Supplemental Figures

Inferring disease progressive stages in single-cell transcriptomics using a weakly-supervised deep learning approach

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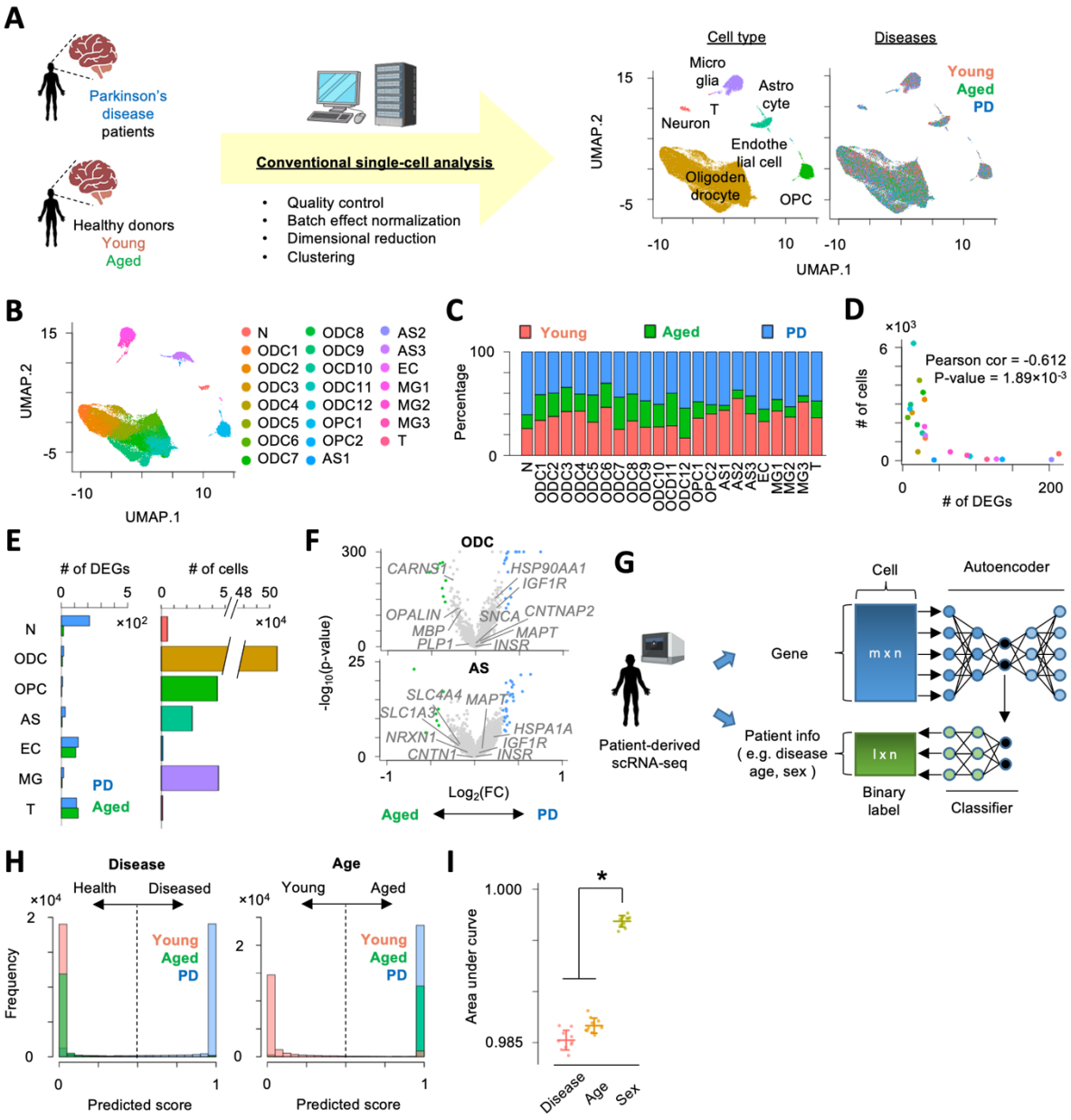
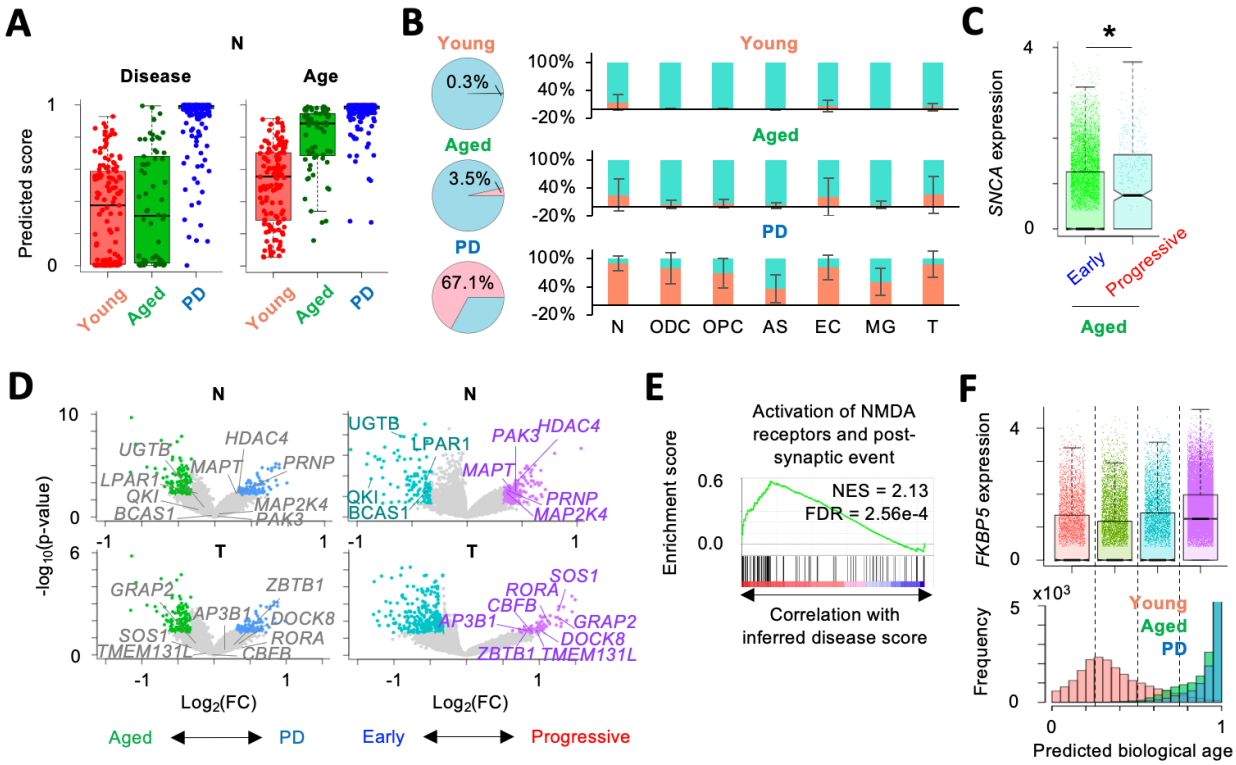


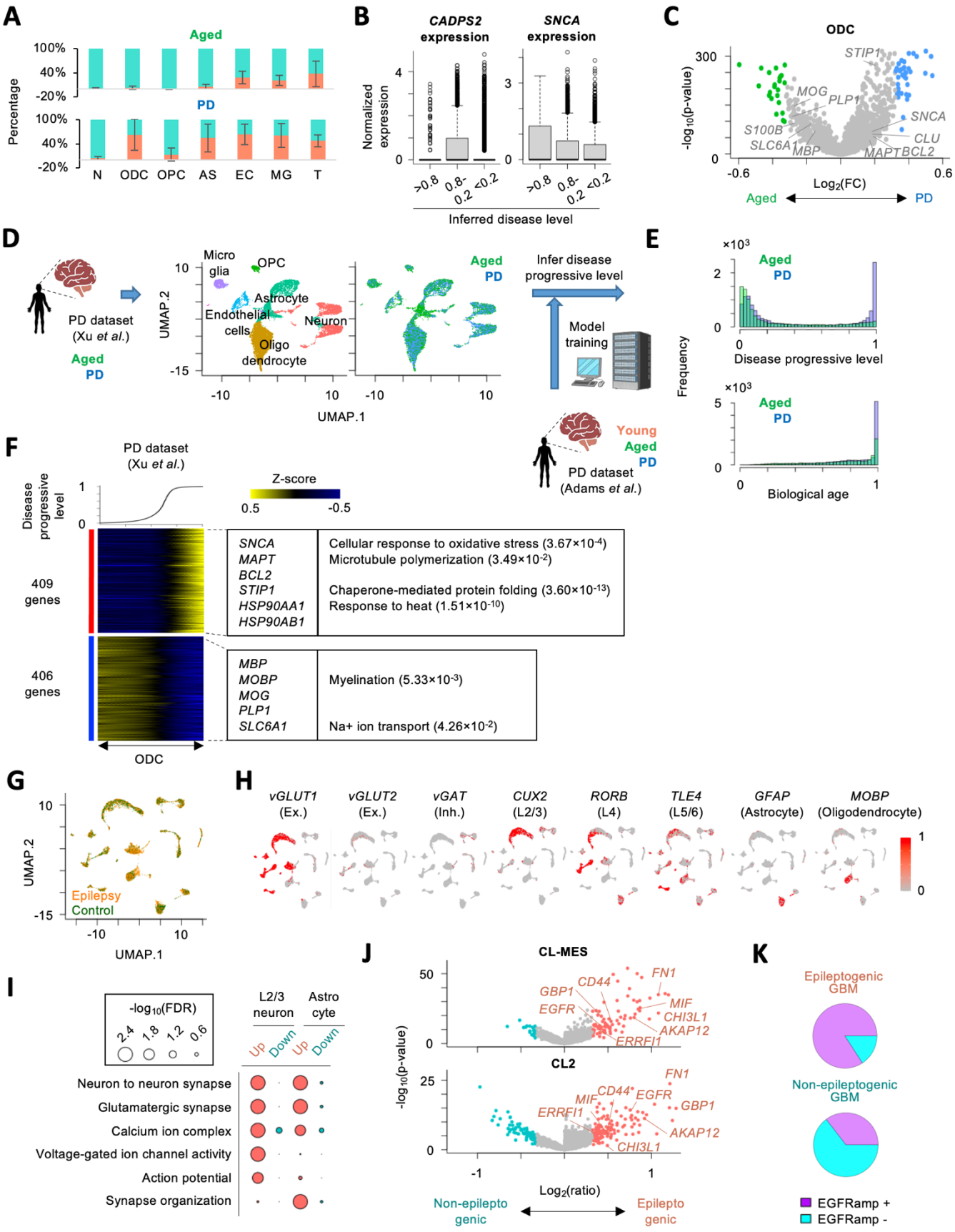
Supplemental Figure 1. Conventional scRNA-seq analysis and application of supervised learning model to single-cell transcriptome profiles of Parkinson's disease patients

A. Conventional single-cell analysis clusters cells into cell types, but not into disease. **B.** UMAP plots of single cells colored by clusters. **C.** Composition of origins of cells in each cluster. **D.** Inverse correlation between the number of differentially-expressed genes and the number of cells in each cluster. **E.** Comparison between (left) the number of differentially-expressed genes (DEGs) between Parkinson's disease patient and age-matched healthy donor and (right) the number of cells in each cell type. **F.** Volcano plots showing differential expression between PD patients and age-matched donors **G.** Supervised deep learning model to discriminate diseased and healthy cells. **H.** Histogram of prediction score (output of deep learning model) of disease and age. **I.** Comparison of area under curve in the prediction of disease, age, and sex. Error bar represents standard deviation (n=10). * p<0.05 by two-sided *t*-test.



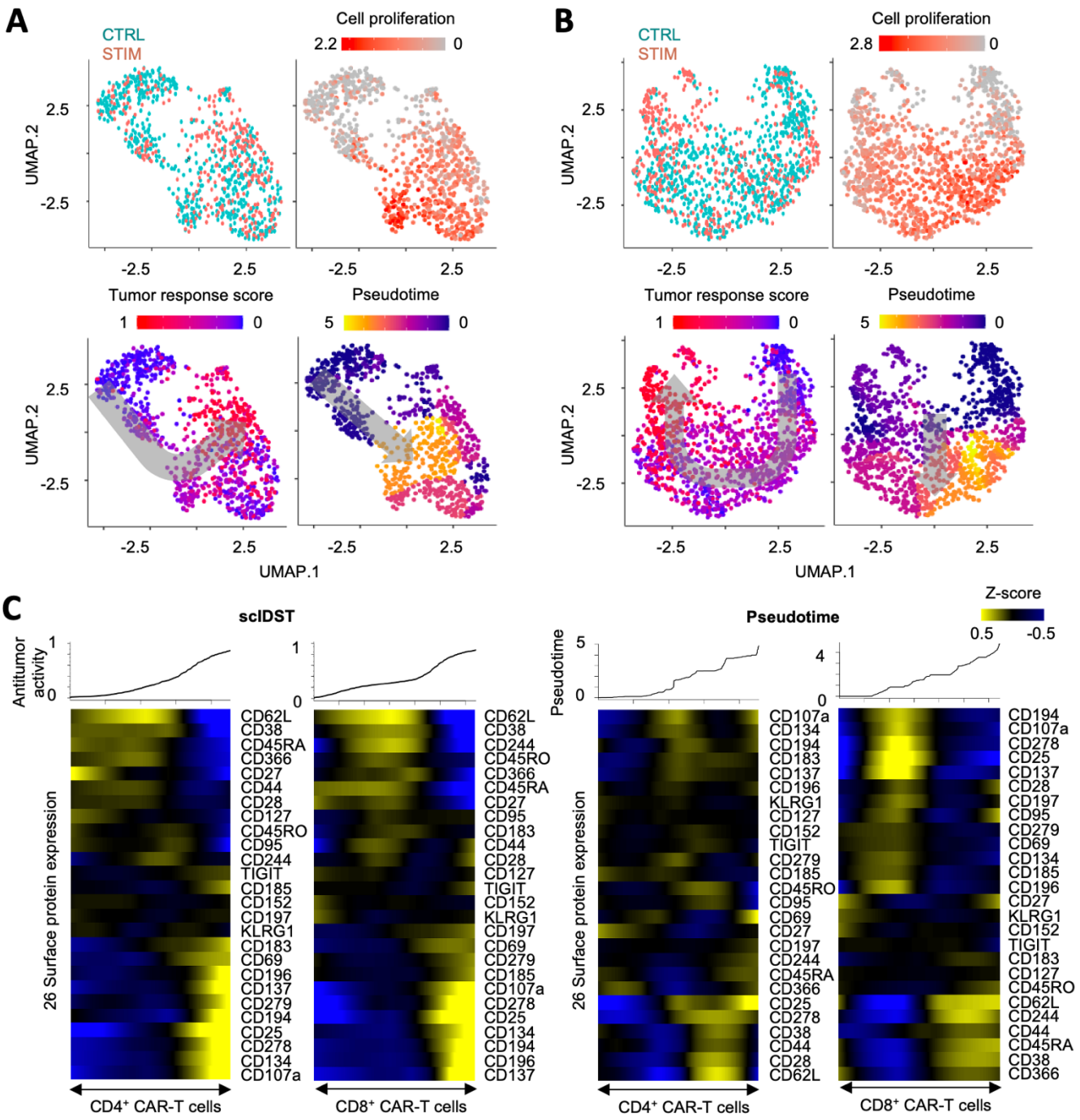
Supplemental Figure 2. Application of weakly-supervised deep learning model to Parkinson's disease patient-derived single-cell transcriptome profiles

A. Comparison of the inferred disease progressive levels and biological ages in neurons across healthy young, aged donors, and Parkinson's disease patients. **B.** Pie charts showing frequency of disease progressive cells. The frequency in each cell type is shown in right panel. Error bars represent standard deviation. **C.** Comparison of *SNCA* expression between early/healthy and disease progressive cells in aged brain. **D.** Volcano plot showing differential expression in neurons and T cells (left) between PD patients and healthy aged donors and (right) between disease progressive stage and early/heathy stage. **E.** Activation of NMDA receptor pathway over the inferred disease process. **F.** Correlation between predicted biological age and *FKBP5* expression. Cells are separated into four groups by predicted biological age. Comparison of *FKBP5* expression across four groups and Histogram of predicted biological age are shown in top and bottom, respectively.


A
Aged
PD
Percentage
100%
40%
-20%
N
ODC
OPC
AS
EC
MG
T
B
CADPS2 expression
SNCA expression
Normalized expression
Inferred disease level
C
ODC
-log10(p-value)
Log2(FC)
Aged
PD
STIP1
MOG
PLP1
SNCA
S100B
SLC6A1
MBP
CLU
MAPT
BCL2
D
PD dataset (Xu et al.)
Micro glia
OPC
Astrocyte
Endothelial cells
Neuron
Oligo dendrocyte
UMAP.1
UMAP.2
Infer disease progressive level
Model training
Young
Aged
PD
PD dataset (Adams et al.)
E
Frequency
Disease progressive level
Biological age
F
PD dataset (Xu et al.)
Disease progressive level
Z-score
409 genes
406 genes
ODC
SNCA
MAPT
BCL2
STIP1
HSP90AA1
HSP90AB1
Cellular response to oxidative stress (3.67×10-4)
Microtubule polymerization (3.49×10-2)
Chaperone-mediated protein folding (3.60×10-13)
Response to heat (1.51×10-10)
MBP
MOBP
MOG
PLP1
SLC6A1
Myelination (5.33×10-3)
Na+ ion transport (4.26×10-2)
G
Epilepsy
Control
UMAP.1
UMAP.2
H
vGLUT1 (Ex.)
vGLUT2 (Ex.)
vGAT (Inh.)
CUX2 (L2/3)
RORB (L4)
TLE4 (L5/6)
GFAP (Astrocyte)
MOBP (Oligodendrocyte)
I
-log10(FDR)
L2/3 neuron
Astro cyte
Up
Down
Neuron to neuron synapse
Glutamatergic synapse
Calcium ion complex
Voltage-gated ion channel activity
Action potential
Synapse organization
J
CL-MES
CL2
-log10(p-value)
Log2(ratio)
Non-epilepto genic
Epilepto genic
FN1
CD44
GBP1
EGFR
MIF
CHI3L1
AKAP12
ERRFI1
K
Epileptogenic GBM
Non-epileptogenic GBM
EGFRamp +
EGFRamp -

Supplemental Figure 3. Application of pre-trained weakly-supervised models to other independent single-cell datasets

A. Frequency of the inferred disease progressive cells in each cell type of Smajic *et al.* dataset. Error bars represent standard deviation. **B.** Comparison of (L) *CADPS2* and (R) *SNCA* expression across three groups that were classified by the inferred disease progressive level. **C.** Volcano plot showing differential expression in oligodendrocyte between PD patients and healthy aged donors in Smajic *et al.* dataset. **D.** Strategy to estimate Parkinson's disease progressive level by the pre-trained model in another scRNA-seq dataset from different brain area. **E.** Histogram of predicted score of disease progressive level and biological age. **F.** Heatmap showing genes, whose expression is highly correlated with inferred disease progressive score (Genes with Pearson correlation > 0.1). Oligodendrocyte cells are sorted by the disease progressive score. Heatmap color represents z-score-normalized gene expression. Representative genes and GO terms are shown in right panel. Statistical significance of GO terms is shown by false discovery rate. **G.** UMAP plot of single cells colored by epilepsy patients (orange) and healthy control donors (darkgreen). **H.** UMAP plot showing representative cell type-specific gene expression in epilepsy patients and healthy donors-derived scRNA-seq dataset. **I.** Representative GO terms overrepresented in differentially-expressed genes between epileptic and non-epileptic cells. Circle size represents $-\log_{10}$(FDR). **J.** Volcano plots showing differential expression between epileptogenic and non-epileptogenic GBM cells in CL-MES and CL2 cluster. Significantly-upregulated ERK1/2 cascade-related genes are also shown. **K.** Pie chart showing ratio of cells with EGFR amplification in the inferred epileptogenic and non-epileptogenic GBM cells.



Supplemental Figure 4. Inference of CD4⁺ and CD8⁺ CAR-T cell activation dynamics to neuroblastoma

A-B. UMAP plots of **(A)** CD4⁺ and **(B)** CD8⁺ individual CAR-T cells colored by the stimulation conditions (top left), cell proliferation-related gene expression (top right), the inferred antitumor activity (bottom left), and pseudotime (bottom right). **C.** Heatmap showing 26 surface protein expression dynamics in CD4⁺ and CD8⁺ CAR-T cells. CAR-T cells were sorted by (left) scIDST or (right) pseudotime. Heatmap color represents z-score-normalized protein expression.