Figure_5

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```r
suppressPackageStartupMessages(source("utils.R"))
```

```
## Warning: package ’tidyr’ was built under R version 4.3.2
```

```
## Warning: package ’ggplot2’ was built under R version 4.3.2
```

```r
metadata = read.csv("data/PAN.A01.v01.raw_count.20210429.NKT_metadata.csv", header=TRUE)
metadata = metadata[metadata$organ=="TH",]
## quality control filtering
metadata = metadata[metadata$mito<.1,]
metadata = metadata[metadata$n_genes>=500,]
```

```r
tcr = readRDS("data/suo_pairedTCRdf.rds")
tcr = tcr[tcr$id %in% metadata$index,]
tcr = label_invariants2(tcr)
tcrscores = tcrpheno::score_tcrs(tcr, chain="ab")
```

```
## hash-2.2.6.3 provided by Decision Patterns
```

```
## [1] "adding CDR1 and CDR2 based on V gene..."
## [1] "identifying amino acids at each position..."
## [1] "converting amino acids into Atchley factors..."
## [1] 13373
## [1] 13373
## [1] "adding interactions between adjacent residues..."
## [1] "TCRs featurized!"
## [1] "scoring TCRs..."
## [1] "all done!"
```

```r
tcrscores$index = rownames(tcrscores)
metadata = left_join(metadata, tcr)
```

```
## Joining with ‘by = join_by(n_counts, n_genes, file, mito, doublet_scores,
## predicted_doublets, old_annotation_uniform, organ, Sort_id, age, method, donor,
## sex, Sample, scvi_clusters, is_maternal_contaminant, anno_lvl_2_final_clean,
## celltype_annotation)‘
```

```
tcrscores = left_join(tcrscores, metadata)
```

```
## Joining with ‘by = join_by(index)‘
```

```
t = table(tcrscores$donor)
tcrscores = tcrscores[tcrscores$donor %in% names(t)[t>=100],]
tcrscores$tphase = "other"
tcrscores$tphase[grepl("DN", tcrscores$celltype_annotation)] = "DN"
tcrscores$tphase[grepl("DP", tcrscores$celltype_annotation)] = "DP"
tcrscores$tphase[tcrscores$celltype_annotation %in% c("CD4+T", "CD8+T", "TREG")] = "SP"

tmp = tcrscores[tcrscores$tphase %in% c("DP", "SP"),]
tmp$Donor = tmp$donor
tmp$lcat = "thymus"
tmp$target4 = tmp$tphase=="SP"
tmp$X4 = tmp$TCR.mem
```

```
plot_TCRscore_validation_bybin(tmp, 4, lcat="thymus", colors = c("turquoise4"), xlab = "TCR-mem score",
```

```
## Joining with ‘by = join_by(qtl)‘
```

```
## # A tibble: 3 x 7
##   effect   group term             estimate std.error statistic      p.value
##   <chr>    <chr> <chr>               <dbl>     <dbl>     <dbl>        <dbl>
## 1 fixed    <NA>  (Intercept)       -0.0169    0.396    -0.0427  0.966
## 2 fixed    <NA>  TCRscore           0.141     0.0267    5.28    0.000000128
## 3 ran_pars Donor sd__(Intercept)    0.966    NA        NA      NA
```

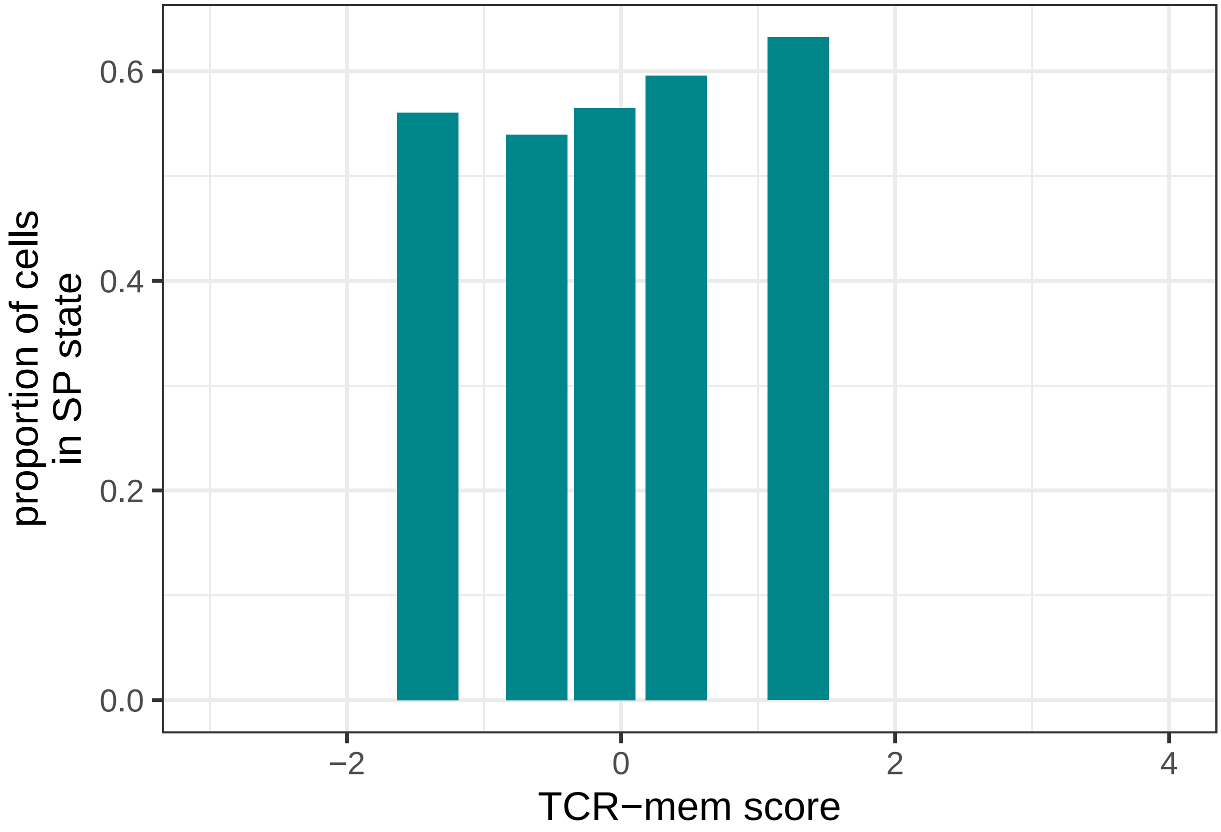
```
## ‘summarise()‘ has grouped output by ’bin’. You can override using the ‘.groups‘
## argument.
```

```
## Warning: Using ‘size‘ aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use ‘linewidth‘ instead.
## This warning is displayed once every 8 hours.
## Call ‘lifecycle::last_lifecycle_warnings()‘ to see where this warning was
## generated.
```

```
## # A tibble: 3 x 2
##   mmp    prop.pos
##   <chr>     <dbl>
## 1 maxptl    0.773
## 2 minptl    0.533
## 3 other     0.577
```

```
## Joining with ‘by = join_by(bin)‘
```

```
## # A tibble: 5 x 9
##      bin    OR lowOR highOR term                 lcat     qtl minbin maxbin
##    <dbl> <dbl> <dbl>  <dbl> <chr>                <chr>  <dbl>  <dbl>  <dbl>
## 1 -1.41   1.08 0.921   1.26 bin-1.40946984417489 thymus    20 -3.82  -0.895
## 2 -0.119  1.14 0.974    1.33 bin-0.118889573644414 thymus    60 -0.357  0.122
## 3  0.402  1.28 1.09     1.50 bin0.40186517804777   thymus    80  0.122  0.706
## 4  1.29   1.50 1.28     1.76 bin1.2933999374633    thymus   100  0.706  3.46
## 5 -0.617  1    1        1    ref                   thymus    40 -0.894 -0.357
```



```
thy = tmp
bld = readRDS("data/Dataset3_scored_wannotations.rds")
df = data.frame(data = c(rep("thymus", nrow(thy)), rep("blood", nrow(bld))), donor = c(thy$donor, bld$D

qu = quantile(df$TCR.mem, probs=seq(0.1, 1, by=0.1))
df$qu = sapply(df$TCR.mem, function(x) names(qu)[min(which(qu>=x))])
gr = df %>% group_by(qu) %>% dplyr::summarise(meanTCRmem = mean(TCR.mem))
df = left_join(df, gr)
```

```
## Joining with ‘by = join_by(qu)‘
```

```
cols = c("bin", "OR", "lowOR", "highOR", "term")
df$bin = df$meanTCRmem
df$Donor = df$donor
df$target = df$target4
st.thy = get_bin_stats(df[df$data=="thymus",], cols, quantile_bin=0.1)
st.bld = get_bin_stats(df[df$data!="thymus",], cols, quantile_bin=0.1)

st.thy = st.thy[,2:5]
colnames(st.thy)[1:3] = paste(colnames(st.thy)[1:3], ".thy", sep="")
```

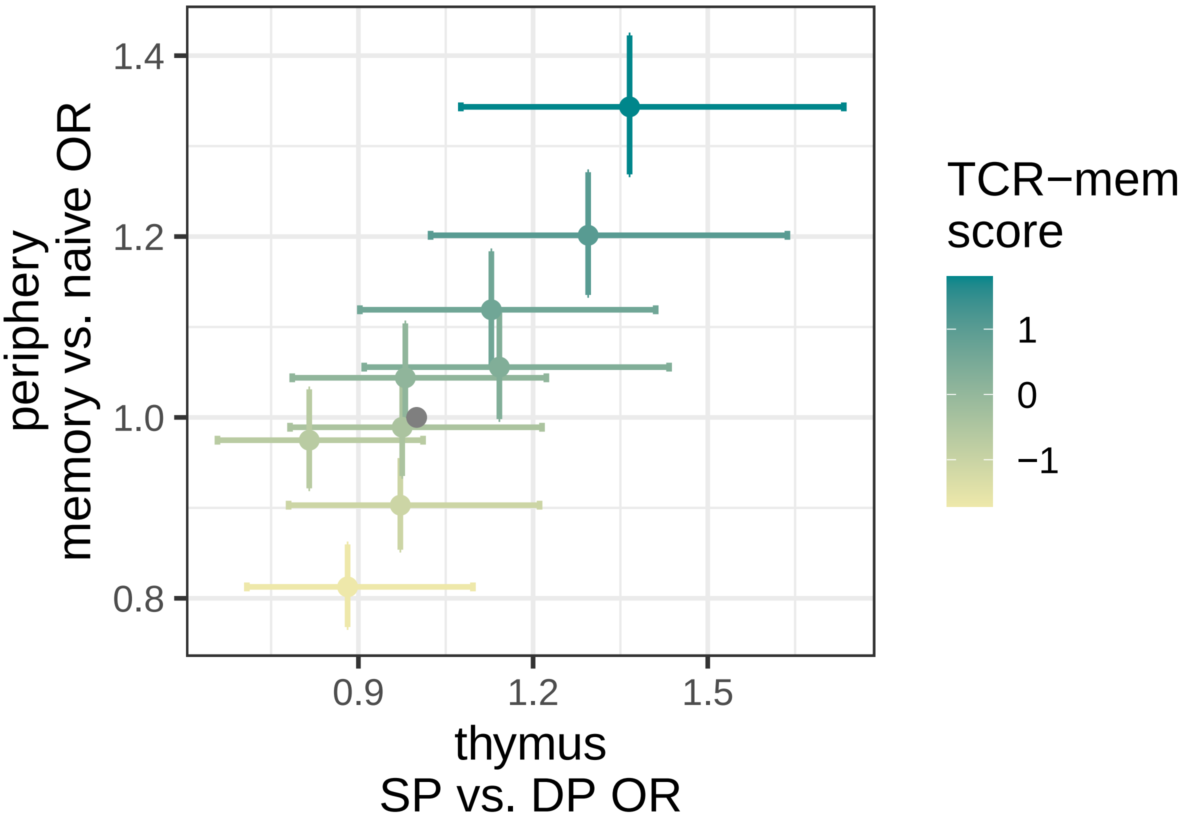
```
st.bld = st.bld[,2:5]
colnames(st.bld)[1:3] = paste(colnames(st.bld)[1:3], ".bld", sep="")

tp = full_join(st.thy, st.bld)
```

```
## Joining with ‘by = join_by(term)‘
```

```
tp = tp[!(grepl("Intercept", as.character(tp$term))),]
tp$meanTCRmem = as.numeric(as.character(gsub("bin", "", as.character(tp$term))))
```

```
## Warning: NAs introduced by coercion
```

```
g = ggplot(tp, aes(OR.thy, OR.bld, color=meanTCRmem))
g = g + geom_errorbar(aes(xmin=lowOR.thy, xmax=highOR.thy), size=1)
g = g + geom_errorbar(aes(ymin=lowOR.bld, ymax=highOR.bld), size=1) + theme_bw(base_size=18)
g =g + geom_point(size=3)
g = g + scale_color_gradient(low="palegoldenrod", high="turquoise4")
g = g + xlab("thymus\nSP vs. DP OR") + ylab("periphery\nmemory vs. naive OR")
g + labs(color="TCR-mem\nscore")
```



```
yi <- c(0.136, 0.141)    # observed effect size estimates
vi <- c(0.00655^2, 0.0267^2) # corresponding sampling variances
rma(yi, vi, method="FE")
```

```
##
## Fixed-Effects Model (k = 2)
##
## I^2 (total heterogeneity / total variability):   0.00%
## H^2 (total variability / sampling variability):  0.03
##
## Test for Heterogeneity:
## Q(df = 1) = 0.0331, p-val = 0.8557
##
## Model Results:
##
## estimate      se     zval    pval   ci.lb   ci.ub
##   0.1363  0.0064  21.4236  <.0001  0.1238  0.1488  ***
##
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

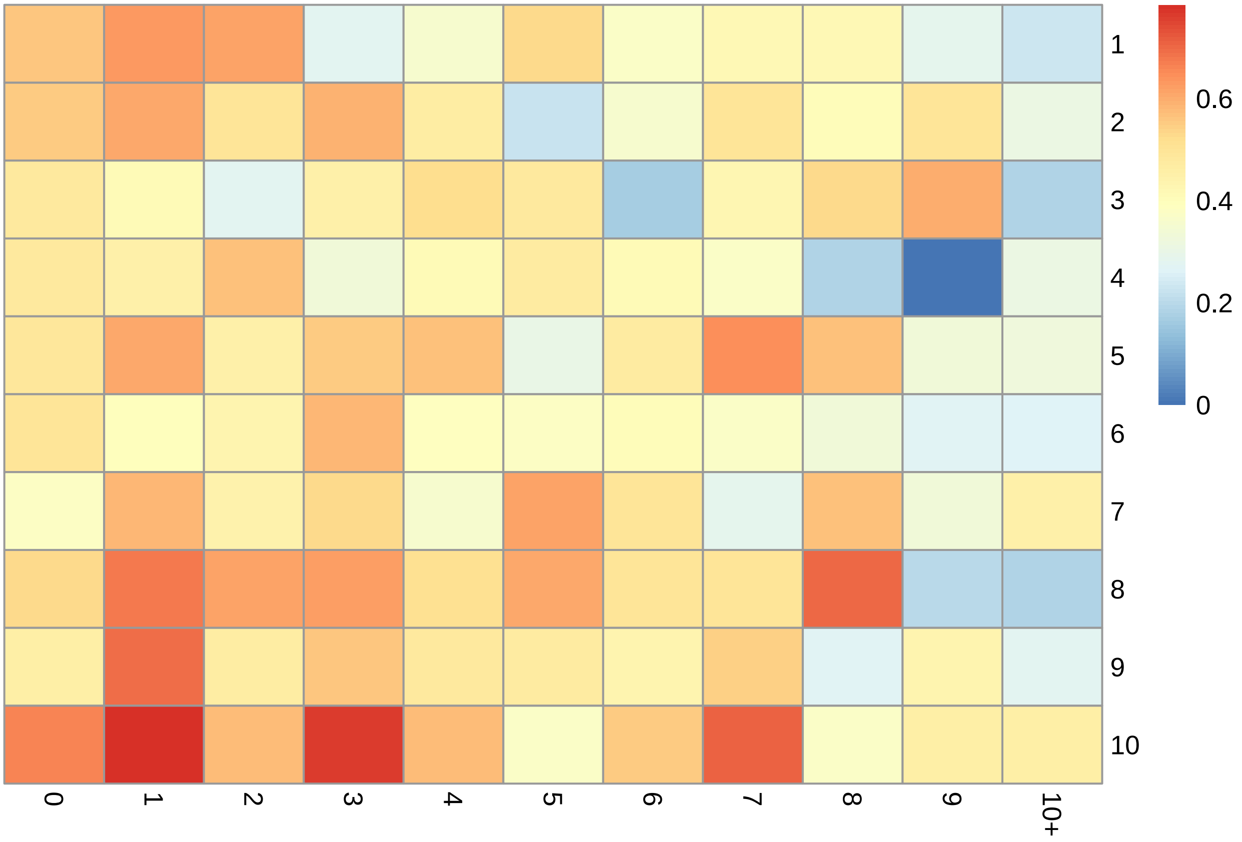
```
igor.thy = process_igor_output("data/suo_igorresults_117.rds")
tcrscores = left_join(tcrscores, igor.thy[,c("id", "ab_ins")], by=c("index"="id"))

tidy(glmer(tphase=="SP" ~ TCR.mem + ab_ins + (1|donor), data=tcrscores[tcrscores$tphase %in% c("SP", "D
```

```
## # A tibble: 4 x 7
##   effect   group term            estimate std.error statistic   p.value
##   <chr>    <chr> <chr>              <dbl>     <dbl>     <dbl>     <dbl>
## 1 fixed    <NA>  (Intercept)      -0.0374    0.424    -0.0882  9.30e- 1
## 2 fixed    <NA>  TCR.mem           0.216     0.0424    5.08    3.78e- 7
## 3 fixed    <NA>  ab_ins           -0.0910    0.0115   -7.94    2.03e-15
## 4 ran_pars donor sd__(Intercept)   1.02     NA        NA       NA
```

```
tcrscores$TCR.mem_decile = convert_to_quantile(tcrscores$TCR.mem, step=0.1)
tcrscores$ab_ins = as.numeric(as.character(tcrscores$ab_ins))
tcrscores$ab_ins2 = as.character(tcrscores$ab_ins)
tcrscores$ab_ins2[tcrscores$ab_ins>=10] = "10+"
ins.gr = tcrscores[!(is.na(tcrscores$TCR.mem_decile)) & tcrscores$tphase %in% c("SP", "DP"),] %>% group
```

```
## `summarise()` has grouped output by 'ab_ins2'. You can override using the
## `.groups` argument.
```

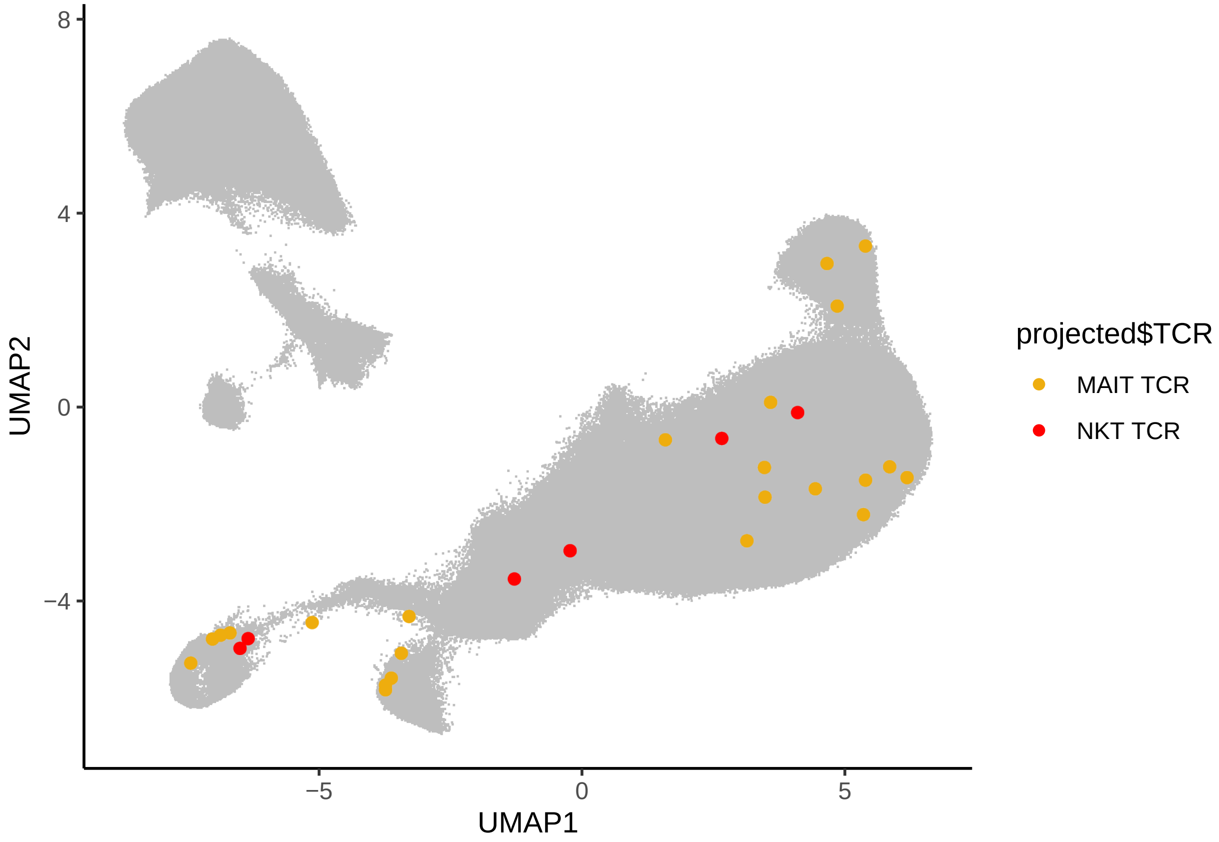
```
tpl = ins.gr[,c("ab_ins2", "TCR.mem_decile", "p")]
tp = data.frame(pivot_wider(tpl, names_from=ab_ins2, values_from=p))
rownames(tp) = tp$TCR.mem_decile
tp = tp[,c(paste("X", seq(0, 9), sep=""), "X10.")]
colnames(tp) = c(as.character(seq(0, 9)), "10+")
rownames(tp) = seq(1, 10)
pheatmap(tp, cluster_rows = FALSE, cluster_cols = FALSE)
```



```r
## mRNA reference constructed with COMBAT data
ref = readRDS("data/COMBAT_metadata.rds")

res = readRDS("data/Dataset6_mapped_to_mRNA_reference.rds")
projected = data.frame(res$umap)
projected$index = res$meta_data$index
projected = projected[projected$index %in% metadata$index[metadata$my_abMAIT==TRUE | metadata$my_abNKT=
projected = left_join(projected, metadata[,c("my_abMAIT", "my_abNKT", "index")])
```

```
## Joining with ‘by = join_by(index)‘
```

```r
projected$TCR = ifelse(projected$my_abMAIT==TRUE, "MAIT TCR", "NKT TCR")

g = ggplot()
g = g + geom_point_rast(aes(x=ref$UMAP1, ref$UMAP2), color="gray", shape=".")
g + geom_point_rast(aes(x=projected$X1, y=projected$X2, color=projected$TCR)) + scale_color_manual(valu
```



```
tmp$target2 = grepl("CD8", tmp$celltype_annotation)
tmp$X2 = tmp$TCR.CD8
plot_TCRscore_validation_bybin(tmp[tmp$tphase=="SP",], 2, lcat="thymus", colors = c("dodgerblue3"), xla
```

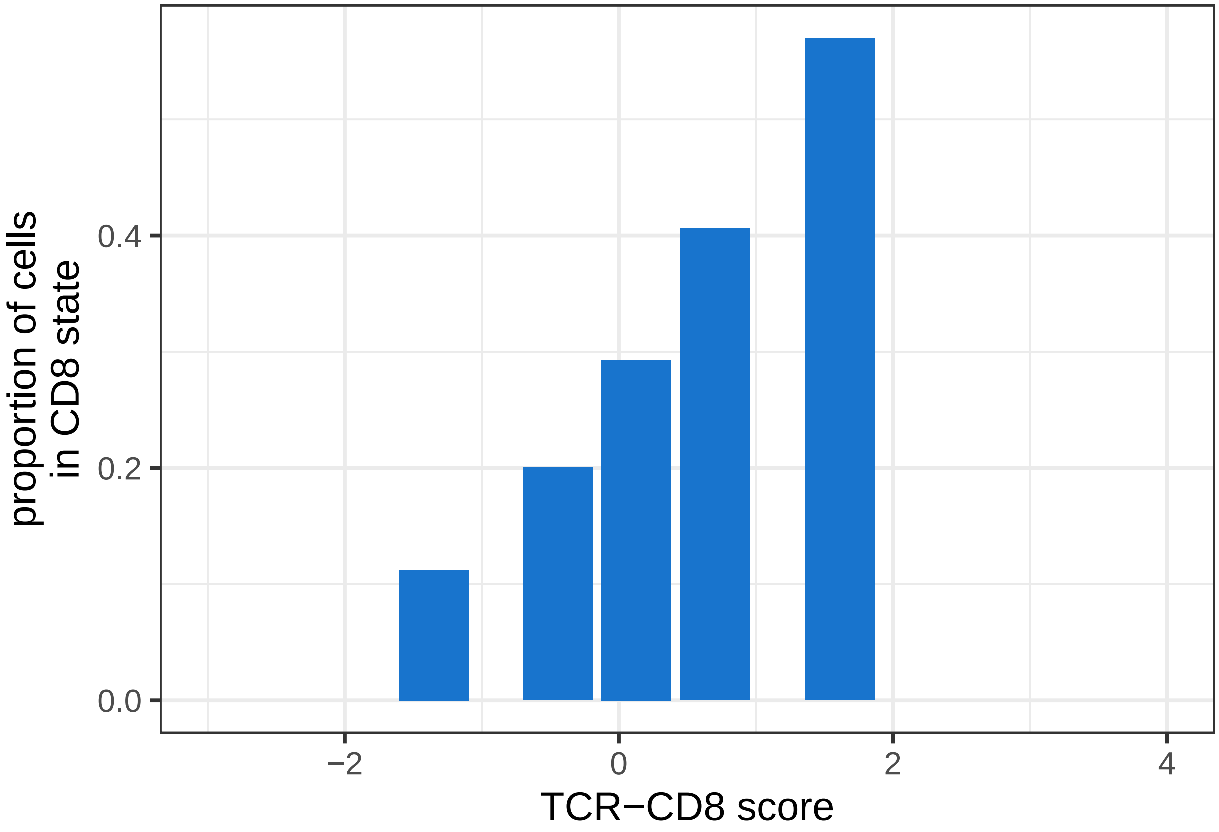
```
## Joining with ‘by = join_by(qtl)‘
```

```
## # A tibble: 3 x 7
##   effect   group term            estimate std.error statistic   p.value
##   <chr>    <chr> <chr>              <dbl>     <dbl>     <dbl>     <dbl>
## 1 fixed    <NA>  (Intercept)       -1.09      0.145     -7.52  5.37e-14
## 2 fixed    <NA>  TCRscore           0.784     0.0375     20.9   4.28e-97
## 3 ran_pars Donor sd__(Intercept)    0.322    NA          NA    NA
```

```
## ‘summarise()‘ has grouped output by ’bin’. You can override using the ‘.groups‘
## argument.
```

```
## # A tibble: 3 x 2
##   mmp    prop.pos
##   <chr>     <dbl>
## 1 maxptl   0.727
## 2 minptl   0.0909
## 3 other    0.315
```

```
## Joining with ‘by = join_by(bin)‘
## # A tibble: 5 x 9
##      bin    OR lowOR highOR term                 lcat     qtl minbin maxbin
##    <dbl> <dbl> <dbl>  <dbl> <chr>                <chr>  <dbl>  <dbl>  <dbl>
## 1 -1.35  0.506 0.386  0.663 bin-1.35160116964468 thymus    20 -3.45  -0.774
## 2  0.127 1.67  1.33   2.09  bin0.126512275429661 thymus    60 -0.155  0.398
## 3  0.704 2.70  2.17   3.35  bin0.703523049094082 thymus    80  0.398  1.03
## 4  1.62  5.11  4.11   6.34  bin1.61775834493033  thymus   100  1.03   3.77
## 5 -0.441 1     1      1     ref                  thymus    40 -0.773 -0.155
```



```
tmp$target3 = grepl("TREG", tmp$celltype_annotation)
tmp$X3 = tmp$TCR.reg
plot_TCRscore_validation_bybin(tmp[tmp$tphase=="SP",], 3, lcat="thymus", colors = c("darkmagenta"), xlal
```

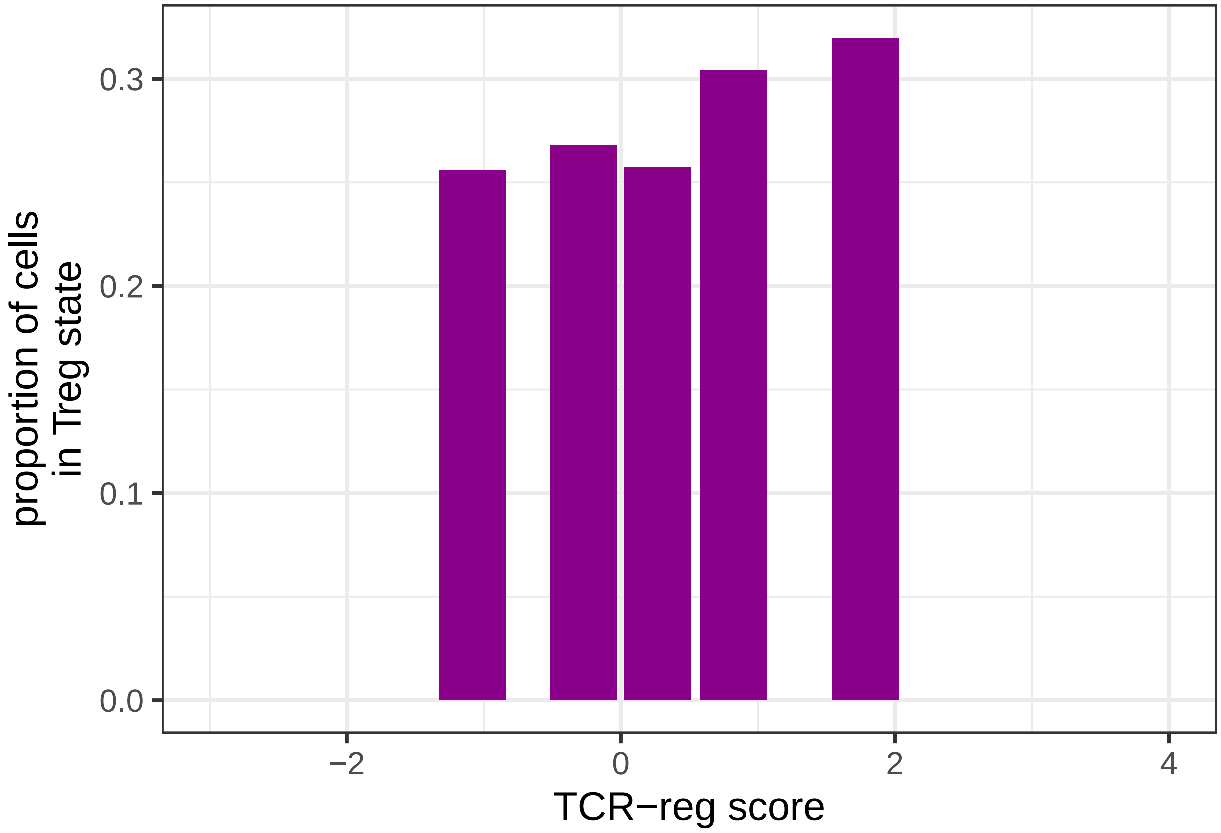
```
## Joining with ‘by = join_by(qtl)‘
## boundary (singular) fit: see help(’isSingular’)
```

```
## # A tibble: 3 x 7
##   effect   group term            estimate std.error statistic    p.value
##   <chr>    <chr> <chr>              <dbl>     <dbl>     <dbl>      <dbl>
## 1 fixed    <NA>  (Intercept)       -0.986    0.0361    -27.3   1.10e-164
## 2 fixed    <NA>  TCRscore           0.139    0.0331      4.19  2.76e-  5
## 3 ran_pars Donor sd__(Intercept)    0       NA          NA    NA
```

```
## boundary (singular) fit: see help(’isSingular’)
## ‘summarise()‘ has grouped output by ’bin’. You can override using the ‘.groups‘ argument.boundary (si
## # A tibble: 3 x 2
##   mmp    prop.pos
##   <chr>     <dbl>
## 1 maxptl    0.477
## 2 minptl    0.205
## 3 other     0.280
```

```
## Joining with ‘by = join_by(bin)‘
```

```
## # A tibble: 5 x 9
##      bin    OR lowOR highOR term                 lcat     qtl  minbin  maxbin
##    <dbl> <dbl> <dbl>  <dbl> <chr>                <chr>  <dbl>   <dbl>   <dbl>
## 1 -1.08  0.940 0.759   1.17 bin-1.08031881407862 thymus    20 -2.98   -0.571
## 2  0.268 0.946 0.763   1.17 bin0.267881944207646 thymus    60  0.0121  0.527
## 3  0.822 1.19  0.968   1.47 bin0.822383871247654 thymus    80  0.528   1.17
## 4  1.79  1.28  1.04    1.58 bin1.78697337205181  thymus   100  1.17    3.80
## 5 -0.275 1     1       1    ref                  thymus    40 -0.571   0.0119
```


proportion of cells
in Treg state
TCR−reg score