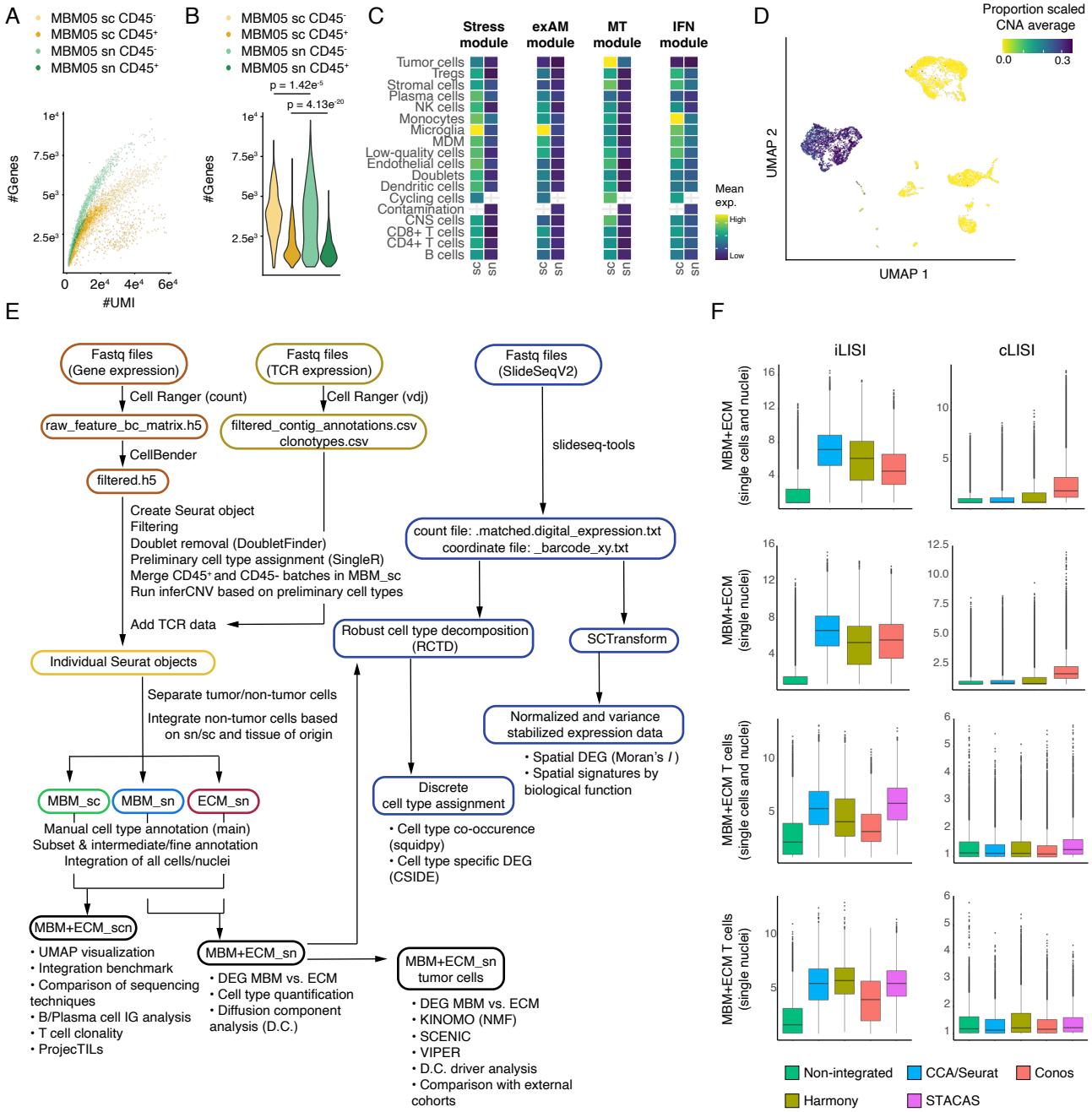
A
MBM05 sc CD45-
MBM05 sc CD45+
MBM05 sn CD45-
MBM05 sn CD45+
#Genes
#UMI
B
p = 1.42e-5
p = 4.13e-20
C
Stress module
exAM module
MT module
IFN module
Tumor cells
Tregs
Stromal cells
Plasma cells
NK cells
Monocytes
Microglia
MDM
Low-quality cells
Endothelial cells
Doublets
Dendritic cells
Cycling cells
Contamination
CNS cells
CD8+ T cells
CD4+ T cells
B cells
sc
sn
Mean exp.
High
Low
D
Proportion scaled CNA average
UMAP 2
UMAP 1
E
Fastq files (Gene expression)
Fastq files (TCR expression)
Fastq files (SlideSeqV2)
Cell Ranger (count)
Cell Ranger (vdj)
raw_feature_bc_matrix.h5
filtered_contig_annotations.csv
clonotypes.csv
slideseq-tools
CellBender
filtered.h5
Create Seurat object
Filtering
Doublet removal (DoubletFinder)
Preliminary cell type assignment (SingleR)
Merge CD45+ and CD45- batches in MBM_sc
Run inferCNV based on preliminary cell types
count file: .matched.digital_expression.txt
coordinate file: _barcode_xy.txt
Add TCR data
Robust cell type decomposition (RCTD)
SCTransform
Individual Seurat objects
Separate tumor/non-tumor cells
Integrate non-tumor cells based on sn/sc and tissue of origin
Normalized and variance stabilized expression data
• Spatial DEG (Moran's I)
• Spatial signatures by biological function
MBM_sc
MBM_sn
ECM_sn
Discrete cell type assignment
• Cell type co-occurence (squidpy)
• Cell type specific DEG (CSIDE)
Manual cell type annotation (main)
Subset & intermediate/fine annotation
Integration of all cells/nuclei
MBM+ECM_scn
• UMAP visualization
• Integration benchmark
• Comparison of sequencing techniques
• B/Plasma cell IG analysis
• T cell clonality
• ProjecTILs
MBM+ECM_sn
• DEG MBM vs. ECM
• Cell type quantification
• Diffusion component analysis (D.C.)
MBM+ECM_sn tumor cells
• DEG MBM vs. ECM
• KINOMO (NMF)
• SCENIC
• VIPER
• D.C. driver analysis
• Comparison with external cohorts
F
iLISI
cLISI
MBM+ECM (single cells and nuclei)
MBM+ECM (single nuclei)
MBM+ECM T cells (single cells and nuclei)
MBM+ECM T cells (single nuclei)
Non-integrated
CCA/Seurat
Conos
Harmony
STACAS