Decoding Intracellular Pathogen of H3N2 at the Single-Cell level using SCKIT

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Contents

Taking the in-vitro experiment of H3N2 infection data (SRA Accession number: SRP239555) as an example, we used PathogenTrack to identify H3N2 infected cells at the single-cell level and used SCKIT to analyze and explore the biological functions that may be related to H3N2 infection.

Install SCKIT from GitHub

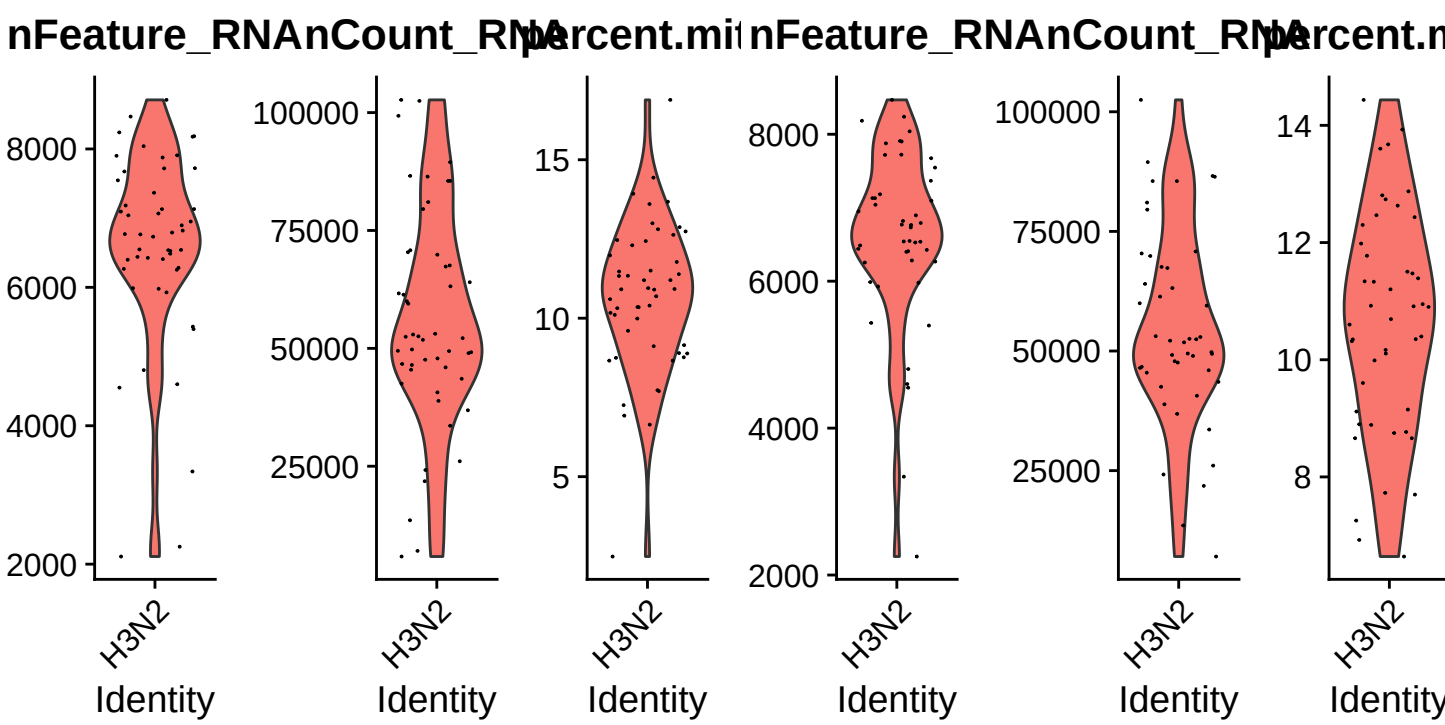
```
if (!requireNamespace("BiocManager", quietly = TRUE))
    install.packages("BiocManager")
if (!requireNamespace("devtools", quietly = TRUE))
    BiocManager::install("devtools")
if (!requireNamespace("Yeskit", quietly = TRUE))
    devtools::install_github("ncrna/Yeskit")
```

Import SCKIT

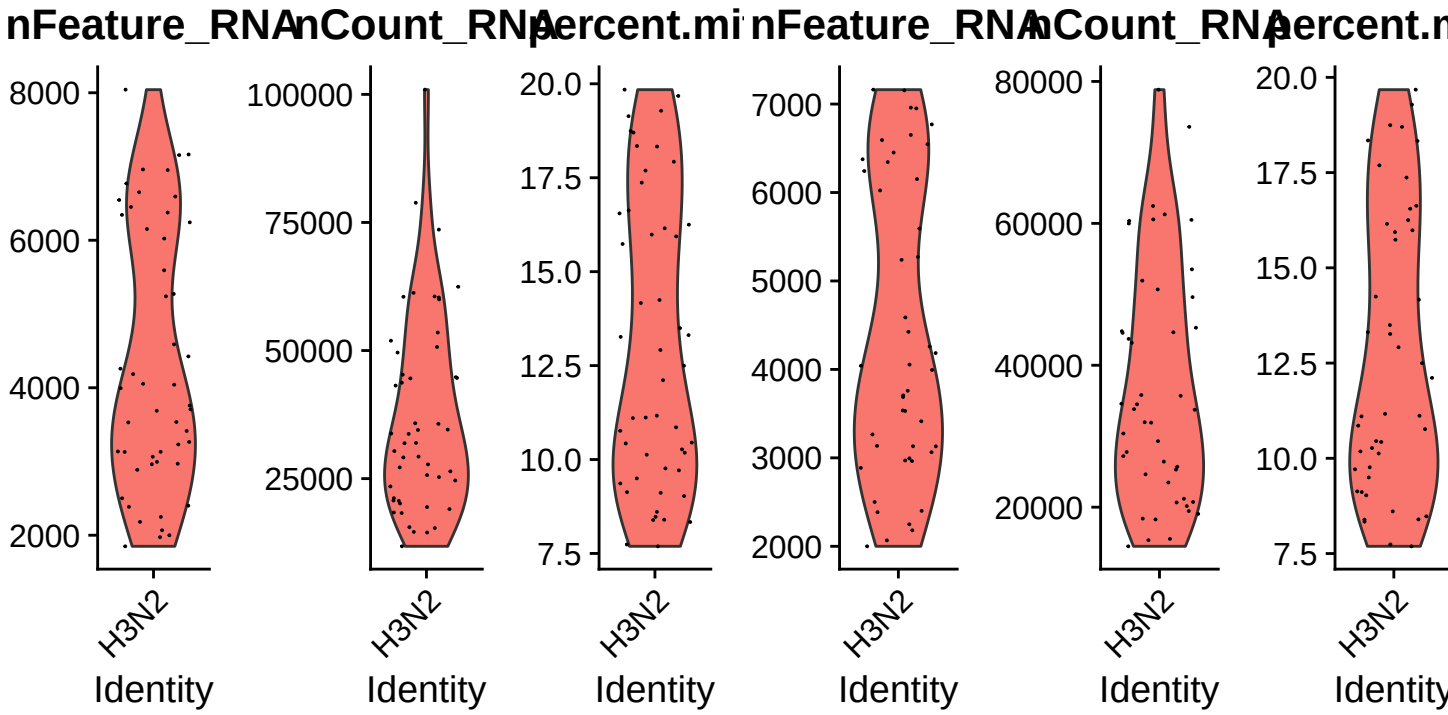
```
library(Yeskit)
library(topGO)
```

Importation

```
Bystander <- scRead(sample_name = "Bystander", data_dir = system.file("extdata/H3N2_10X_matrix/Bystande
```

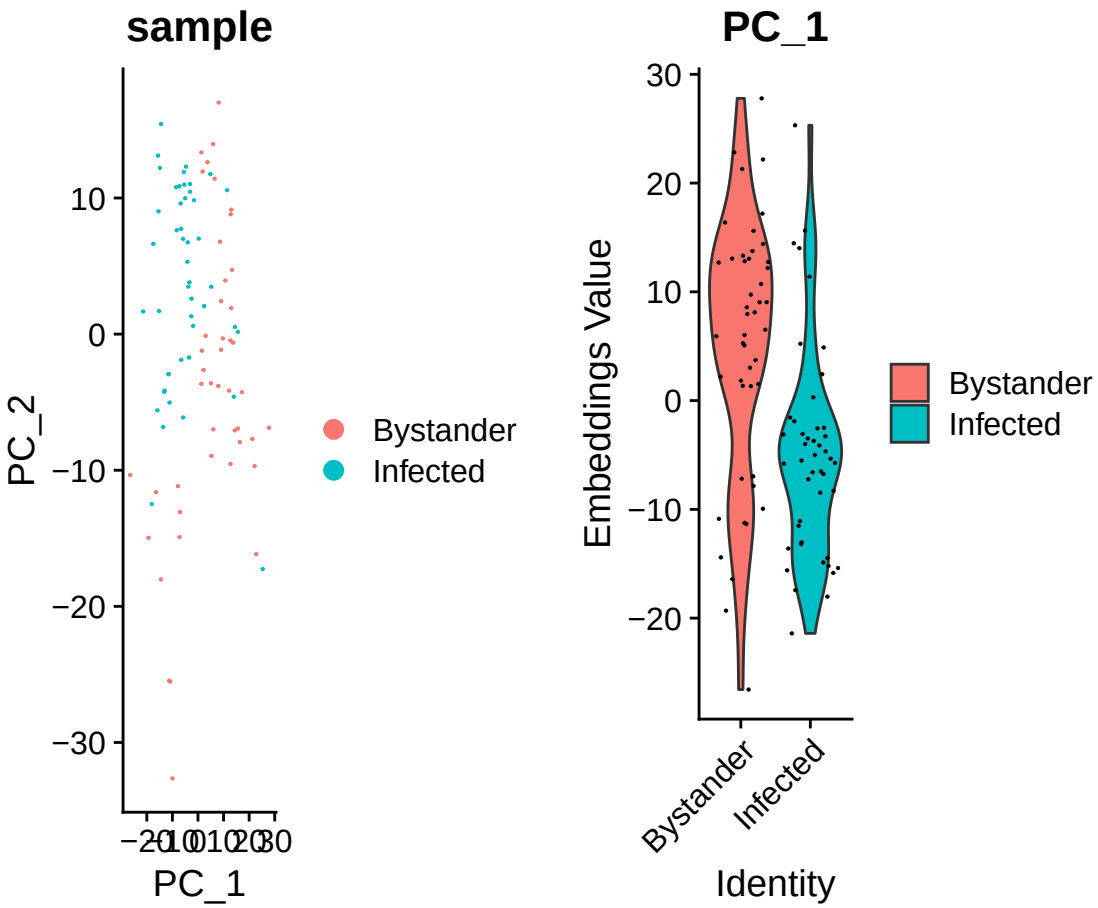



```
Infected <- scRead(sample_name = "Infected", data_dir = system.file("extdata/H3N2_10X_matrix/Infected/"
```



Integration

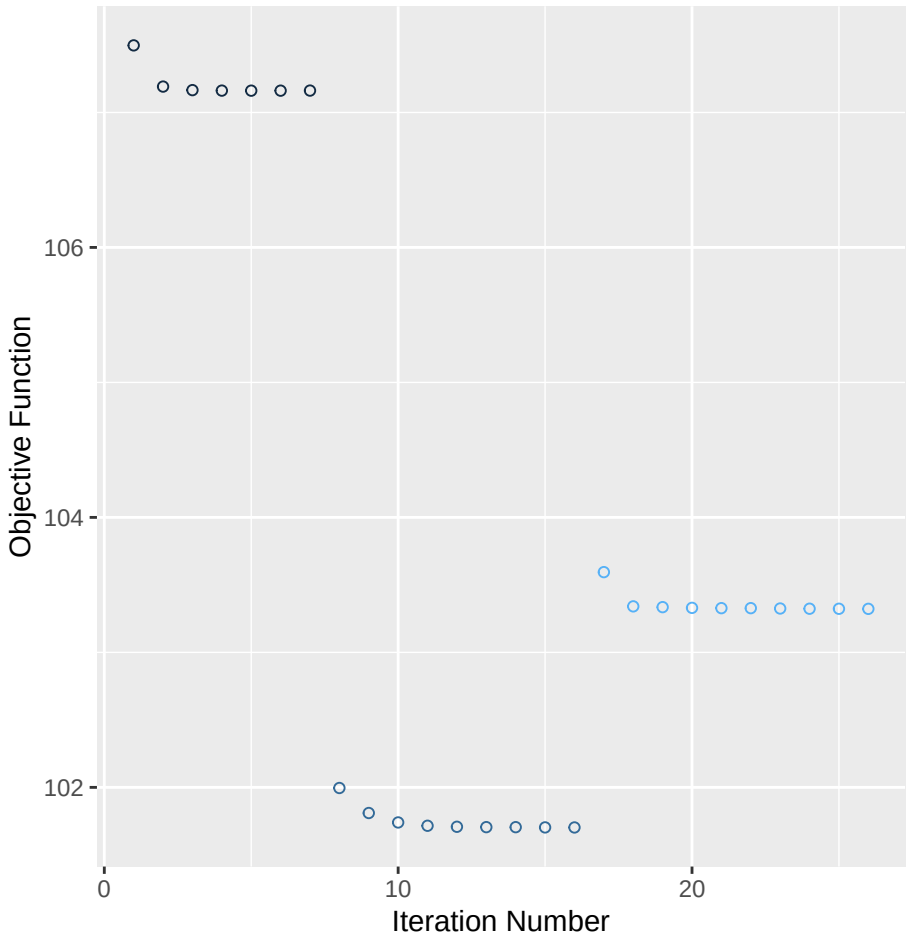
```
H3N2_integrated <- scIntegrate(object.list=list(Bystander, Infected), object.names = c("Bystander", "In
```

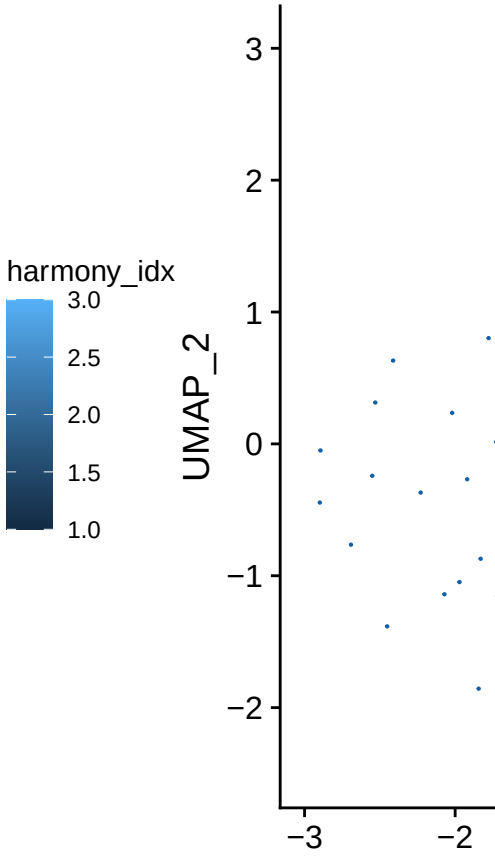



```
## Warning: Invalid name supplied, making object name syntactically valid. New
## object name is Seurat..ProjectDim.RNA.harmony; see ?make.names for more details
## on syntax validity
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 92
## Number of edges: 3839
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.3562
## Number of communities: 2
## Elapsed time: 0 seconds
```

```
## Warning: The default method for RunUMAP has changed from calling Python UMAP via reticulate to the R
## To use Python UMAP via reticulate, set umap.method to 'umap-learn' and metric to 'correlation'
## This message will be shown once per session
```





FindMarkers

```
H3N2_integrated@misc$Markers <- Seurat::FindAllMarkers(object = H3N2_integrated, assay = 'RNA', test.us
```

Differential analysis

Differential analysis between Infected and Bystander

```
H3N2_integrated@misc$Infected_vs_Bystander <- scDGE(object = H3N2_integrated, comparison = c("Infected"
```

```
## ### Comparing cluster-0 between Infected and Bystander ...
##  done.
## ### Comparing cluster-1 between Infected and Bystander ...
##  done.
```

Differential analysis between H3N2_positive and H3N2_negative

```
H3N2_integrated@misc$H3N2 <- scPathogenDGE(object = H3N2_integrated, species.by = "H3N2", min.cells = 5
```

```
## Warning in scPathogenDGE(object = H3N2_integrated, species.by = "H3N2", : All clusters will be evalua
```

```
## ### Analysis feature H3N2 ...
```

```
## ====== Analysis cluster-0 ... done.
## ====== Analysis cluster-1 ... done.
```

GO annotation

GO annotation of Markers

```
H3N2_integrated@misc$Markers.GO <- scGO(object = H3N2_integrated, key = "Markers", logFC = 0.25, only.p
```

```
## [1] "Running cluster 0"
```

```
## Warning in scGO(object = H3N2_integrated, key = "Markers", logFC = 0.25, : No
## up-regulated genes left to perform GO analysis!
```

```
## [1] "Running cluster 1"
```

```
## Warning in scGO(object = H3N2_integrated, key = "Markers", logFC = 0.25, : No
## up-regulated genes left to perform GO analysis!
```

GO annotation of DGEs between Infected and Bystander

```
H3N2_integrated@misc$Infected_vs_Bystander.GO <- scGO(object = H3N2_integrated, key = "Infected_vs_Byst
```

```
## [1] "Running cluster 0"
## [1] "Running cluster 1"
```

```
## Warning in scGO(object = H3N2_integrated, key = "Infected_vs_Bystander", : No
## up-regulated genes left to perform GO analysis!
```

```
## Warning in scGO(object = H3N2_integrated, key = "Infected_vs_Bystander", : No
## down-regulated genes left to perform GO analysis!
```

GO annotation of DGEs between H3N2_positive and H3N2_negative

```
H3N2_integrated@misc$H3N2.GO <- scPathogenGO(object = H3N2_integrated, key = "H3N2", clusters = NULL, s
```

```
## Warning in scPathogenGO(object = H3N2_integrated, key = "H3N2", clusters = NULL, : All clusters will
```

```
## [1] "Running H3N2 in cluster-0 ... "
## [1] "done.\n"
## [1] "Running H3N2 in cluster-1 ... "
## [1] "done.\n"
```

MSigDB scoring

```
H3N2_integrated <- scMsigdbScoring(object = H3N2_integrated, category = "H", geneSets = NULL)
```

```
## Warning: The following features are not present in the object: CD83, CCL20,
## ICAM1, EGR3, IL1B, BCL2A1, IL1A, TRAF1, F3, IL6, TNF, PTX3, CCL4, DUSP2, CXCL10,
## EGR2, PTGER4, SERPINB2, CXCL6, TNFAIP6, INHBA, CD69, IL7R, CCL5, OLR1, CXCL11,
## GPR183, TLR2, CSF2, PLEK, CD80, FUT4, CCRL2, SIK1, IL12B, SLC16A6, IL23A,
## IL15RA, ICOSLG, TNC, not searching for symbol synonyms
```

```
## Warning: The following features are not present in the object: HK2, ALDOC, LOX,
## GCK, PPFIA4, PYGM, F3, ALDOB, PGF, GAPDHS, CHST2, TMEM45A, BCAN, GPC3, PKLR,
## NOCT, EDN2, RORA, DTNA, HS3ST1, GPC4, BGN, BRS3, TKTL1, MT1E, SDC3, PKP1, PGAM2,
```

```
## CXCR4, B4GALNT2, HAS1, SDC2, PDGFB, SLC2A5, DCN, PLAC8, FBP1, PCK1, INHA, IL6,
## KIF5A, LALBA, NCAN, TGFB3, not searching for symbol synonyms

## Warning: The following features are not present in the object: ALDOC, LPL, ADH4,
## CBS, GSTM2, AVPR1A, MAL2, not searching for symbol synonyms

## Warning: The following features are not present in the object: ARHGAP4, OPHN1,
## TIAM1, SHROOM2, SORBS2, RAPGEF5, not searching for symbol synonyms

## Warning: The following features are not present in the object: DLL1, NOTCH4,
## LEF1, NKD1, WNT5B, WNT6, DKK4, WNT1, CCND2, HEY2, HEY1, not searching for symbol
## synonyms

## Warning: The following features are not present in the object: ENG, LEFTY2, NOG,
## not searching for symbol synonyms

## Warning: The following features are not present in the object: CSF2RB, IL2RG,
## TLR2, IL6, IL1R2, IL3RA, CSF3R, ITGB3, REG1A, A2M, IL15RA, EBI3, INHBE, CRLF2,
## TNFRSF1B, CD14, IL1B, IL18R1, TNF, CXCL13, CXCL10, IL9R, ITGA4, ACVRL1, PIK3R5,
## DNTT, CSF2, IL2RA, IL12RB1, CCR1, CNTFR, PLA2G2A, CXCL9, IL7, CXCL11, CCL7, LTB,
## IL17RB, CD36, not searching for symbol synonyms

## Warning: The following features are not present in the object: POLR1H, HCLS1,
## NPR2, not searching for symbol synonyms

## Warning: The following features are not present in the object: CDC25A, H2AX,
## H2AZ1, H2BC12, EGF, PTTG3P, DMD, H2AZ2, not searching for symbol synonyms

## Warning: The following features are not present in the object: IL1B, DIABLO,
## IL1A, TNF, FASLG, EGR3, PRF1, SATB1, PDGFRB, CCND2, TIMP3, LEF1, CASP1, GPX1,
## AIFM3, HGF, LUM, IFNB1, BCL2L10, CD2, PLAT, DCN, BMF, CD14, CD69, PLCB2,
## PLPPR4, H1-0, GSTM1, BGN, GNA15, F2, IL6, FEZ1, NEFH, CCNA1, GUCY2D, AVPR1A, not
## searching for symbol synonyms

## Warning: The following features are not present in the object: DTX1, DLL1, WNT2,
## DTX4, HEYL, ST3GAL6, not searching for symbol synonyms

## Warning: The following features are not present in the object: FABP4, ADIPOQ,
## LPL, CD36, SORBS1, ADIG, ACADL, ORM1, ITGA7, MRAP, SULT1A1, LEP, LTC4S, SPARCL1,
## FZD4, RETN, CYP4B1, COL15A1, PTGER3, MTARC2, ITIH5, OMD, ENPP2, CIDEA, ANGPT1,
## not searching for symbol synonyms

## Warning: The following features are not present in the object: GREB1, MYB,
## ANXA9, NPY1R, PDZK1, EGR3, LRIG1, PGR, RET, ABAT, MSMB, OVOL2, RASGRP1, CXCL12,
## TMPRSS3, IL17RB, CBFA2T3, TFF3, FCMR, RAPGEFL1, DHRS2, KCNK15, TGIF2, REEP1,
## MPPED2, TIAM1, CLDN7, OLFML3, SLC1A1, KLK10, P2RY2, CISH, PDLIM3, SLC37A1,
## CALCR, ESRP2, KRT13, LAD1, TBC1D30, SOX3, SLC24A3, KCNK5, RPS6KA2, CLIC3, ADCY1,
## not searching for symbol synonyms

## Warning: The following features are not present in the object: PDZK1, ANXA9,
## PGR, RET, MYB, EGR3, NPY1R, IL17RB, TFF3, GPER1, CALCR, TIAM1, CXCL12, TMPRSS3,
## SERPINA3, PDLIM3, GJB3, PRLR, SERPINA5, OVOL2, SOX3, KLK10, RAPGEFL1, PCP4,
## CACNA2D2, ASCL1, CISH, KRT13, CLIC3, KLK11, PLAC1, CCNA1, IGSF1, DHRS2, S100A9,
## SLC24A3, TPSAB1, GFUS, FGFR3, BATF, MAPK13, KCNK5, CXCL14, TRIM29, RPS6KA2,
## SERPINA1, TNNC1, HMGCS2, PTGER3, SNX10, ST14, TH, CHST8, LTF, DUSP2, ST6GALNAC2,
## not searching for symbol synonyms

## Warning: The following features are not present in the object: KLK3, KLK2,
## TMPRSS2, MAF, ALDH1A3, AZGP1, SELENOP, MAK, STEAP4, GUCY1A1, MERTK, ADAMTS1,
## H1-0, not searching for symbol synonyms
```

```
## Warning: The following features are not present in the object: ACTA1, TNNI2,
## MYL1, TNNC1, TNNC2, MYH3, MYLPF, TNNT3, TNNT2, CASQ2, ACTC1, MYOM1, MYL4,
## MYBPH, MYH7, MYH8, ACTN2, TNNI1, SGCG, HRC, DES, MYOZ1, CSRP3, ATP2A1, CKM,
## MYL2, CKMT2, MYH1, FXYD1, DMD, ACTN3, TCAP, MYL3, PYGM, LDB3, COX6A2, FABP3,
## MYL7, CHRNA1, COL3A1, GJA5, MYF6, MYH2, PGAM2, MYOG, MYH4, AEBP1, MEF2C, NOS1,
## CACNG1, PPFIA4, HSPB2, SGCA, COX7A1, CTF1, COL15A1, KCNH2, SORBS1, VIPR1, CASQ1,
## ANKRD2, LAMA2, DENND2B, NCAM1, COL6A3, MYH11, IGF1, PVALB, ACHE, CHRNG, PKIA,
## COL1A1, CAV3, CDH13, LSP1, REEP1, MYBPC3, ITGA7, GNAO1, PTGIS, SH3BGR, CAMK2B,
## EPHB3, APLNR, SOD3, FOXO4, EFS, DTNA, SLN, CD36, APOD, not searching for symbol
## synonyms

## Warning: The following features are not present in the object: SH3GL2, DOP1A,
## ATP6V1B1, not searching for symbol synonyms

## Warning: The following features are not present in the object: RSAD2, IFI27,
## CXCL10, BST2, RTP4, CXCL11, CASP1, DHX58, WARS1, EPSTI1, UBA7, LAMP3, GBP4,
## CD74, GMPR, IL7, LPAR6, SELL, BATF2, CCRL2, not searching for symbol synonyms

## Warning: The following features are not present in the object: OAS2, RSAD2,
## MX2, CXCL10, BST2, XAF1, GBP4, IRF8, IFI27, CXCL9, IL10RA, EPSTI1, IDO1, CXCL11,
## ITGB7, RTP4, CASP1, ICAM1, WARS1, FCGR1A, MARCHF1, TNFAIP6, VCAM1, CD274, CIITA,
## SELP, GPR18, FPR1, SERPING1, CCL5, PELI1, CD86, HLA-DQA1, CCL7, DHX58, FGL2,
## SECTM1, IL15RA, HLA-DRB1, GBP6, LCP2, HLA-G, KLRK1, BANK1, IL7, CD69, IRF4,
## BATF2, SLAMF7, P2RY14, IL6, GZMA, CD74, CSF2RB, CMKLR1, XCL1, IL2RB, VAMP5,
## STAT4, not searching for symbol synonyms

## Warning: The following features are not present in the object: CLDN7, ACTN3,
## CLDN19, COL17A1, NECTIN1, CLDN9, CDH3, SHROOM2, CLDN14, PTPRC, THY1, CADM3,
## CLDN8, JAM3, ICAM1, CLDN18, NRAP, COL16A1, ACTN2, LAYN, CLDN6, CDH8, ATP1A3,
## MYL9, SYK, CADM2, ALOX15B, CX3CL1, DMP1, CDSN, MAPK13, RAC2, DSC1, CD34, ICAM4,
## ACTC1, TRAF1, ARHGEF6, PDZD3, PCDH1, SLIT2, KRT31, ADAMTS5, ICAM2, CD274, CDH15,
## MPZL2, WNK4, NFASC, SDC3, FLNC, COL9A1, NRXN2, ICAM5, VWF, CD209, VCAM1, ACTG2,
## NEGR1, KCNH2, DSC3, MMP9, TNFRSF11B, AMIGO1, NLGN3, ACTA1, PECAM1, ITGA9, CD86,
## TRO, CLDN5, not searching for symbol synonyms

## Warning: The following features are not present in the object: RHCG, MAL, LYPD3,
## PKHD1, ATP6V0A4, CRYBG1, SHROOM2, THY1, GHRL, GAS1, SLC34A3, SLC2A4, IL2RB,
## RTN4RL1, IL2RG, CD160, SLC22A12, NTNG1, SCUBE1, CX3CL1, not searching for symbol
## synonyms

## Warning: The following features are not present in the object: SHH, SCG2, AMOT,
## UNC5C, HEY1, THY1, SLIT1, HEY2, L1CAM, PLG, NKX6-1, RTN1, CRMP1, CNTFR, ACHE,
## OPHN1, not searching for symbol synonyms

## Warning: The following features are not present in the object: C2, MMP14,
## SERPING1, F5, MMP13, F7, PLG, C1QA, CASP1, GZMA, C1QC, DPP4, KLK1, KLKB1,
## CR2, SPOCK2, F10, SERPINA1, CTSO, CD40LG, C4BPB, PLEK, GP9, F2, CR1, SERPINB2,
## CD36, APOBEC3G, FCN1, GZMK, PDGFB, S100A9, WAS, PLAT, MT3, PRSS36, GZMB, CASP5,
## TMPRSS6, RASGRP1, LCP2, GP1BA, LCK, OLR1, ZFPM2, ACTN2, ITGAM, LTF, KCNIP2,
## HPCAL4, C9, L3MBTL4, F3, PLA2G7, S100A12, PHEX, GNGT2, MMP12, KCNIP3, MMP8,
## FCER1G, PIK3R5, SERPINC1, GNG2, SCG3, CCL5, APOA4, IL6, NOTCH4, ADRA2B, CDH13,
## ITIH1, PIK3CG, not searching for symbol synonyms

## Warning: The following features are not present in the object: IARS1, TARS1,
## H2AX, DNAJA4, not searching for symbol synonyms

## Warning: The following features are not present in the object: CAMK4, GNGT1,
## ADCY2, FGF6, FGF17, TIAM1, PRKCB, GNA14, FGF22, IL4, SLA, MAPK10, IL2RG, FASLG,
```

```
## DAPP1, LCK, NGF, CXCR4, not searching for symbol synonyms

## Warning: The following features are not present in the object: HK2, CORO1A,
## WARS1, SLA, FGL2, ITGB2, CFP, CXCR4, EPRS1, CDC25A, DAPP1, not searching for
## symbol synonyms

## Warning: The following features are not present in the object: H2AZ1, CDC25A,
## H2AX, CDKN2A, not searching for symbol synonyms

## Warning: The following features are not present in the object: U2AF1, IARS1,
## KARS1, H2AZ1, EPRS1, not searching for symbol synonyms

## Warning: The following features are not present in the object: HK2, DUSP2, not
## searching for symbol synonyms

## Warning: The following features are not present in the object: COL3A1, COL1A1,
## COL6A3, COL1A2, LUM, LOX, THBS2, COL16A1, FAP, BGN, POSTN, THY1, TNC, MMP3,
## LOXL1, FBLN5, ELN, COMP, IL32, TIMP3, VCAM1, EDIL3, MAGEE1, ECM1, LAMA2, INHBA,
## MGP, COL5A3, SNTB1, LRRC15, TNFRSF11B, EFEMP2, ABI3BP, LAMA1, COL8A2, MMP14,
## MYL9, PRRX1, FMOD, IL6, FBLN2, PTX3, SNAI2, DCN, MFAP5, SFRP4, RGS4, SGCG,
## ITGB3, PDLIM4, CTHRC1, ECM2, CRLF1, GAS1, PRSS2, OXTR, SCG2, CXCL6, MMP1, MXRA5,
## NTM, CXCL12, PDGFRB, SLIT2, GPX7, not searching for symbol synonyms

## Warning: The following features are not present in the object: CXCL10, CCL5,
## FPR1, CCL20, IL1A, CCL7, CCL22, CXCL11, CCR7, CXCL9, IL6, IL1B, TLR2, CD69,
## ICAM1, CCRL2, AQP9, C3AR1, GNA15, CMKLR1, PTGER4, OPRK1, ITGB8, INHBA, OSM,
## IL12B, CXCL6, ACVR2A, F3, BST2, CALCRL, CSF3, GPR132, NLRP3, IL15RA, OLR1,
## PTGER2, CSF3R, TNFAIP6, IL7R, NMUR1, IL2RB, TLR1, MMP14, P2RX7, SELL, P2RY2,
## FFAR2, PROK2, TACR1, CYBB, SCARF1, EBI3, CCL17, TLR3, APLNR, IL10RA, GPR183,
## TNFRSF1B, GPC3, HPN, CD48, CXCR6, SLC1A2, GP1BA, RGS16, SLAMF1, LCK, NPFFR2,
## ICOSLG, RASGRP1, KCNJ2, IL18R1, IL10, KCNA3, LAMP3, VIP, RGS1, KCNMB2, MEFV,
## CCL24, LCP2, PDPN, SGMS2, MARCO, CD14, ROS1, NDP, MSR1, RNF144B, PCDH7, IL18RAP,
## RTP4, CHST2, ITGB3, SELE, NOD2, CLEC5A, TACR3, SLC4A4, MEP1A, LTA, PIK3R5,
## STAB1, ICAM4, CX3CL1, SLC28A2, not searching for symbol synonyms

## Warning: The following features are not present in the object: CYP1A1, CYP1A2,
## GSTA3, CYP2J2, CYP27A1, ADH1C, CYP2C18, LCAT, FMO3, SLC22A1, ARG1, CDO1, FMO1,
## NDRG2, UPB1, AOX1, HGFAC, F11, CYP26A1, SERPINA6, GNMT, MAOA, SLC6A12, HSD11B1,
## KARS1, FBP1, GCKR, ADH7, VNN1, FETUB, GAD1, IGF1, CD36, CES1, TMEM176B, GSTT2,
## PLG, XDH, CYP4F2, ESR1, TDO2, FABP1, ABCD2, G6PC, DDC, CRP, HRG, TYR, SLC46A3,
## PTGDS, AQP9, TTPA, MAN1A1, ITIH4, CYP17A1, REG1A, CCL25, F10, TAT, MARCHF6,
## MBL2, ENPEP, HSD17B2, ANGPTL3, CYP2E1, IRF8, ACSM1, ITIH1, not searching for
## symbol synonyms

## Warning: The following features are not present in the object: ACADL, CYP4A11,
## FABP1, FABP2, VNN1, EHHADH, CA4, ADH1C, ADH7, MAOA, HAO2, HMGCS2, GPD1, RDH16,
## INMT, CD36, ACSL5, CD1D, TDO2, CA6, FMO1, AOC3, CIDEA, LTC4S, XIST, CYP4A22,
## AQP7, CYP1A1, H2AZ1, GAPDHS, GAD2, IL4I1, CEL, not searching for symbol synonyms

## Warning: The following features are not present in the object: MAOB, not
## searching for symbol synonyms

## Warning: The following features are not present in the object: ALDOB, HK2,
## PFKFB1, FBP2, PPFIA4, B3GAT1, CHST6, PGAM2, CHST1, GFUS, GAPDHS, CXCR4, GPC4,
## MIOX, GPC3, TKTL1, CHST2, LCT, DSC2, HS6ST2, SDC2, RARS1, SDC3, CLDN9, TFF3,
## GYS2, B3GNT3, DCN, GPR87, NDST3, MERTK, ARTN, CHST4, not searching for symbol
## synonyms

## Warning: The following features are not present in the object: PRDX2, MPO, LSP1,
```

```
## IPCEF1, not searching for symbol synonyms

## Warning: The following features are not present in the object: CDKN2B, CDKN2A,
## RRAD, CCND2, SERPINB5, KLK8, CASP1, CDH13, H2AW, H2AJ, RGS16, DEF6, VWA5A, ABAT,
## ALOX15B, TCN2, ZNF365, IL1A, ST14, TCHH, TNNI1, FGF13, CLCA2, KRT17, ZBTB16,
## GLS2, NHLH2, IRAG2, H1-2, not searching for symbol synonyms

## Warning: The following features are not present in the object: C4BPB, ICAM1,
## RASGRP1, CDKN2B, H2AX, MMP14, ABCB1, HLA-F, RRAD, IL6, RET, PDLIM3, EPCAM,
## SULT1A1, CYP1A1, CDO1, HTR7, TARS1, NPTX2, PLCL1, FMO1, KCNH2, COL2A1, MAOA,
## TCHH, SLC6A12, PTPRD, MAPK8IP2, ONECUT1, TACR3, CCK, not searching for symbol
## synonyms

## Warning: The following features are not present in the object: MMP16, SDC2,
## ADGRL2, RGS4, AMPH, COL1A2, F3, COL3A1, CELF2, ACVR2A, COL1A1, PDGFRB, KALRN,
## PTGFR, FBLN5, ITGB3, SNAI2, KIT, MT1E, not searching for symbol synonyms

## Warning: The following features are not present in the object: POSTN, LPL,
## CCND2, SERPINA5, KCNJ8, COL3A1, OLR1, SLCO2A1, PGLYRP1, PRG2, LUM, CXCL6, not
## searching for symbol synonyms

## Warning: The following features are not present in the object: ALAS2, GYPB, RHD,
## GYPA, GYPE, GYPC, EPB42, RHCE, RHAG, TAL1, MARCHF8, SPTB, HBQ1, OSBP2, ANK1,
## NFE2, AHSP, SPTA1, CLIC2, KEL, TRIM10, TSPO2, XK, SNCA, CA1, KLF1, H1-0, HBD,
## CROCCP2, TMCC2, GATA1, PRDX2, CTSE, HBB, ACSL6, SLC30A10, HBZ, ACKR1, H4C3,
## ICAM4, FTCD, MYL4, ALDH1L1, HBBP1, TENT5C, TRIM58, LMO2, MARCHF2, SYNJ1, ACP5,
## TYR, not searching for symbol synonyms

## Warning: The following features are not present in the object: F2, MMP14, F10,
## PLG, SERPING1, C2, F9, CTSO, TMPRSS6, MMP9, PROZ, MMP1, VWF, F11, MMP11, HPN,
## MBL2, F13B, MMP8, A2M, MMP10, MASP2, FGG, C8A, PLAT, OLR1, RGN, C8B, C9, F2RL2,
## GP9, CTSK, GDA, KLK8, SERPINC1, ITIH1, F3, CTSE, ITGB3, KLKB1, MMP3, MEP1A,
## GP1BA, APOA1, SERPINA1, TF, COMP, HRG, C8G, APOC2, SERPINB2, RAPGEF3, CPN1,
## DPP4, PDGFB, PECAM1, CPB2, DCT, LEFTY2, C1QA, HMGCS2, PLEK, APOC3, P2RY1, TIMP3,
## S100A1, not searching for symbol synonyms

## Warning: The following features are not present in the object: CISH, IL2RA,
## TNFRSF4, HK2, IL2RB, CTLA4, CD83, IKZF2, IL10, TNFRSF18, ECM1, PTGER2, ITIH5,
## CD79B, TNFRSF1B, TIAM1, S100A1, PENK, IRF4, CST7, TLR7, GBP4, RGS16, IL13,
## CSF2, FLT3LG, CCND2, TRAF1, IL3RA, FGL2, PTH1R, CAPN3, IL1R2, SYT11, BATF,
## GPR65, CCR4, GATA1, HOPX, GPR83, CD48, DRC1, SELP, TNFSF11, LRRC8C, SH3BGRL2,
## ICOS, LTB, ENPP1, IL1RL1, IL18R1, TNFRSF8, RHOH, CXCL10, IRF6, IL10RA, MAP6,
## SELL, SERPINC1, RORA, F2RL2, ABCB1, IRF8, GUCY1B1, SHE, LRIG1, AGER, CD86, not
## searching for symbol synonyms

## Warning: The following features are not present in the object: AKR1D1, ABCD2,
## CYP8B1, CYP7B1, CYP27A1, AGXT, CYP39A1, CYP7A1, PIPOX, HSD3B1, HAO1, ABCG8,
## AQP9, NR1H4, CYP46A1, BBOX1, APOA1, ABCG4, NR3C2, ABCA6, GNMT, ABCA9, ACSL5,
## ABCA8, DIO1, TTR, KLF1, LCK, SULT1B1, NR1I2, GC, ABCA4, DIO2, RXRG, SERPINA6,
## CH25H, SOAT2, TFCP2L1, NR0B2, not searching for symbol synonyms

## Warning: The following features are not present in the object: EHHADH, ABCD2,
## HAO2, ABCB4, ABCB1, ACSL5, NR1I2, DIO1, ABCC8, ALB, RXRG, UGT2B17, SERPINA6,
## HSD11B2, TTR, CRABP2, CRABP1, SCGB1A1, FABP6, CACNA1B, CEL, ESR2, not searching
## for symbol synonyms

## Warning: The following features are not present in the object: PTPRC, IL12B,
## IL12A, CD3E, CD3D, CD28, HCLS1, CRTAM, IFNG, CD3G, CD86, IL10, CD4, LCK, C2,
## ITGB2, HLA-DQA1, CD1D, CD80, HLA-DRA, THY1, TLR1, HLA-G, HLA-DMB, IL7, IL4, TNF,
```

```
## CD247, IL2, IRF4, INHBA, TLR3, ZAP70, CD74, LTB, CCR5, CD40LG, HLA-DOA, IL6,
## CD2, CCL5, FASLG, ELANE, SPI1, PRF1, IL12RB1, LCP2, CDKN2A, STAT4, CD7, HLA-DOB,
## CD8A, ICAM1, CCL4, GZMB, STAB1, IL2RA, NLRP3, SIT1, HDAC9, CARTPT, TRAT1, CCL22,
## IL1B, KRT1, WARS1, CCR2, MMP9, IL16, CFP, WAS, ITGAL, KLRD1, RARS1, TLR2, CCND2,
## IL2RG, ITK, NCR1, MAP4K1, CCL19, CD8B, F2, LY86, FCGR2B, GZMA, AARS1, CD96,
## ICOSLG, CCL11, GPR65, ACHE, IGSF6, IL13, DARS1, ACVR2A, CXCR3, PRKCB, CXCL9,
## NCF4, CCR1, MBL2, IRF8, CCL7, IL2RB, FGR, IL18RAP, CXCL13, CCL13, RPL3L, LY75,
## NOS2, IL9, not searching for symbol synonyms

## Warning: The following features are not present in the object: PDHA2, TSSK2,
## TNP1, ZPBP, DPEP3, ADAM2, ACTL7B, GAPDHS, TUBA3C, DDX25, PRM2, TCP11, PAPOLB,
## OAZ3, ODF1, ACRBP, YBX2, NAA11, TULP2, ADAD1, SYCP1, DDX4, TEKT2, PGK2, ACRV1,
## CRISP2, AKAP4, CCNA1, PACRG, GSG1, THEG, CST8, DCC, HSPA1L, DNAJB8, IL13RA2,
## CAMK4, SLC2A5, NOS1, ART3, ZNRF4, SNAP91, TNP2, HBZ, POMC, TKTL1, H1-6, MTNR1A,
## DMC1, CHRM4, NEFH, SEPTIN4, NPY5R, SHE, GPR182, ACE, HTR5A, CLVS1, CNIH2, GRM8,
## ADCYAP1, ALOX15, MEP1B, SCG3, GFI1, ELOVL3, IL12RB2, DMRT1, GAD1, CFTR, JAM3,
## HOXB1, not searching for symbol synonyms

## Warning: The following features are not present in the object: HSD11B1, ITGB2,
## PPBP, GABRA3, IRF8, FGF9, INHBA, ADAM8, PRKG2, MMP11, MMP10, GALNT3, MMP9,
## WNT7A, CLEC4A, CCND2, PCP4, CFHR2, ALDH1A2, NR0B2, ALDH1A3, SATB1, GUCY1A1,
## CSF2, APOD, CMKLR1, TMEM176B, ADGRA2, LAPTM5, CD37, CIDEA, IL1B, GYPC, LY96,
## FLT4, SPON1, IGF2, NR1H4, ACE, PRRX1, C3AR1, TRAF1, TLR8, TMEM100, GADD45G,
## NAP1L2, PLAT, SCG3, ANO1, IL1RL2, CXCL10, HDAC9, PEG3, TNNT2, PTPRR, CCL20,
## ARG1, RETN, PDCD1LG2, H2BC3, HOXD11, LAT2, RELN, TMEM176A, MAP4K1, IL33, MAFB,
## LCP1, NGF, SERPINA3, RGS16, SPARCL1, ABCB1, USH1C, IL2RG, IL10RA, MPZL2, PIGR,
## VWA5A, F13A1, ADGRL4, EPB41L3, PLVAP, ADAMDEC1, IL7R, ENG, CXCR4, IKZF1, MYCN,
## FCER1G, PECAM1, SNAP91, TNFRSF1B, TPH1, not searching for symbol synonyms

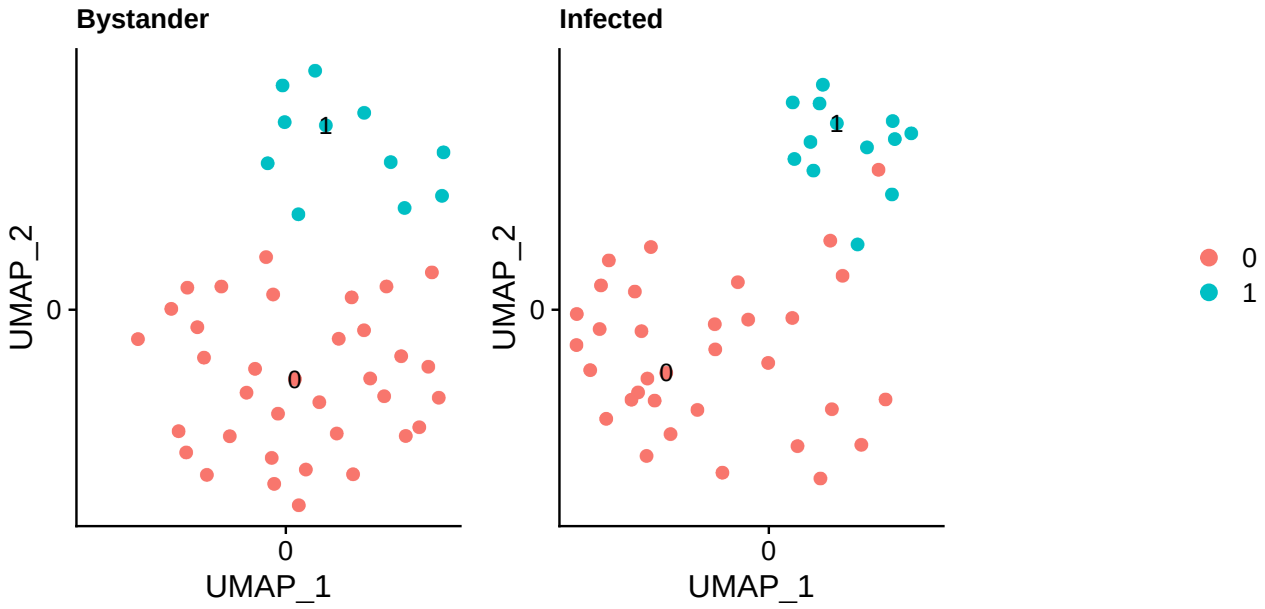
## Warning: The following features are not present in the object: CDH16, FGFR3,
## NOS1, PDE6B, SIDT1, NTF3, SCN10A, SLC6A3, CLDN8, TGM1, PCDHB1, RYR2, GDNF, AMBN,
## SERPINA10, ALOX12B, CALCB, FGGY, SPRR3, ATP6V1B1, PRODH, GPR19, EDN2, EDAR,
## KCNMB1, TENT5C, STAG3, KRT4, INSL5, GP1BA, ABCG4, MYH7, BRDT, PTGFR, KCNN1,
## SELENOP, PROP1, LGALS7, DCC, SNCB, COL2A1, UGT2B17, NPY4R, WNT16, SLC6A14,
## ITGB1BP2, SLC12A3, YBX2, CKM, CPB1, ARHGDIG, CALML5, KRT1, ABCB11, MYOT, KLK7,
## THNSL2, CHRNG, CD40LG, KRT13, GP2, CD207, CCR8, ZBTB16, PRKN, CPA2, MEFV, CCNA1,
## SLC38A3, KLK8, CYP39A1, KCNQ2, CHST2, ITIH3, EGF, TEX15, CLPS, CYP11B2, CLDN16,
## HSD11B2, HNF1A, FGF22, TFCP2L1, OXT, KCND1, MACROH2A2, NRIP2, RGS11, CPEB3, TG,
## NUDT11, FSHB, TFF2, SSTR4, PDCD1, KCNE2, KLHDC8A, CNTFR, IL5, NPHS1, SCGB1A1,
## PAX3, VPREB1, TSHB, CACNA1F, P2RX6, PAX4, GPR3, GRID2, TCL1A, PLAG1, PKP1,
## IRS4, IL12B, EPHA5, SOX10, CACNG1, CAPN9, SMPX, LYPD3, PNMT, IFNG, ACTC1, RSAD2,
## HTR1B, FGF16, TENM2, RIBC2, NGB, MYO15A, SLC5A5, KRT5, TFAP2B, CD80, ATP4A,
## ARPP21, SERPINB2, TLX1, EFHD1, P2RY4, not searching for symbol synonyms

## Warning: The following features are not present in the object: NEUROD1, ISL1,
## NKX2-2, PCSK1, NKX6-1, SLC2A2, DCX, GCK, MAFB, INS, PDX1, ABCC8, IAPP, NEUROG3,
## GCG, DPP4, PAX4, SCGN, HNF1A, CHGA, PCSK2, INSM1, SST, G6PC2, PKLR, LMO2, not
## searching for symbol synonyms
```

Visualization

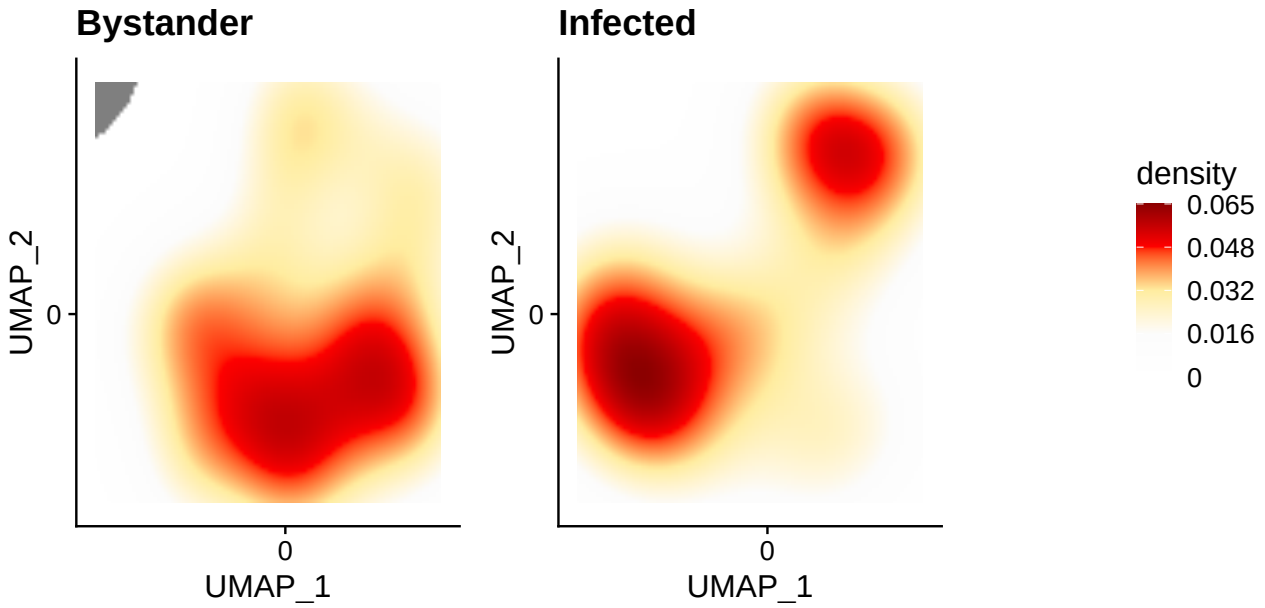
Visualization of cell clusters by scDimPlot

```
scDimPlot(object = H3N2_integrated, reduction = "umap", cols = NULL, split.by = "sample", ncol = 2, pt.
```



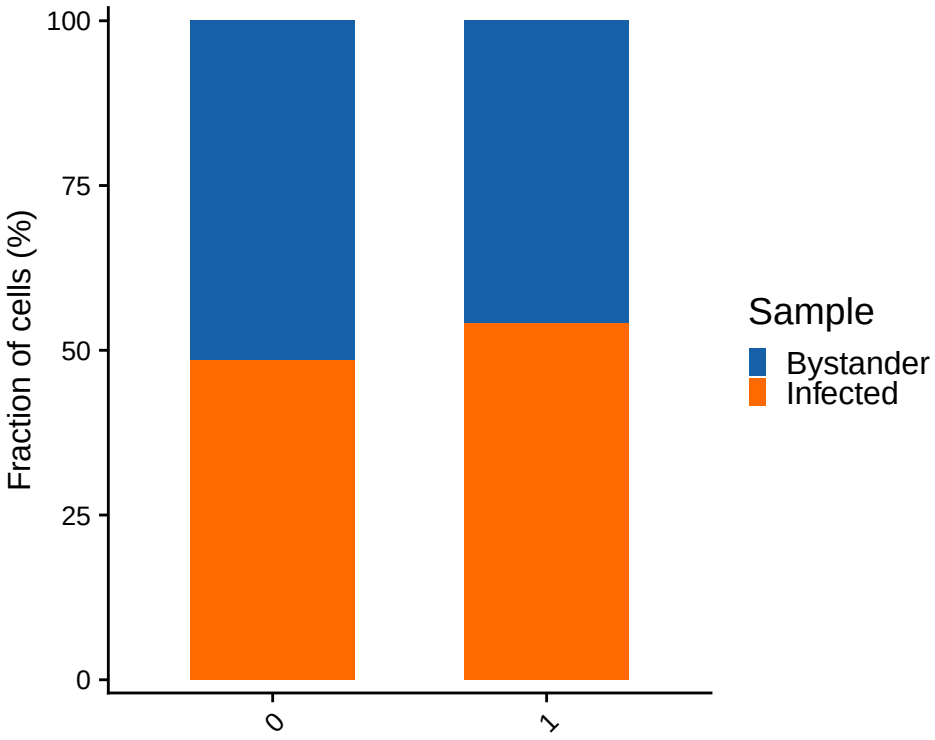
Visualization of cell densities by scDensityPlot

```
scDensityPlot(object = H3N2_integrated, reduction = "umap", split.by = "sample", ncol = 2)
```



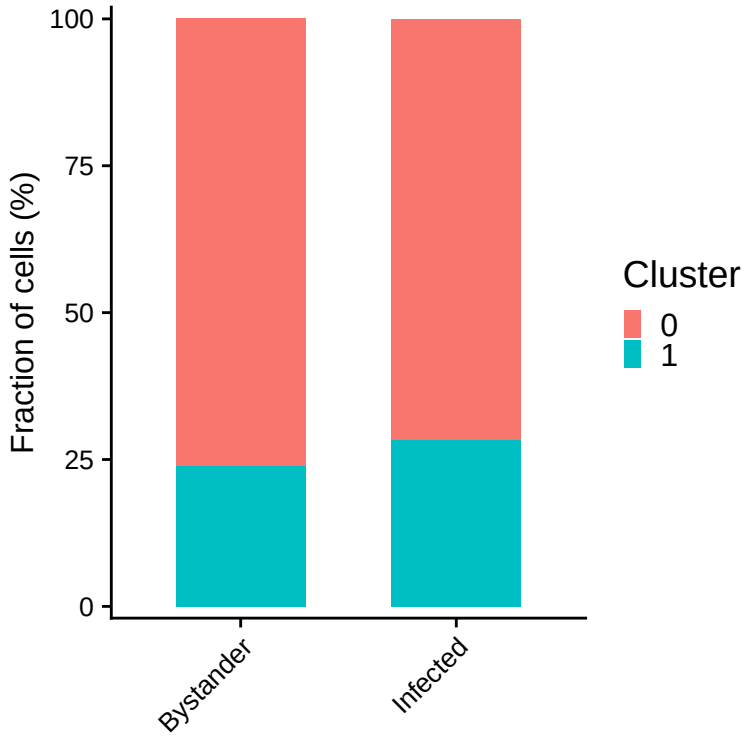
Visualization of cell population fractions by scPopulationPlot, the x axis stands for clusters

```
scPopulationPlot(object = H3N2_integrated, by = "cluster", cols = "sc", order = c("Bystander", "Infecte
```



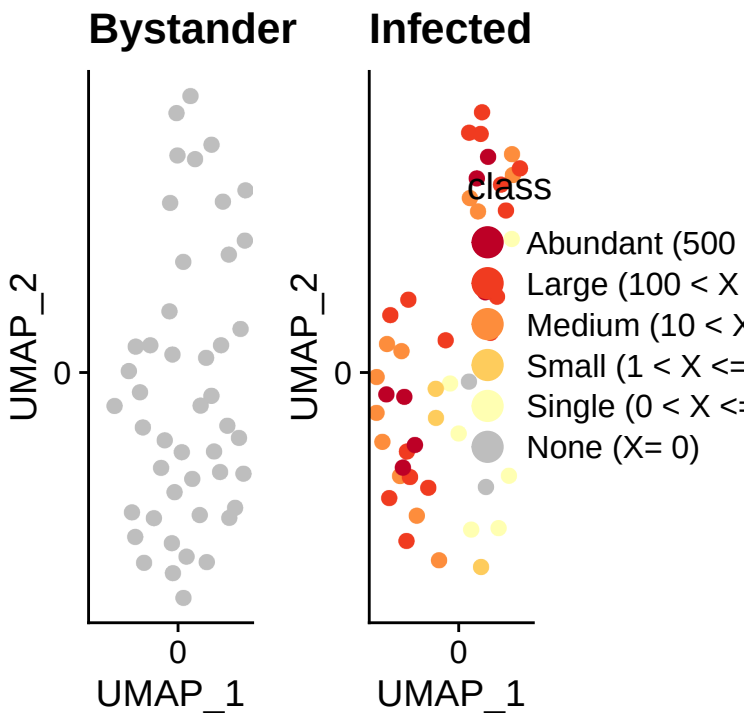
Visualization of cell population fractions by scPopulationPlot, the x axis stands for samples

```
scPopulationPlot(object = H3N2_integrated, by = "sample", order = c("Bystander", "Infected"))
```



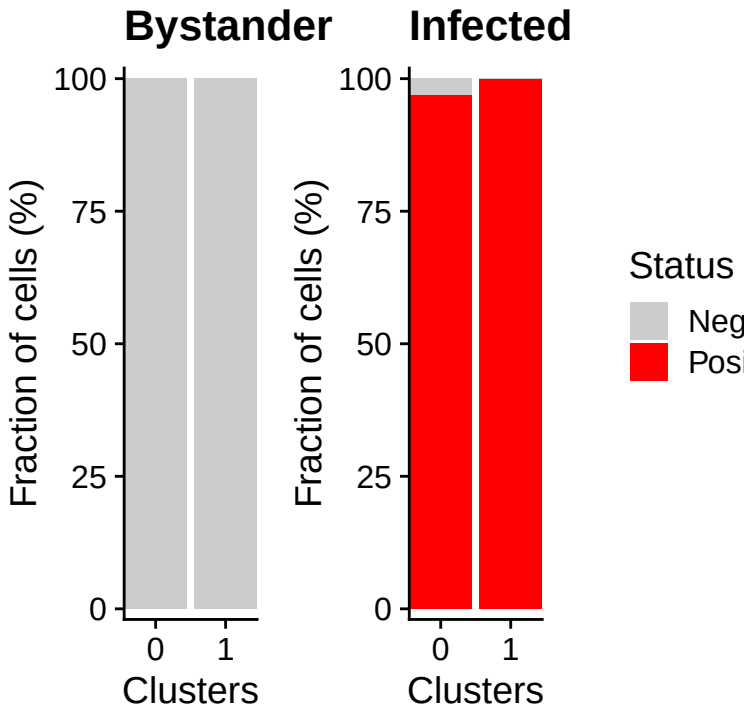
Visualization of meta data by scVizMeta

```
scVizMeta(object = H3N2_integrated, reduction = "umap", signature="H3N2", title = "H3N2", raster = TRUE
```



Visualization of H3N2-infected cell fractions by scPathogenRatioPlot

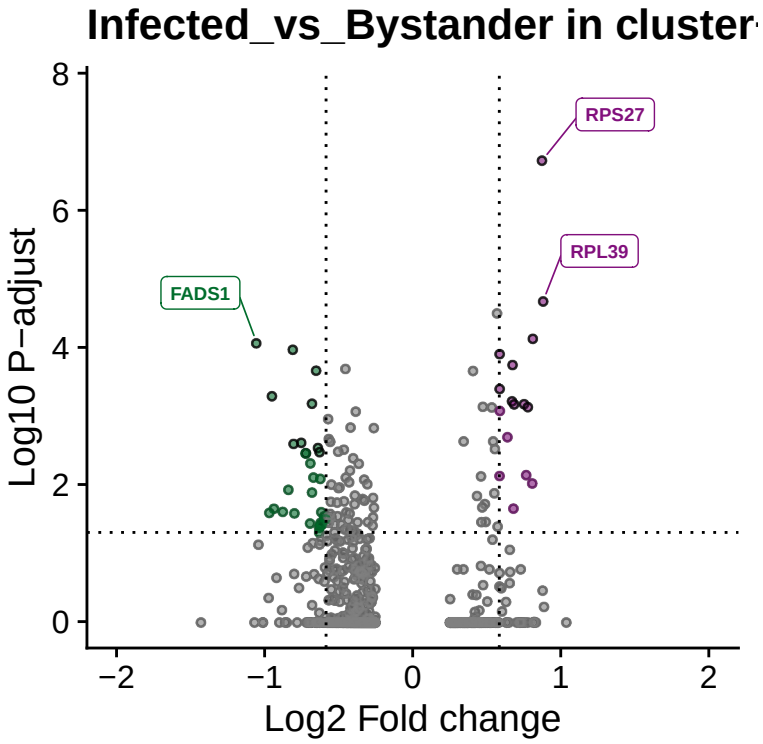
```
scPathogenRatioPlot(object = H3N2_integrated, species = "H3N2", split.by = "sample", ncol = 2)
```



Visualization of DGEs by scVolcanoPlot

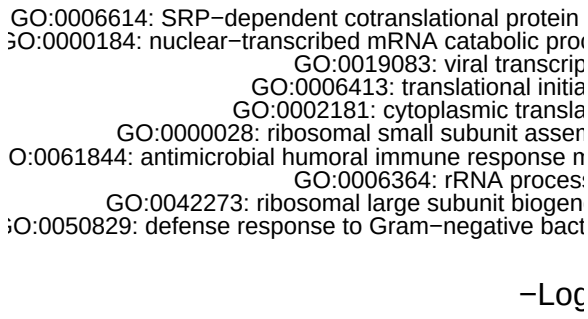
```
scVolcanoPlot(H3N2_integrated, key = "Infected_vs_Bystander", cluster = "0", top_n = 10)
```

```
## Warning: ggrepel: 17 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```



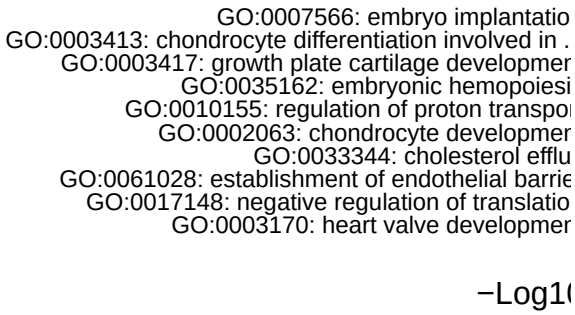
Visualization of enriched GO terms for up-regulated genes by scGOBarPlot

```
scGOBarPlot(object = H3N2_integrated, key = "Infected_vs_Bystander.GO", ont = "BP", top_n = 10, directi
```



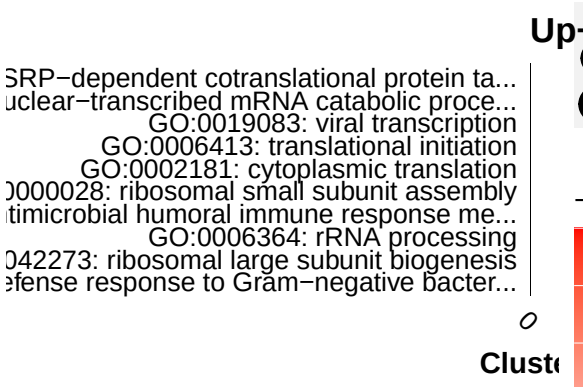
Visualization of enriched GO terms for down-regulated genes by scGOBarPlot

```
scGOBarPlot(object = H3N2_integrated, key = "Infected_vs_Bystander.GO", ont = "BP", top_n = 10, directi
```



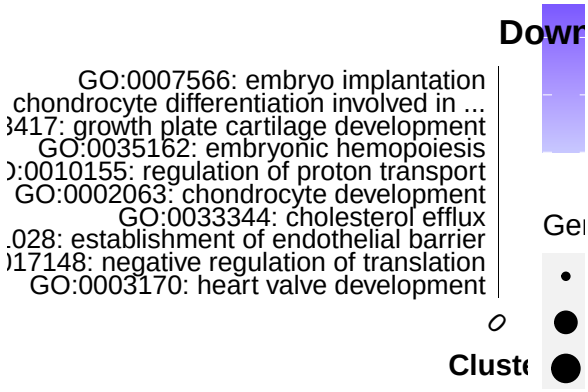
Visualization of enriched GO terms for up-regulated genes by scGODotPlot

```
scGODotPlot(object = H3N2_integrated, key = "Infected_vs_Bystander.GO", ont = "BP", direction = "up", t
```



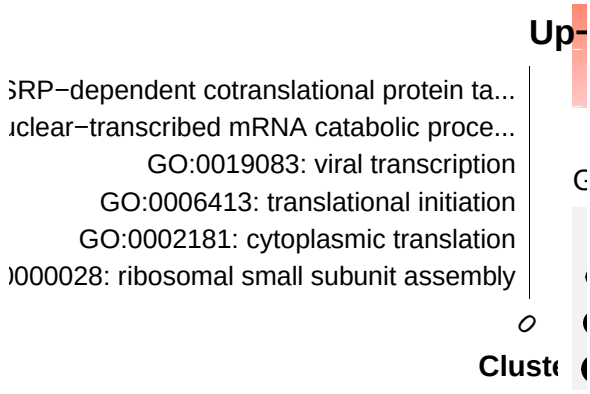
Visualization of enriched GO terms for down-regulated genes by scGODotPlot

```
scGODotPlot(object = H3N2_integrated, key = "Infected_vs_Bystander.GO", ont = "BP", direction = "down",
```



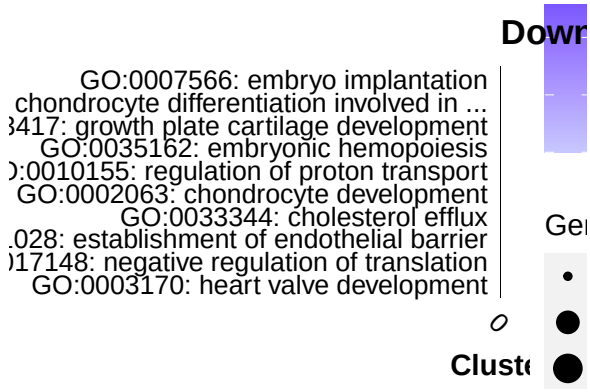
Visualization of up-regulated GO terms in cluster-0

```
scGODotPlot(object = H3N2_integrated, key = "Infected_vs_Bystander.GO", ont = "BP", direction = "up", t
```



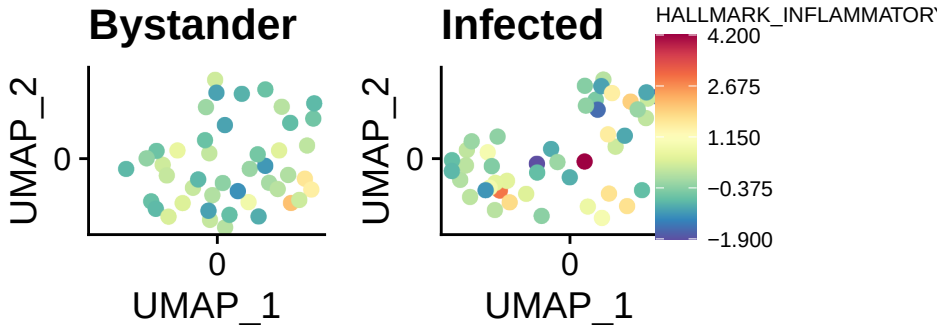
Visualization of down-regulated GO terms in cluster-0

```
scGODotPlot(object = H3N2_integrated, key = "Infected_vs_Bystander.GO", ont = "BP", direction = "down",
```



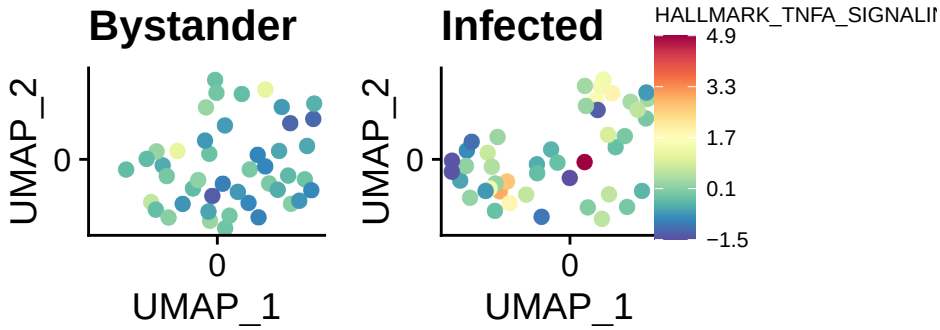
Visualization of HALLMARK_INFLAMMATORY_RESPONSE pathway

```
scScoreDimPlot(H3N2_integrated, signature = "HALLMARK_INFLAMMATORY_RESPONSE", split.by="sample", ncol =
```



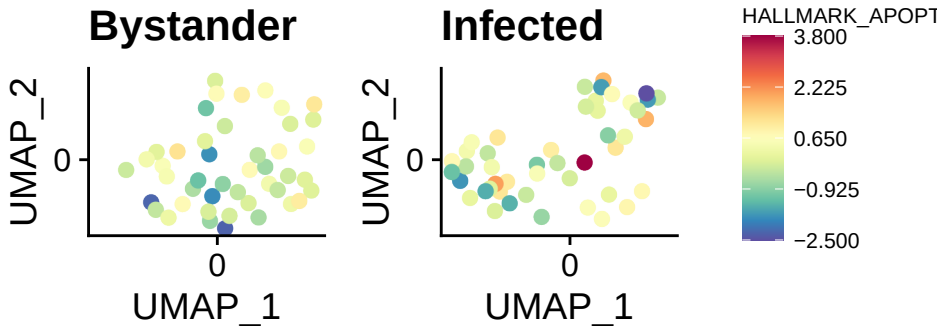
Visualization of HALLMARK_TNFA_SIGNALING_VIA_NFKB pathway

```
scScoreDimPlot(H3N2_integrated, signature = "HALLMARK_TNFA_SIGNALING_VIA_NFKB", split.by="sample", ncol
```



Visualization of HALLMARK_APOPTOSIS pathway

```
scScoreDimPlot(H3N2_integrated, signature = "HALLMARK_APOPTOSIS", split.by="sample", ncol = 2, pt.size =
```



Session Information

```
## R version 4.1.1 (2021-08-10)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats4    parallel  stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] org.Hs.eg.db_3.13.0  topGO_2.44.0         SparseM_1.81
##  [4] GO.db_3.13.0         AnnotationDbi_1.54.1 IRanges_2.26.0
##  [7] S4Vectors_0.30.0     Biobase_2.52.0       graph_1.70.0
## [10] BiocGenerics_0.38.0  Yeskit_0.99.0
##
## loaded via a namespace (and not attached):
```

```
##   [1] utf8_1.2.2                  reticulate_1.20
##   [3] tidyselect_1.1.1            RSQLite_2.2.8
##   [5] htmlwidgets_1.5.4           grid_4.1.1
##   [7] Rtsne_0.15                  devtools_2.4.2
##   [9] munsell_0.5.0               codetools_0.2-18
##  [11] ica_1.0-2                   future_1.22.1
##  [13] miniUI_0.1.1.1              withr_2.4.2
##  [15] colorspace_2.0-2            highr_0.9
##  [17] knitr_1.33                  rstudioapi_0.13
##  [19] Seurat_4.0.4                SingleCellExperiment_1.14.1
##  [21] ROCR_1.0-11                 tensor_1.5
##  [23] listenv_0.8.0               MatrixGenerics_1.4.3
##  [25] labeling_0.4.2              GenomeInfoDbData_1.2.6
##  [27] harmony_0.1.0               polyclip_1.10-0
##  [29] bit64_4.0.5                 farver_2.1.0
##  [31] rprojroot_2.0.2             parallelly_1.28.1
##  [33] vctrs_0.3.8                 generics_0.1.0
##  [35] xfun_0.25                   R6_2.5.1
##  [37] GenomeInfoDb_1.28.4         ggbeeswarm_0.6.0
##  [39] bitops_1.0-7                spatstat.utils_2.2-0
##  [41] cachem_1.0.6                DelayedArray_0.18.0
##  [43] promises_1.2.0.1            scales_1.1.1
##  [45] beeswarm_0.4.0              gtable_0.3.0
##  [47] Cairo_1.5-12.2              globals_0.14.0
##  [49] processx_3.5.2              goftest_1.2-2
##  [51] rlang_0.4.11                splines_4.1.1
##  [53] lazyeval_0.2.2              spatstat.geom_2.2-2
##  [55] yaml_2.2.1                  reshape2_1.4.4
##  [57] abind_1.4-5                 httpuv_1.6.3
##  [59] tools_4.1.1                 usethis_2.0.1
##  [61] ggplot2_3.3.5               ellipsis_0.3.2
##  [63] spatstat.core_2.3-0         RColorBrewer_1.1-2
##  [65] sessioninfo_1.1.1           ggridges_0.5.3
##  [67] Rcpp_1.0.7                  plyr_1.8.6
##  [69] progress_1.2.2              zlibbioc_1.38.0
##  [71] purrr_0.3.4                 RCurl_1.98-1.4
##  [73] ps_1.6.0                    prettyunits_1.1.1
##  [75] rpart_4.1-15                deldir_0.2-10
##  [77] pbapply_1.5-0               cowplot_1.1.1
##  [79] zoo_1.8-9                   SeuratObject_4.0.2
##  [81] SummarizedExperiment_1.22.0 ggrepel_0.9.1
##  [83] cluster_2.1.2               fs_1.5.0
##  [85] magrittr_2.0.1              data.table_1.14.0
##  [87] RSpectra_0.16-0             scattermore_0.7
##  [89] lmtest_0.9-38               RANN_2.6.1
##  [91] fitdistrplus_1.1-5          matrixStats_0.61.0
##  [93] pkgload_1.2.1               hms_1.1.0
##  [95] patchwork_1.1.1             mime_0.11
##  [97] evaluate_0.14               xtable_1.8-4
##  [99] gridExtra_2.3               testthat_3.0.4
## [101] compiler_4.1.1              tibble_3.1.4
## [103] KernSmooth_2.23-20          crayon_1.4.1
## [105] htmltools_0.5.2             mgcv_1.8-36
## [107] later_1.3.0                 tidyr_1.1.3
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## [109] DBI_1.1.1                  MASS_7.3-54
## [111] MAST_1.18.0                Matrix_1.3-4
## [113] cli_3.0.1                  igraph_1.2.6
## [115] GenomicRanges_1.44.0       pkgconfig_2.0.3
## [117] plotly_4.9.4.1             spatstat.sparse_2.0-0
## [119] vipor_0.4.5                XVector_0.32.0
## [121] stringr_1.4.0              callr_3.7.0
## [123] digest_0.6.28              sctransform_0.3.2
## [125] RcppAnnoy_0.0.19           spatstat.data_2.1-0
## [127] Biostrings_2.60.2          rmarkdown_2.10
## [129] leiden_0.3.9               uwot_0.1.10
## [131] shiny_1.6.0                lifecycle_1.0.0
## [133] nlme_3.1-152               jsonlite_1.7.2
## [135] desc_1.3.0                 viridisLite_0.4.0
## [137] fansi_0.5.0                pillar_1.6.2
## [139] lattice_0.20-44            ggrastr_0.2.3
## [141] KEGGREST_1.32.0            fastmap_1.1.0
## [143] httr_1.4.2                 pkgbuild_1.2.0
## [145] survival_3.2-13            glue_1.4.2
## [147] remotes_2.4.0              png_0.1-7
## [149] bit_4.0.4                  stringi_1.7.4
## [151] blob_1.2.2                 memoise_2.0.0
## [153] dplyr_1.0.7                irlba_2.3.3
## [155] future.apply_1.8.1
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