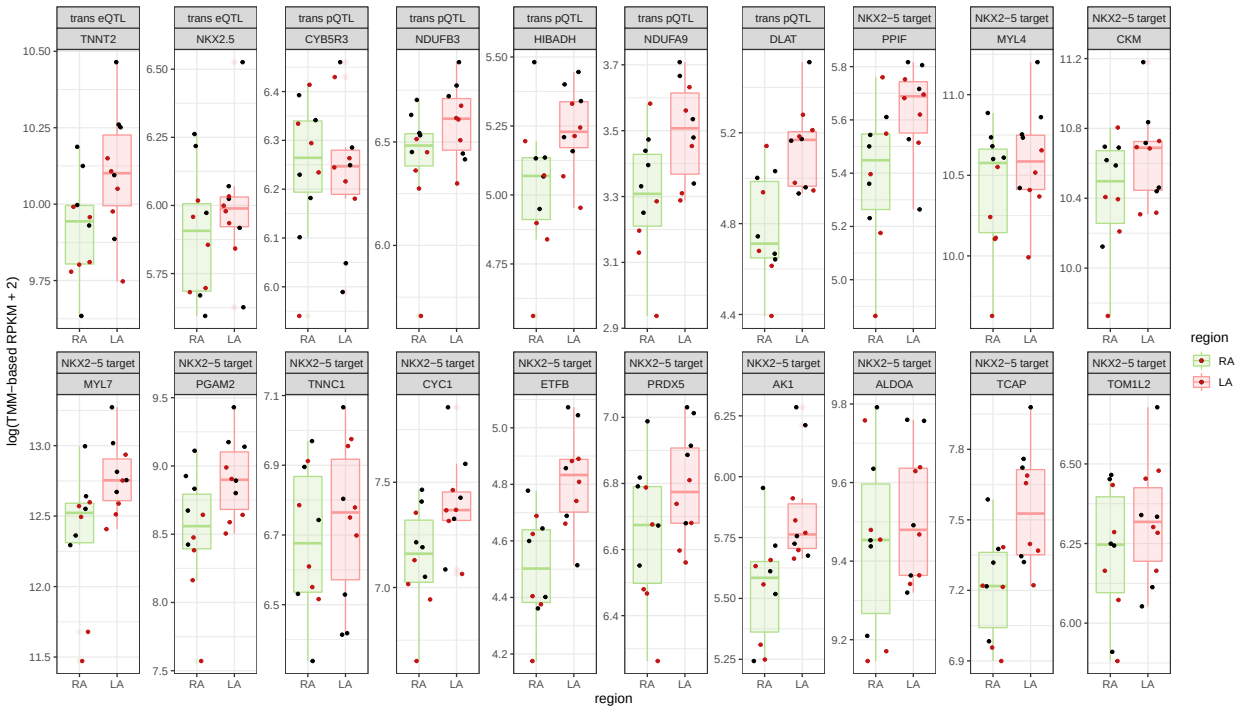
No huge differences in library size:



After correction for library size (TMM−based RPKMs):







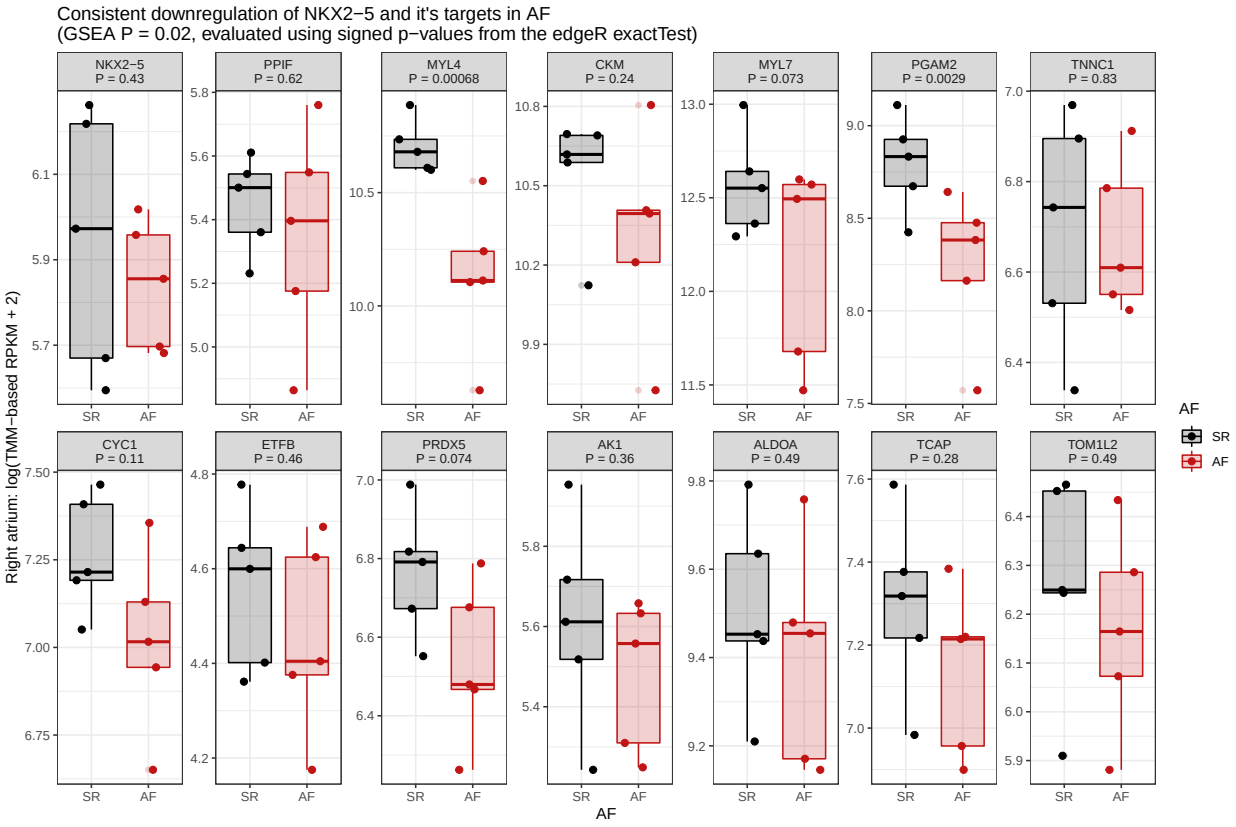

```
## [1] "Right atrium:"
```

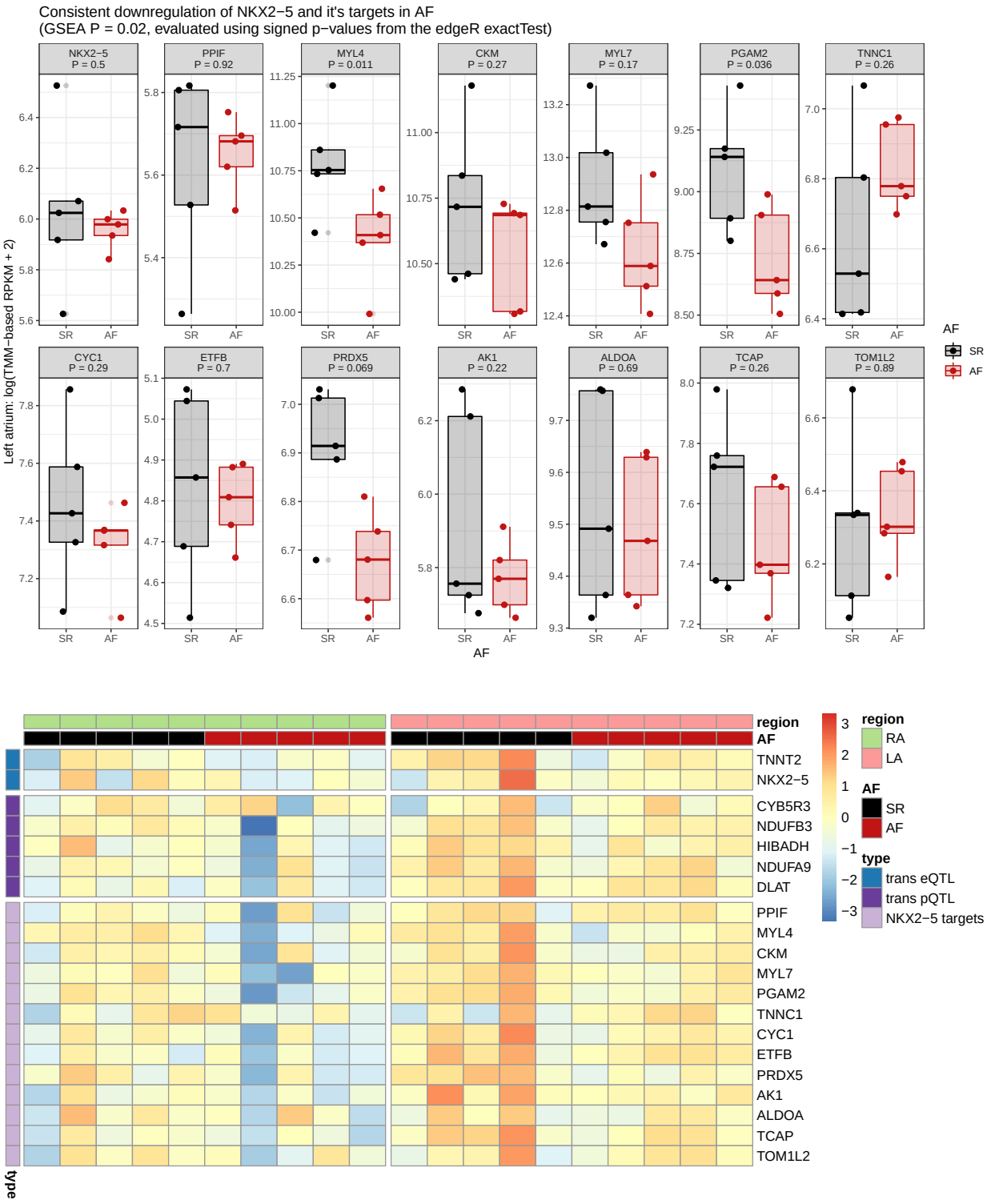
```
##         symbol       logFC    logCPM       PValue       FDR symbol2
## NKX2.5 NKX2-5 -0.12083565  6.893973 0.4309282300 1.00000000  NKX2.5
## PPIF     PPIF -0.07457071  6.672741 0.6152418823 1.00000000    PPIF
## MYL4     MYL4 -0.54877653 10.508736 0.0006810827 0.05457129    MYL4
## CKM       CKM -0.20800205 11.199817 0.2389393567 0.94110738     CKM
## MYL7     MYL7 -0.35170922 12.340329 0.0730553758 0.65779021    MYL7
## PGAM2   PGAM2 -0.52095482  8.508459 0.0029233794 0.12858532   PGAM2
```

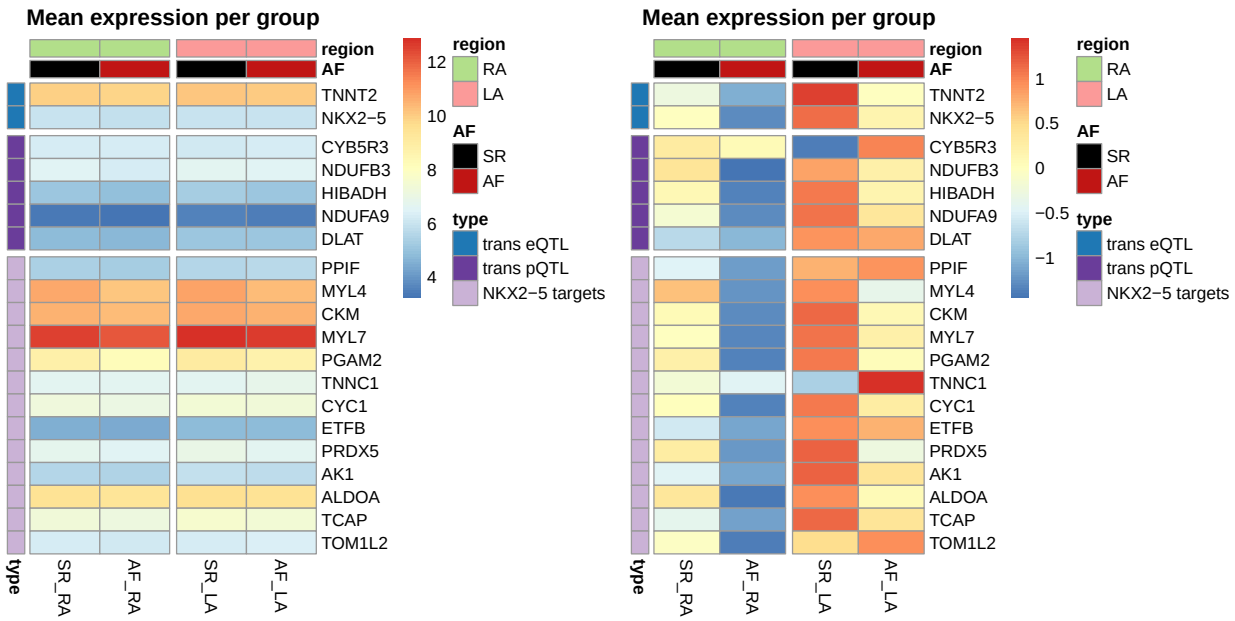
```
## TNNC1    TNNC1 -0.03108355  9.051189 0.8333231964 1.00000000   TNNC1
## CYC1      CYC1 -0.23659353  7.538934 0.1104059337 0.76952662    CYC1
## ETFB      ETFB -0.09987551  6.566480 0.4602519723 1.00000000    ETFB
## PRDX5    PRDX5 -0.22579814  6.515512 0.0739769141 0.66018049   PRDX5
## AK1        AK1 -0.13623868  7.029380 0.3569448794 0.99750456     AK1
## ALDOA    ALDOA -0.09920934 10.710710 0.4921777069 1.00000000   ALDOA
## TCAP      TCAP -0.16364452  9.867318 0.2770849081 0.96690773    TCAP
## TOM1L2 TOM1L2 -0.09799518  8.834506 0.4931498320 1.00000000  TOM1L2
```

```
## [1] "Left atrium:"
```

```
##         symbol       logFC    logCPM     PValue       FDR symbol2
## NKX2.5 NKX2-5 -0.10387860  6.893973 0.49832557 1.0000000  NKX2.5
## PPIF     PPIF  0.01451649  6.672741 0.92231160 1.0000000    PPIF
## MYL4     MYL4 -0.41171128 10.508736 0.01071171 0.3381628    MYL4
## CKM       CKM -0.19433722 11.199817 0.27118284 1.0000000     CKM
## MYL7     MYL7 -0.27127273 12.340329 0.16659110 1.0000000    MYL7
## PGAM2   PGAM2 -0.36680728  8.508459 0.03588102 0.5963547   PGAM2
## TNNC1   TNNC1  0.16665729  9.051189 0.25925345 1.0000000   TNNC1
## CYC1     CYC1 -0.15774362  7.538934 0.28692925 1.0000000    CYC1
## ETFB     ETFB -0.05129415  6.566480 0.70432490 1.0000000    ETFB
## PRDX5   PRDX5 -0.22992992  6.515512 0.06877527 0.7636373   PRDX5
## AK1       AK1 -0.17956565  7.029380 0.22456629 1.0000000     AK1
## ALDOA   ALDOA -0.05677004 10.710710 0.69428462 1.0000000   ALDOA
## TCAP     TCAP -0.17004265  9.867318 0.25872121 1.0000000    TCAP
## TOM1L2 TOM1L2  0.02051197  8.834506 0.88599894 1.0000000  TOM1L2
```




Consistent downregulation of NKX2−5 and it's targets in AF
(GSEA P = 0.02, evaluated using signed p−values from the edgeR exactTest)
NKX2−5 P = 0.5
PPIF P = 0.92
MYL4 P = 0.011
CKM P = 0.27
MYL7 P = 0.17
PGAM2 P = 0.036
TNNC1 P = 0.26
CYC1 P = 0.29
ETFB P = 0.7
PRDX5 P = 0.069
AK1 P = 0.22
ALDOA P = 0.69
TCAP P = 0.26
TOM1L2 P = 0.89
Left atrium: log(TMM−based RPKM + 2)
SR
AF
AF
region
RA
LA
AF
SR
AF
type
trans eQTL
trans pQTL
NKX2−5 targets
TNNT2
NKX2−5
CYB5R3
NDUFB3
HIBADH
NDUFA9
DLAT
PPIF
MYL4
CKM
MYL7
PGAM2
TNNC1
CYC1
ETFB
PRDX5
AK1
ALDOA
TCAP
TOM1L2
type



2.2 GSEA analysis

```
## [1] "GSEA: exact test on logFC:"
```

```
##                             pathway size
## 1:              Atrial_fibrillation   26
## 2: AF_trans_core_genes_and_targets   19
## 3:               NKX2-5_and_targets   14
## 4:                   NKX2-5_targets   13
## 5:                       Core_genes    6
## 6:           Proteomics_core_genes    4
## 7:                        Cis_genes    7
## 8:      Transcriptomics_core_genes    2
```

```
## [1] "GSEA: exact test on logFC (right atrium):"
```

```
##                             pathway size
## 1:              Atrial_fibrillation   26
## 2: AF_trans_core_genes_and_targets   19
## 3:               NKX2-5_and_targets   14
## 4:                   NKX2-5_targets   13
## 5:                       Core_genes    6
## 6:           Proteomics_core_genes    4
## 7:                        Cis_genes    7
## 8:      Transcriptomics_core_genes    2
```

```
## [1] "GSEA: exact test on signed pvalue (right atrium):"
```

```
##                             pathway size
## 1:              Atrial_fibrillation   26
## 2: AF_trans_core_genes_and_targets   19
## 3:               NKX2-5_and_targets   14
## 4:                   NKX2-5_targets   13
## 5:           Proteomics_core_genes    4
## 6:                       Core_genes    6
```

```
## 7:                       Cis_genes    7
## 8:      Transcriptomics_core_genes    2
```

```
## [1] "GSEA: exact test on logFC (left atrium):"
```

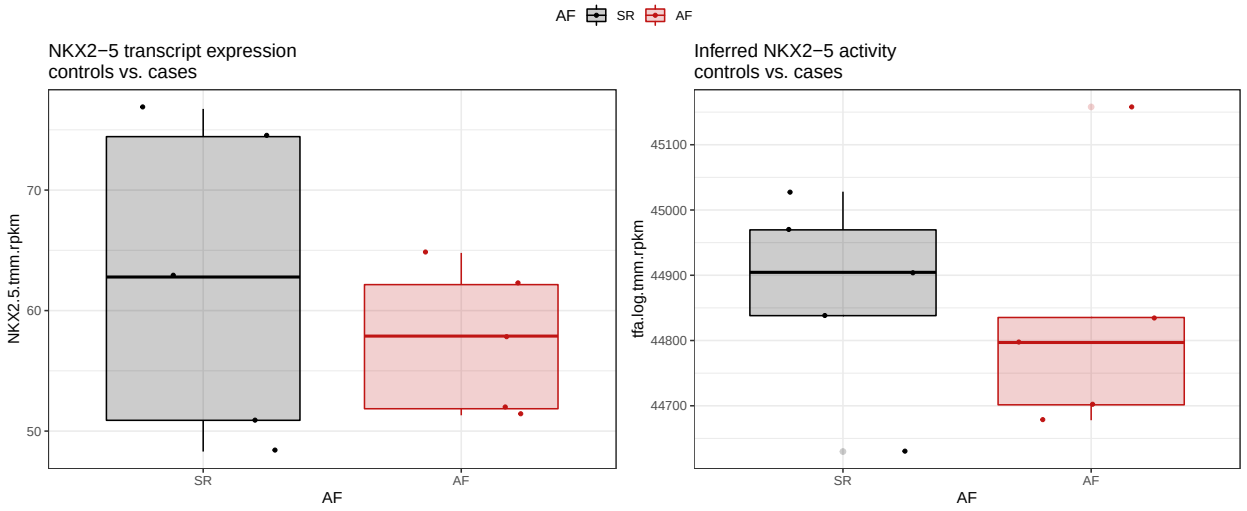
```
##                            pathway size
## 1:                  NKX2-5_targets   13
## 2: AF_trans_core_genes_and_targets   19
## 3:             Atrial_fibrillation   26
## 4:              NKX2-5_and_targets   14
## 5:                       Core_genes    6
## 6:      Transcriptomics_core_genes    2
## 7:                       Cis_genes    7
## 8:           Proteomics_core_genes    4
```

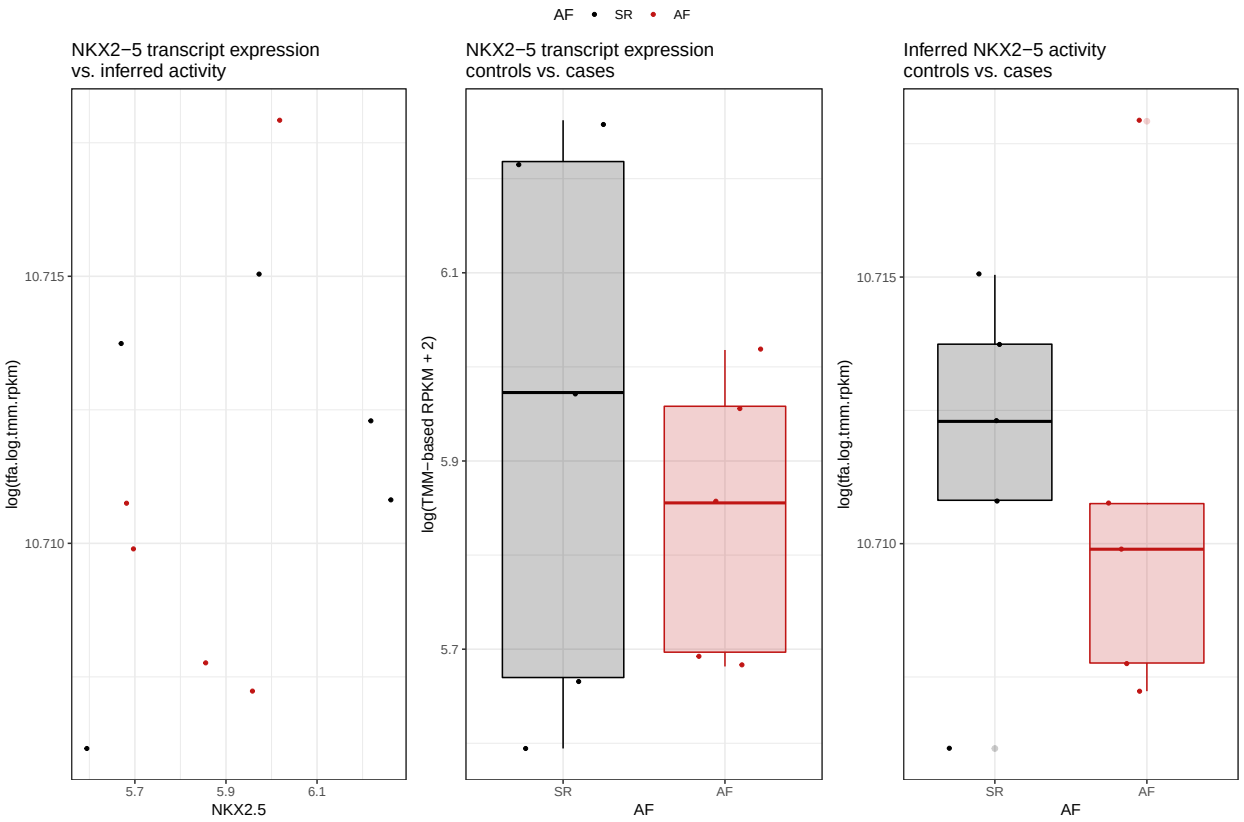
```
## [1] "GSEA: exact test on signed pvalue (left atrium):"
```

```
##                            pathway size
## 1:                  NKX2-5_targets   13
## 2:              NKX2-5_and_targets   14
## 3: AF_trans_core_genes_and_targets   19
## 4:             Atrial_fibrillation   26
## 5:                       Core_genes    6
## 6:      Transcriptomics_core_genes    2
## 7:           Proteomics_core_genes    4
## 8:                       Cis_genes    7
```

3 NKX2-5 activity

3.1 Estimate NKX2-5 transcription factor activity



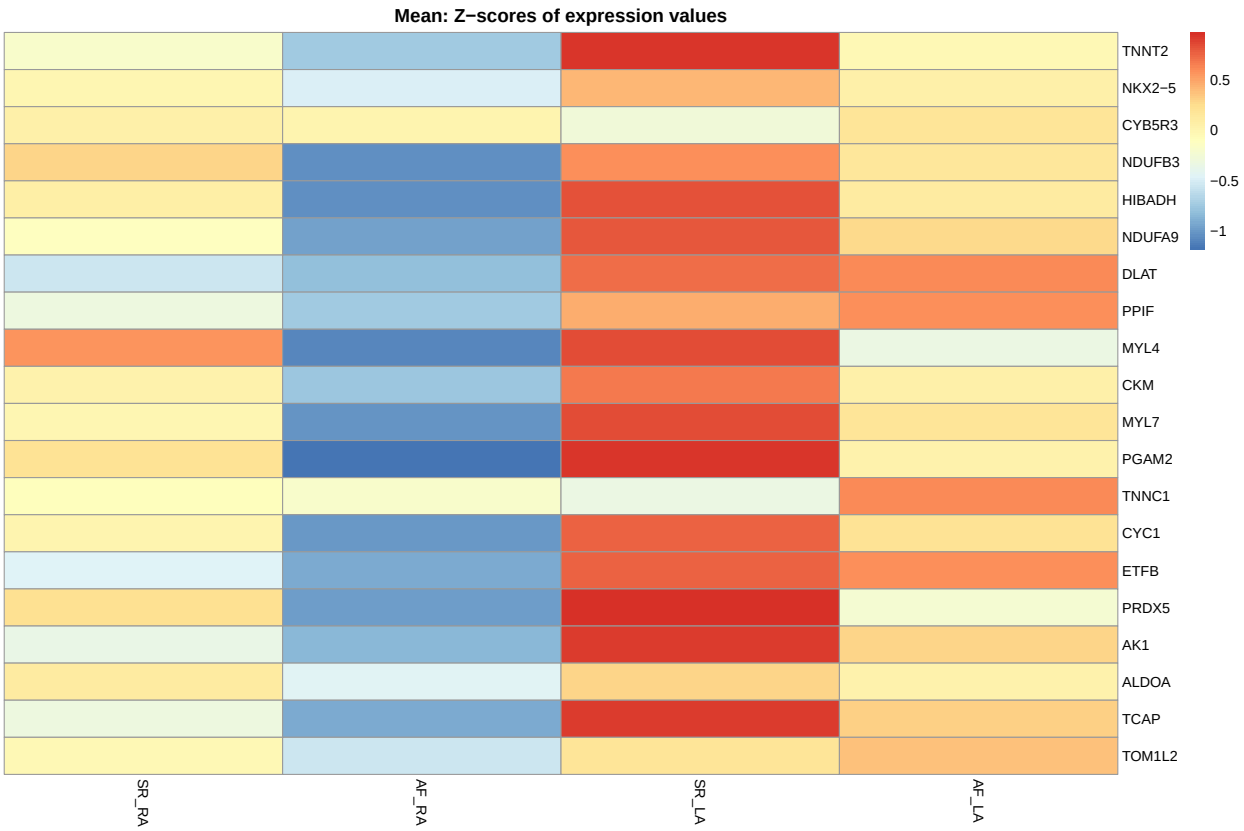


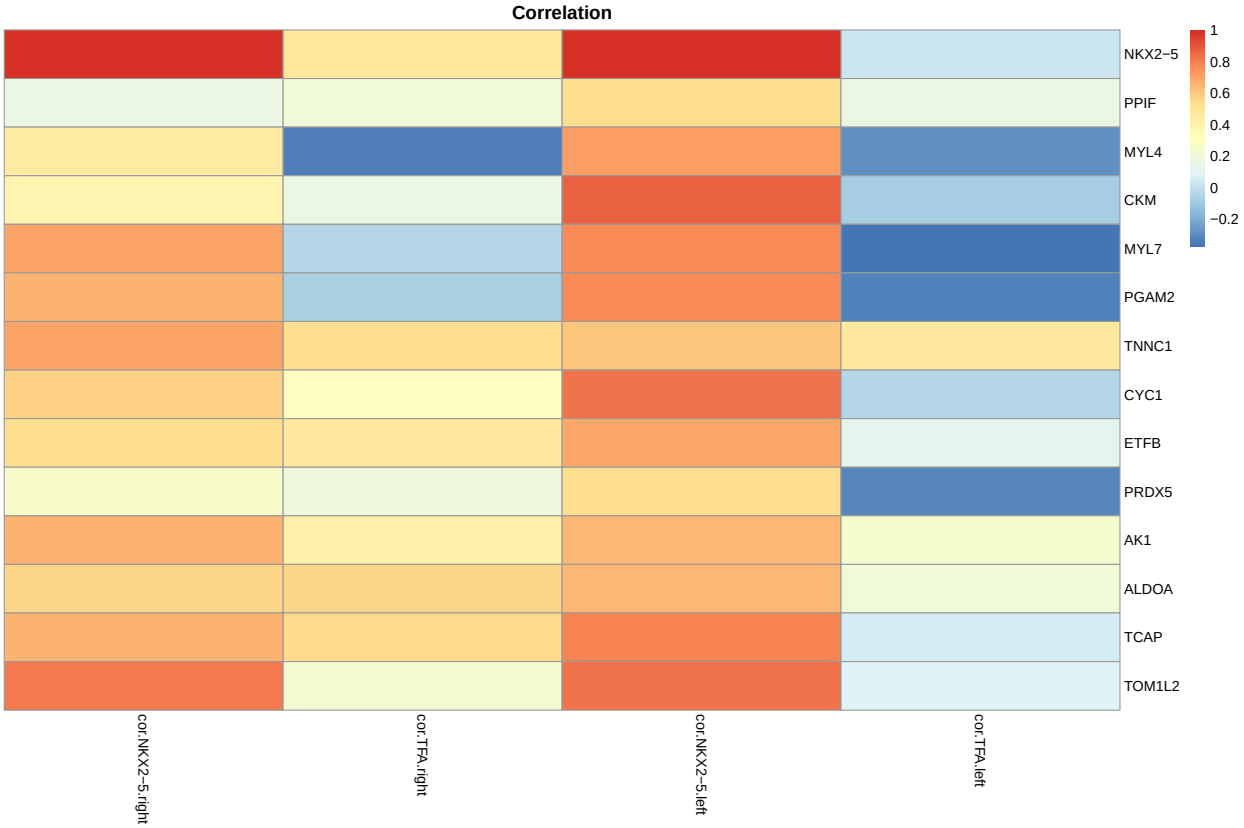
3.2 Correlation between NKX2-5 targets, NKX2-5 expression and NKX2-5 activity

```
##          NKX2-5         PPIF        MYL4         CKM       MYL7       PGAM2     TNNC1
## NKX2-5 1.000000  0.161905366  0.46027872  0.38276650 0.69773192  0.65730476 0.7038942
## tfa    0.306451 -0.009398003 -0.06556531 -0.03917326 0.07563738 -0.04500414 0.4911046
##             CYC1      ETFB      PRDX5       AK1      ALDOA      TCAP      TOM1L2
## NKX2-5 0.5767759 0.5338863 0.2645012 0.6563410 0.56070900 0.6620147  0.81400252
## tfa    0.1209265 0.1428526 0.1229414 0.1878614 0.04022938 0.1049924 -0.07922953
```

```
##           NKX2-5       PPIF       MYL4        CKM       MYL7       PGAM2      TNNC1
## NKX2-5  1.0000000  0.5384664  0.7159745  0.8690235  0.7782552  0.7671259  0.6018266
## tfa    -0.2254459 -0.4774340 -0.2730175 -0.3459742 -0.2530855 -0.3459211 -0.1043365
##              CYC1       ETFB       PRDX5        AK1       ALDOA       TCAP      TOM1L2
## NKX2-5  0.8271903  0.6949080  0.5387839  0.65480827  0.6435109  0.7876978  0.8269131
## tfa    -0.3742567 -0.4635427 -0.3600200 -0.07161581 -0.3671474 -0.4393346 -0.4378656
```

4 Overall summary


Mean: Z−scores of expression values
TNNT2
NKX2−5
CYB5R3
NDUFB3
HIBADH
NDUFA9
DLAT
PPIF
MYL4
CKM
MYL7
PGAM2
TNNC1
CYC1
ETFB
PRDX5
AK1
ALDOA
TCAP
TOM1L2
0.5
0
−0.5
−1
SR_RA
AF_RA
SR_LA
AF_LA


Correlation
NKX2−5
PPIF
MYL4
CKM
MYL7
PGAM2
TNNC1
CYC1
ETFB
PRDX5
AK1
ALDOA
TCAP
TOM1L2
1
0.8
0.6
0.4
0.2
0
−0.2
cor.NKX2−5.right
cor.TFA.right
cor.NKX2−5.left
cor.TFA.left