

Counterfactual Confounder Adjustment for Single-cell Differential Expression Analysis

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Results

Cell type annotation



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PsychENCODE's marker gene selection might be imperfect since some "marker" genes are shared by different cell types. However, it has enough discriminatory power. Using this initial dictionary of marker genes, we can start analyzing 10x data, which consist of these three files:

- Sparse count matrix:

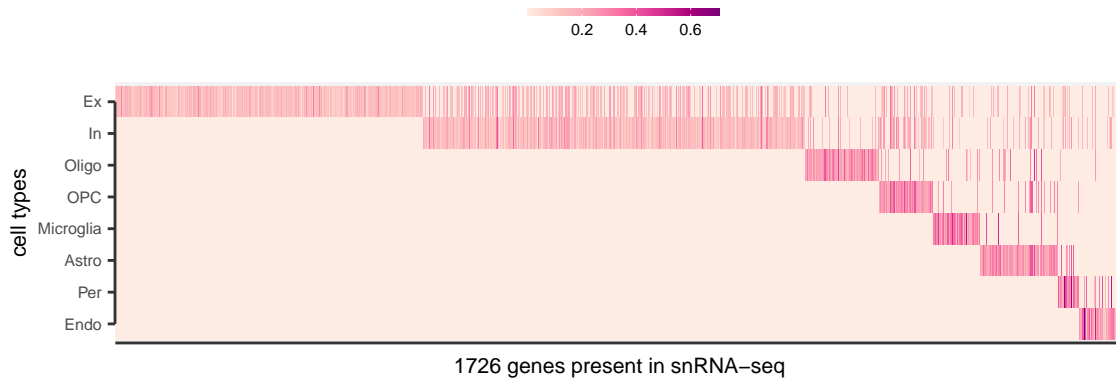
```
## %%MatrixMarket matrix coordinate integer general
## 17926 70634 119696200
## 56 1 1
## 139 1 1
## 270 1 1
## 324 1 1
## 375 1 1
## 389 1 1
## 420 1 1
## 557 1 1
```

- Cell barcode list:

```
## AAACGGGAGATCCCGC.1
## AAATGCCTCCAATGGT.1
## AACCATGTCAGTGCAT.1
## AACCATGTCTGTACGA.1
## AACCGCGTCCGCATAA.1
## AACGTTGGTTCAGGCC.1
## AACTGGTGTACCGGCT.1
## AAGACCTAGTTAACGA.1
## AAGGAGCAGCAATCTC.1
## AAGGAGCTCTGCTGCT.1
```

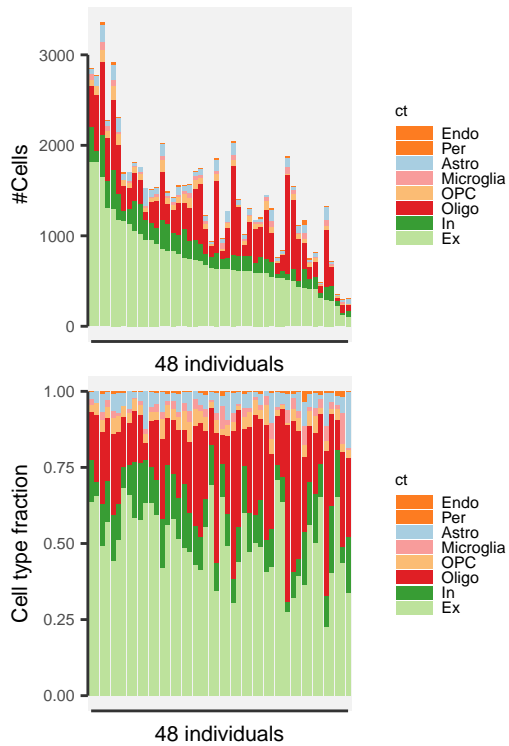
- Gene/feature list (these names should exist in the marker file):

```
## F0538757.2
## SAMD11
## NOC2L
## KLHL17
## PLEKHN1
## PERM1
## HES4
## ISG15
## AGRN
## RNF223
```



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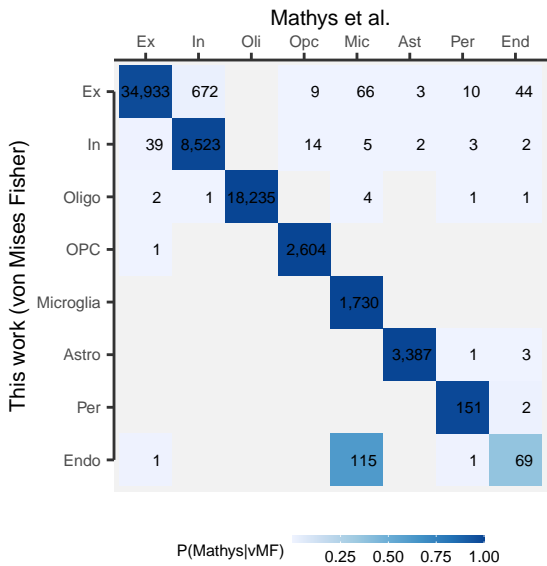
Basic statistics for cell type annotation



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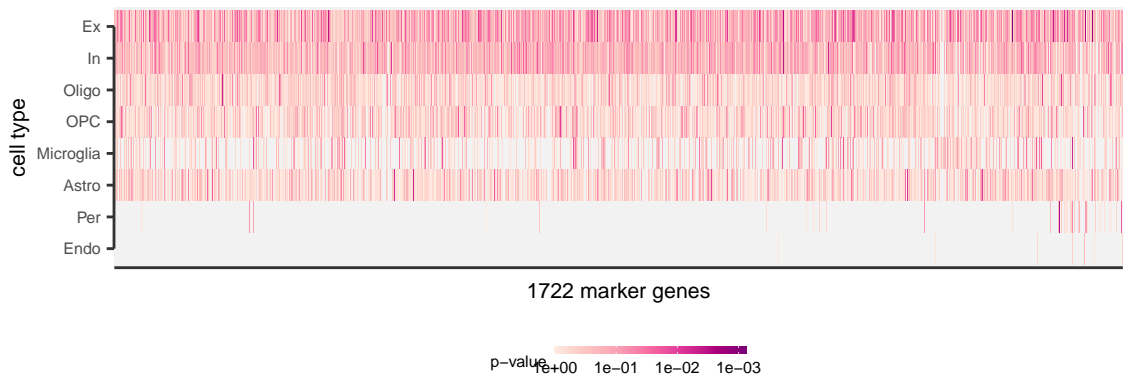
- 1,472 cells per individual: 50.9% ($N=745 \pm 388$) cells for Ex, 12.23% ($N=179 \pm 103$) cells for In, 3.54% ($N= 54 \pm 35$) cells for OPC, 0.33% ($N= 4 \pm 5$) cells for Endo, 25.22% ($N=380 \pm 252$) cells for Oligo, 2.61% ($N= 36 \pm 25$) cells for Microglia, 4.95% ($N= 71 \pm 47$) cells for Astro, 0.34% ($N= 4 \pm 4$) cells for Per on average.

Correlation with the annotations of Mathys *et al.*

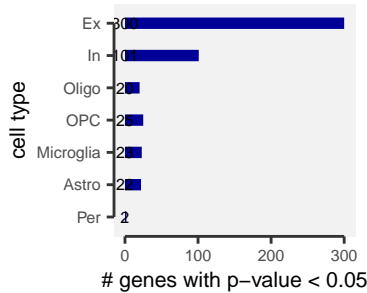


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Marker genes are associated with the AD phenotype



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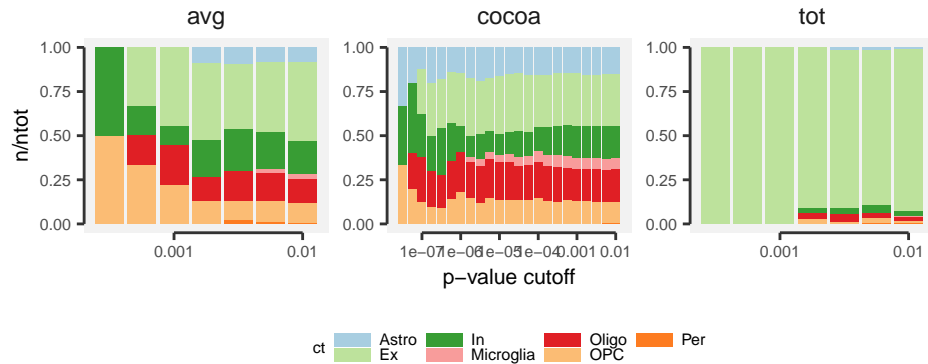


PDF

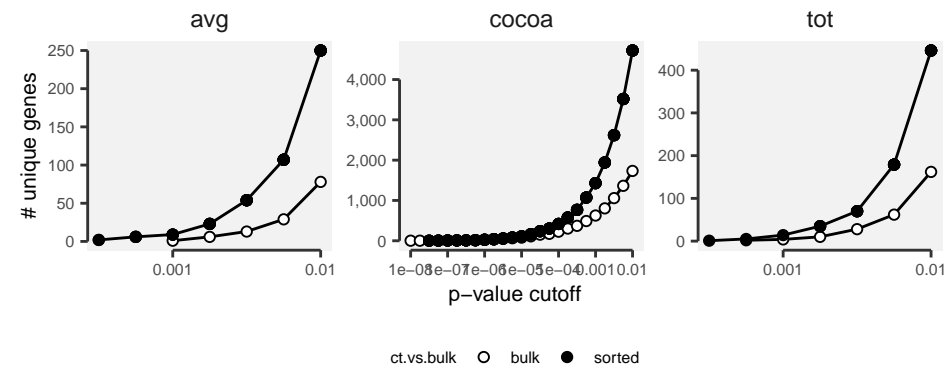
Differential expression analysis

What if cell types were combined? Cell-type-sorted pseudo-bulk analysis improves statistical power

```
## Joining, by = "ct"
```



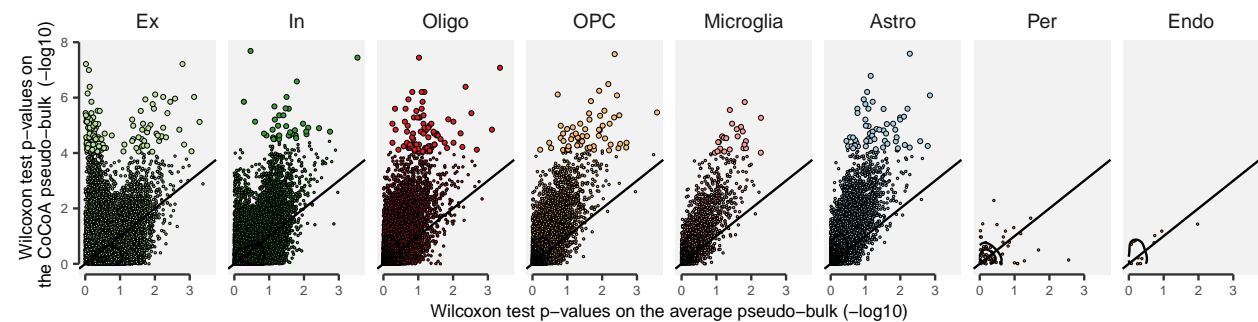
PDF



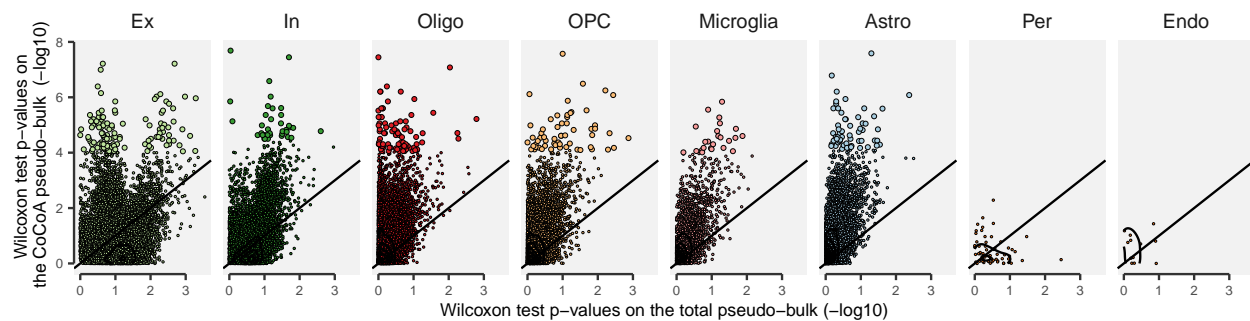
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CoCoA identified 377 genes are differentially-regulated between different AD conditions

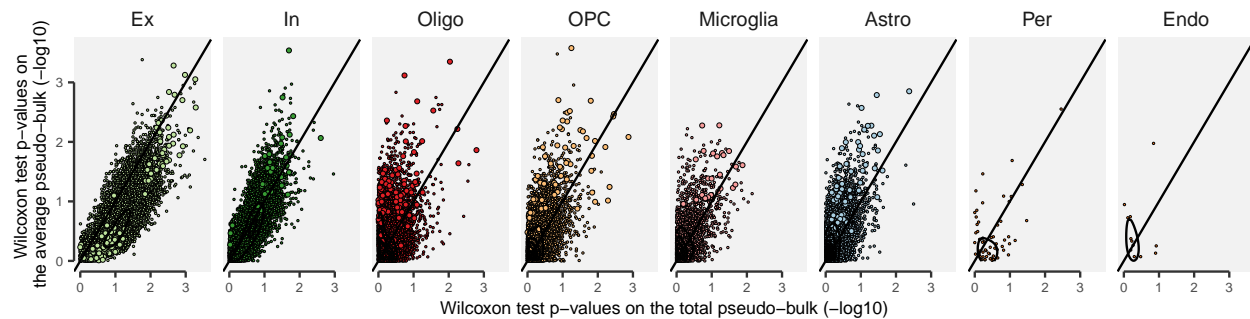
- 377 genes (2.72%) consist of 123 found in Ex, 36 found in In, 86 found in Oligo, 58 found in OPC, 24 found in Microglia, 57 found in Astro



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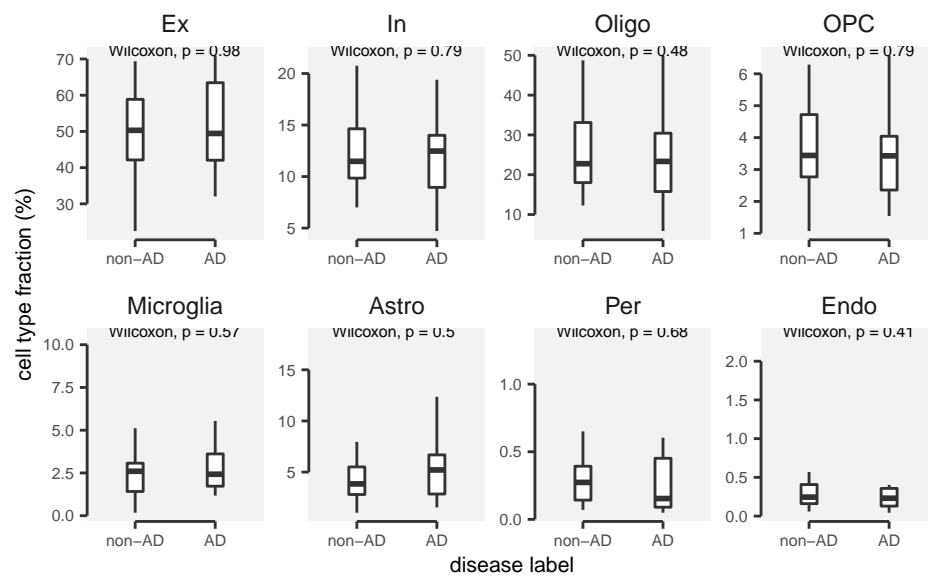
```
## pdf
## 2
```

p-value distribution

Why do we find more genes with CoCoA?

Cell type information is not particularly associated with phenotypes

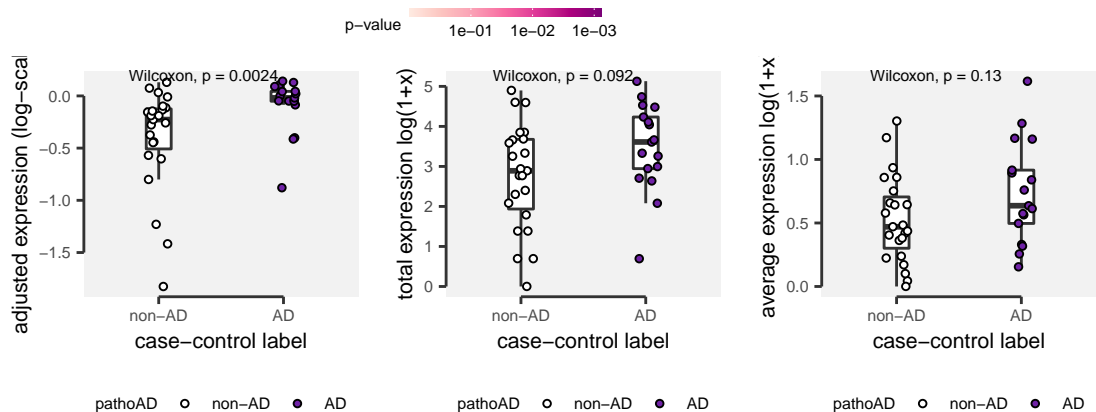
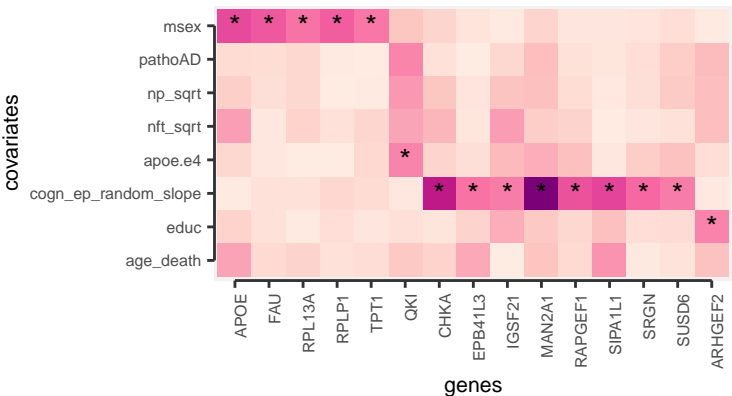
```
## Joining, by = "projid"
```



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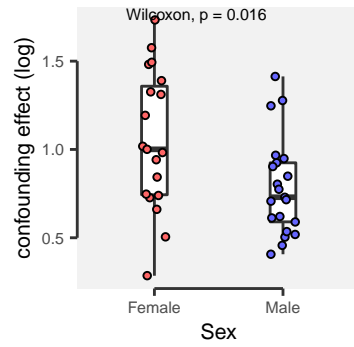
Take the examples of Microglia genes

```
## Loading required package: cba
## Loading required package: proxy
##
## Attaching package: 'proxy'
##
## The following objects are masked from 'package:stats':
##
##   as.dist, dist
##
## The following object is masked from 'package:base':
##
##   as.matrix
```



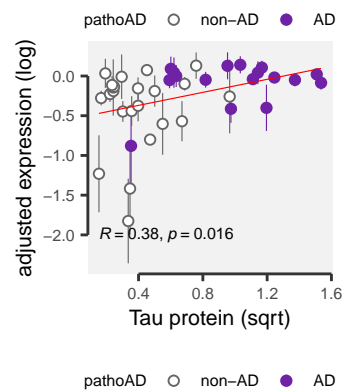
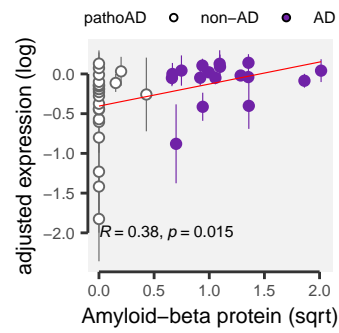
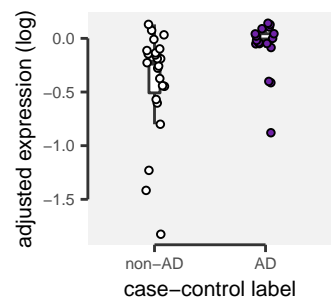
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APOE



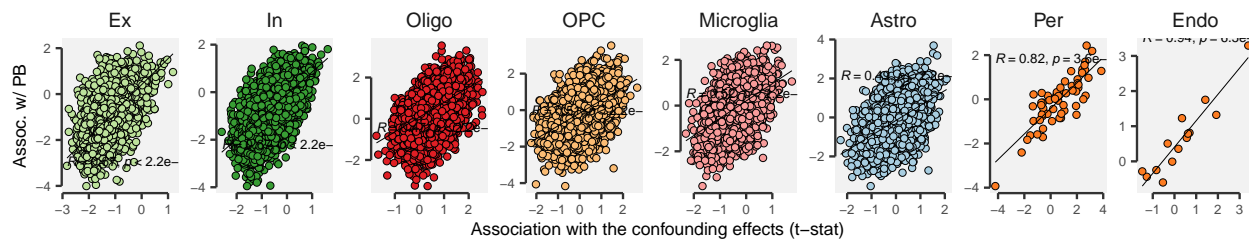
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APOE ($p = 3.6e-03$)



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Confounding effects prevalent...

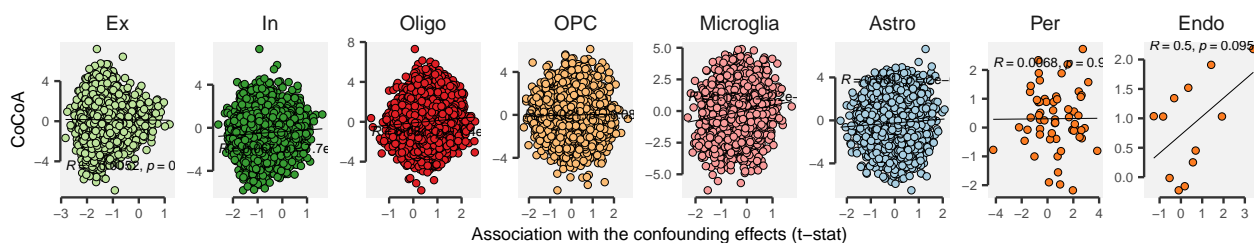


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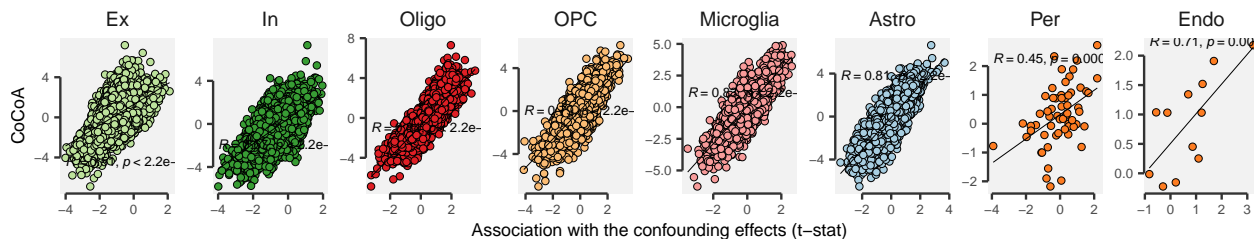
```
## Warning: Removed 4702 rows containing non-finite values (stat_smooth).
```

```
## Warning: Removed 4702 rows containing non-finite values (stat_cor).
```

```
## Warning: Removed 4702 rows containing missing values (geom_point).
```

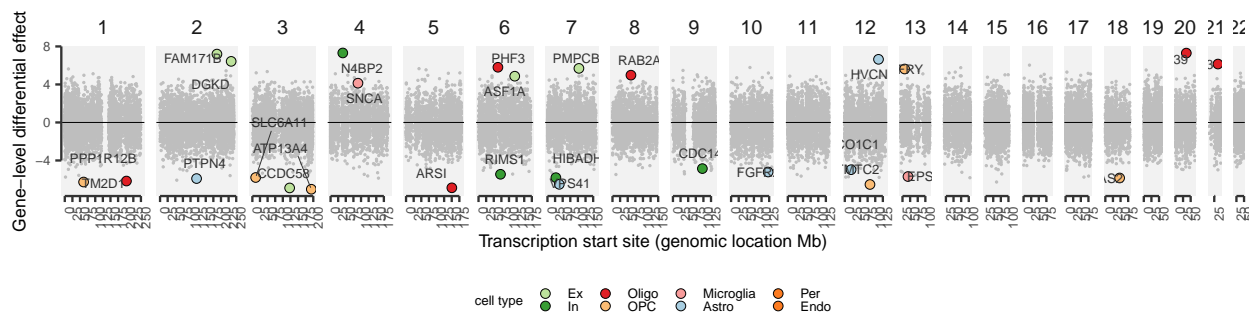


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A genomic view combining all the cell types

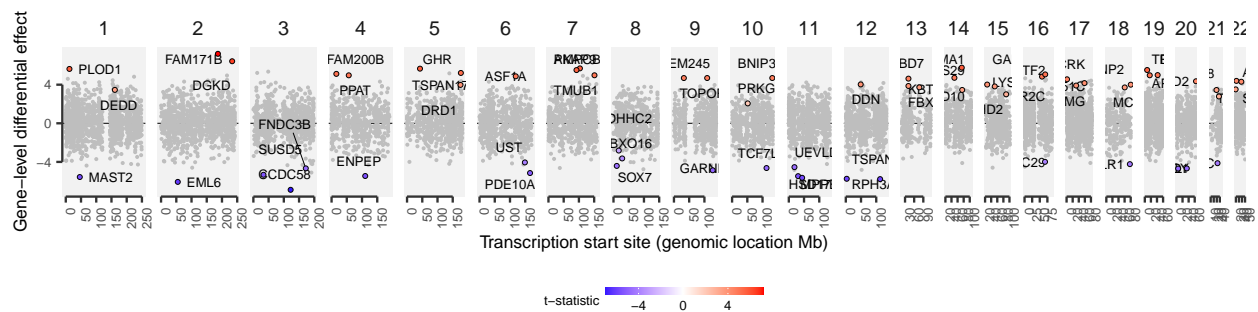


Combined genomic view

- PMPCB @ Ex
- ASF1A @ Ex
- CCDC58 @ Ex
- FAM171B @ Ex

- DGKD @ Ex
- CDC14B @ In
- N4BP2 @ In
- HIBADH @ In
- RIMS1 @ In
- TTC3 @ Oligo
- RBM39 @ Oligo
- RAB2A @ Oligo
- PHF3 @ Oligo
- ARSI @ Oligo
- PPP1R12B @ Oligo
- FRY @ OPC
- PIAS2 @ OPC
- SLC6A11 @ OPC
- TMTC2 @ OPC
- TM2D1 @ OPC
- ATP13A4 @ OPC
- EPSTI1 @ Microglia
- SNCA @ Microglia
- VPS41 @ Astro
- SLCO1C1 @ Astro
- FGFR2 @ Astro
- HVCN1 @ Astro
- PTPN4 @ Astro

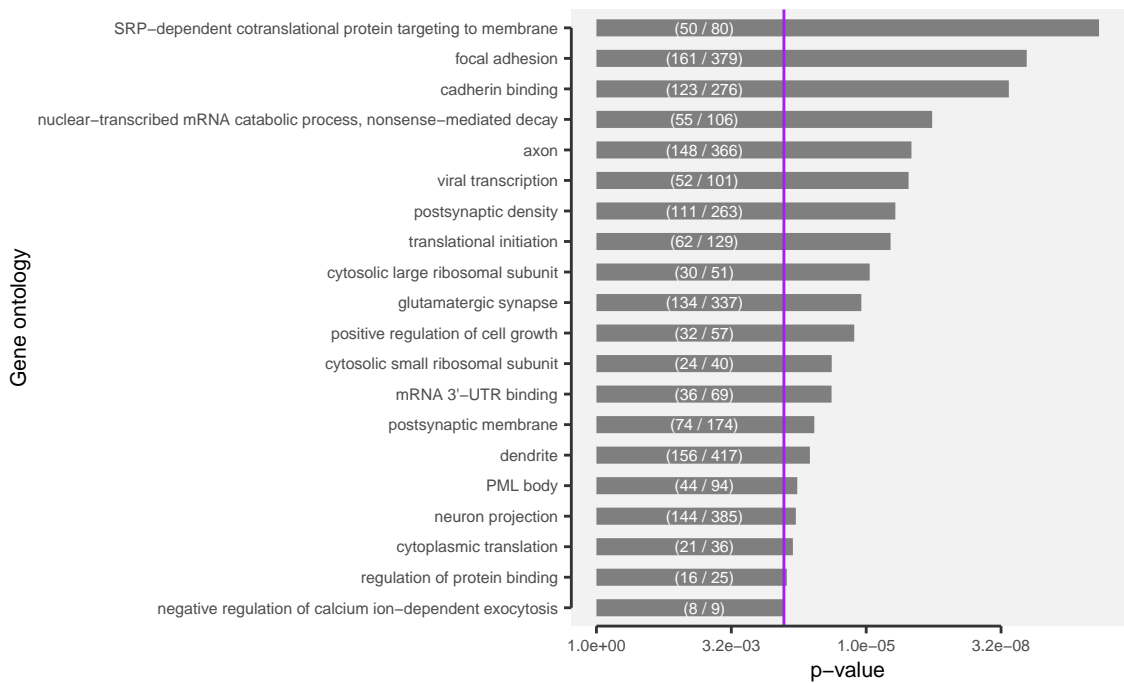
Genomic views for each cell type



Ex

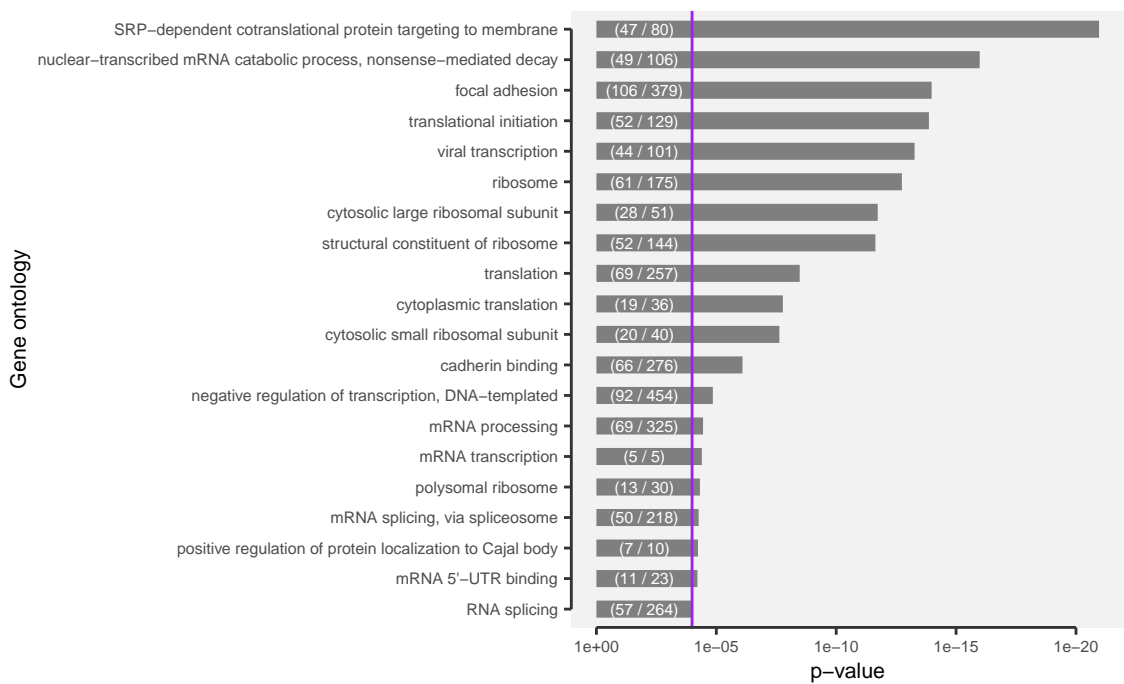
Ex top genes

Gene ontology enrichment



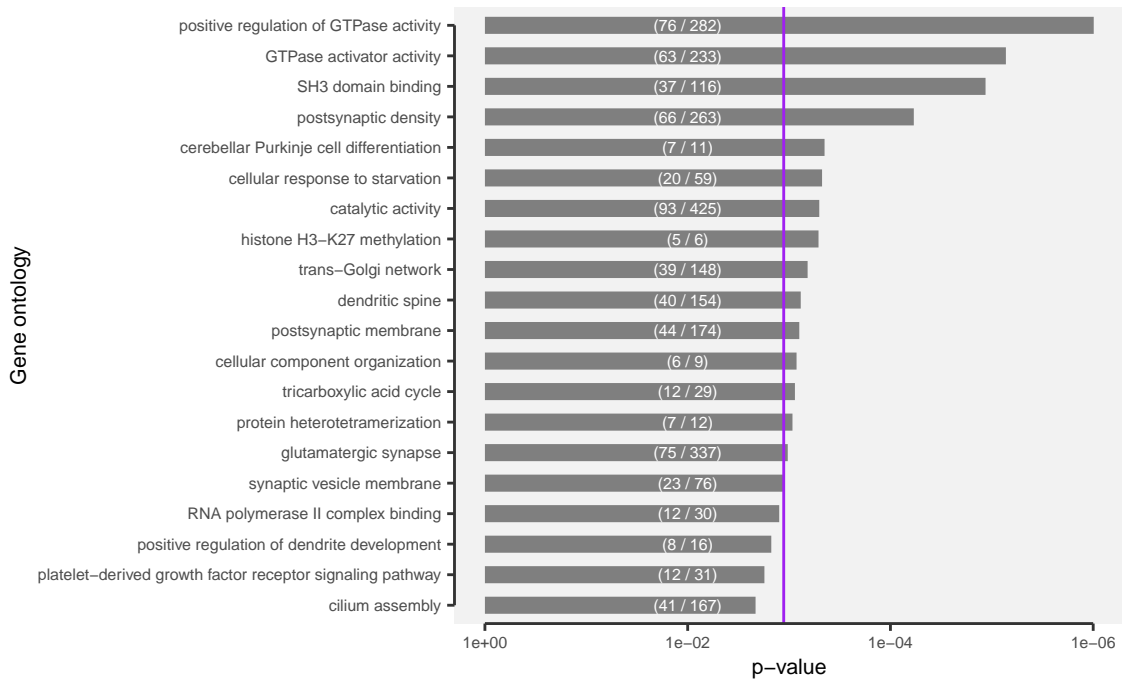
PDF

Up-regulated DEGs (2,092 genes)



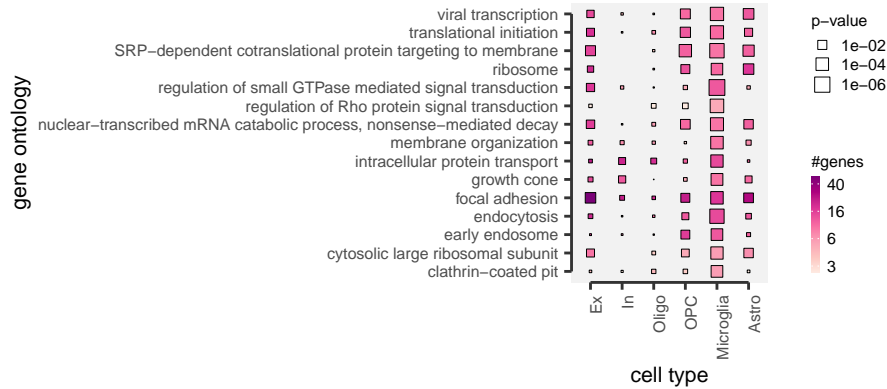
PDF

Down-regulated DEGs (2,317 genes)



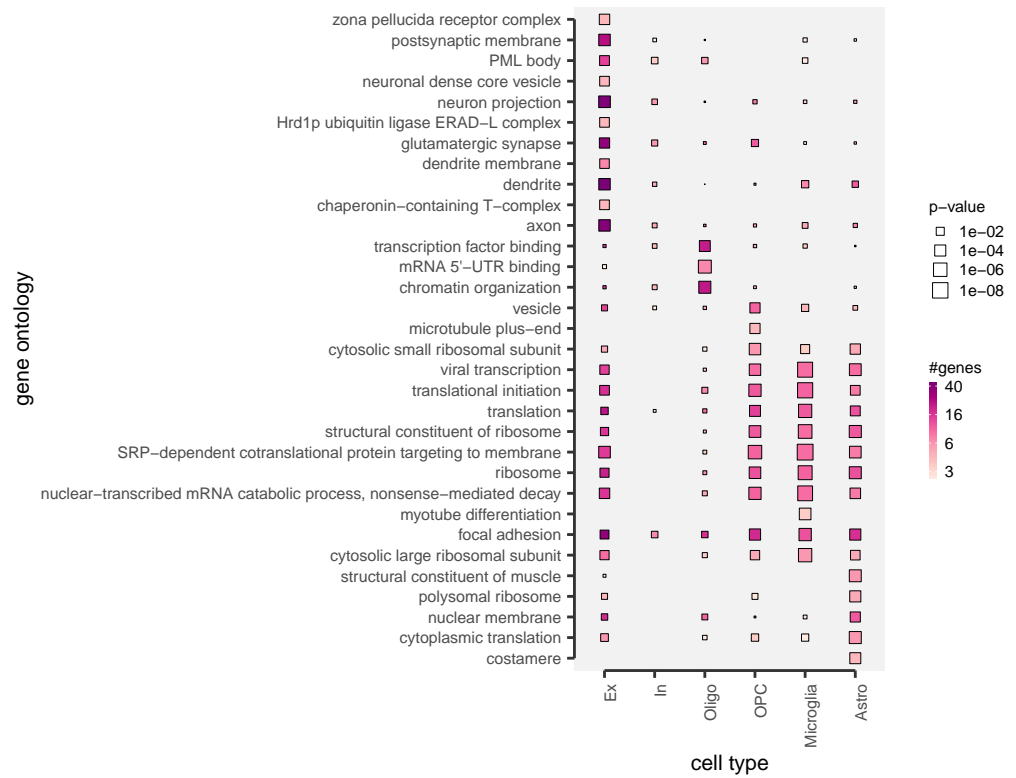
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GO enrichment for each cell type



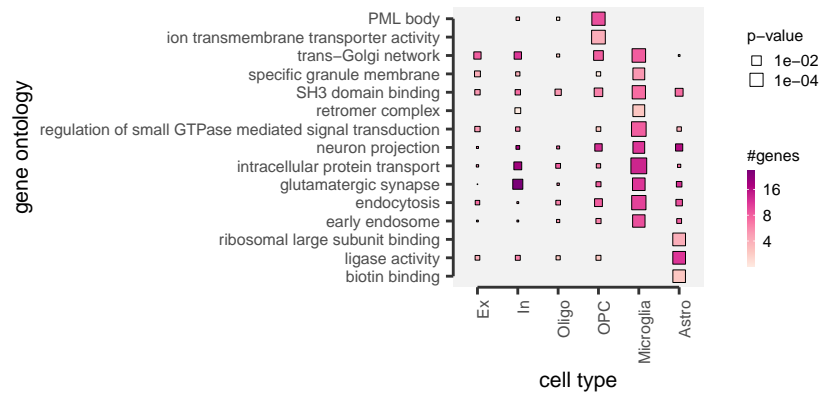
DEGs combined

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DEGs up-regulated

PDF



DEGs down-regulated

PDF