Use of primary conjugated antibodies to model the heterogeneity of GBM's
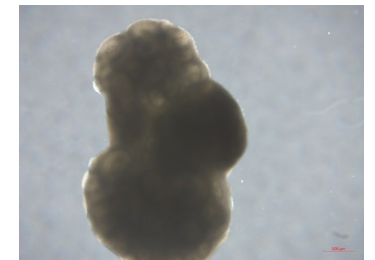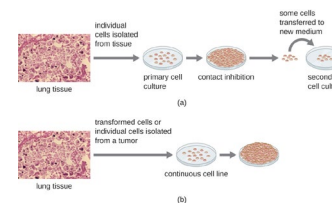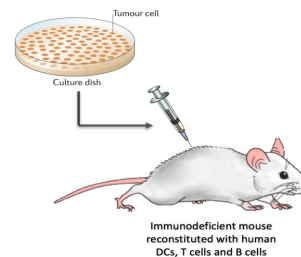
Ryan Gensler

NSSSP 2019
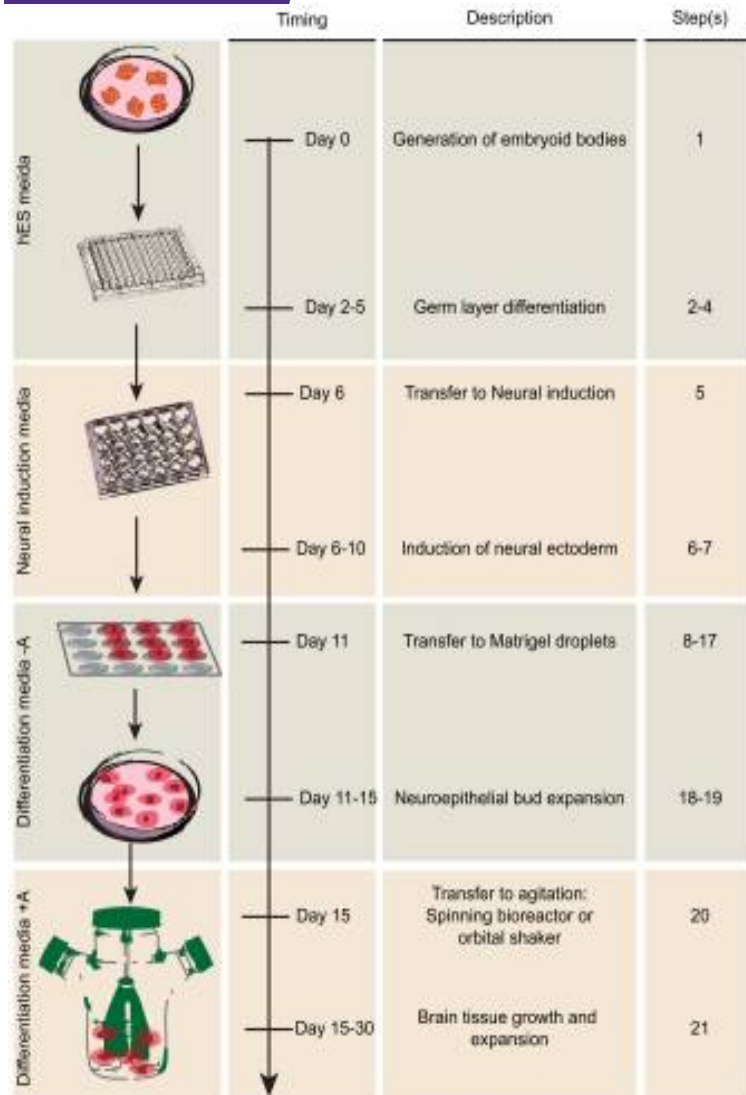
UNIVERSITY *of* WASHINGTON

3 Models for GBM

Model Type	Xenografts	Cell Lines	Organoids
Pros	• Heterogeneity • 3D morphology • In vivo	• Cheap • Grow quickly	• Heterogeneity • Cheap • 3D morphology • Act as patient avatars
Cons	• Expensive • Slow tumor growth	• Lack heterogeneity • In vitro	• In vitro • Not yet verified

Tumour cell
Culture dish
Immunodeficient mouse reconstituted with human DCs, T cells and B cells

individual cells isolated from tissue
lung tissue
primary cell culture
contact inhibition
some cells transferred to new medium
secondary cell culture
(a)

transformed cells or individual cells isolated from a tumor
lung tissue
continuous cell line
(b)

What/How are organoids grown?

Timing	Description	Step(s)
Day 0	Generation of embryoid bodies	1
Day 2-5	Germ layer differentiation	2-4
Day 6	Transfer to Neural induction	5
Day 6-10	Induction of neural ectoderm	6-7
Day 11	Transfer to Matrigel droplets	8-17
Day 11-15	Neuroepithelial bud expansion	18-19
Day 15	Transfer to agitation: Spinning bioreactor or orbital shaker	20
Day 15-30	Brain tissue growth and expansion	21

Lancaster and Knoblich, Nat Protoc 2014

➢ **Organoid Morphology:**
  > **3D**
  > **Populated by neuronal, glial, and NSCs**
  > **Organized tissue with temporal patterning**

➢ **Start from ESCs and correct factors cause neural differentiation**

➢ **At day 30, inject tumor cells into the organoid**

➢ **Day 60-90: Organoid sectioned, stained, imaged**

Aim of Project

- **Create primary conjugated antibodies**

- **Use these 1° conjugated antibodies to avoid the lengthy TSA-IHC protocol**

- **Co-stain tissue**

- **Use these co-stains to understand the heterogeneity of the GBMs**
  - > **I.e. what proteins GBMs are expressing for a specific patient tumor**

- **Big Picture: Prove that GBM injected tumors cells are initiating GBM growth in organoid models**

- **Compare organoid models to heterogeneity of the patient tumor**

Methods

Methods

Staining via TSA-IHC

Co-staining via conj. 1º antibodies

Overnight with only one unconjugated 1º antibody

Overnight with multiple conj. 1º antibodies

1º now conj. with fluorescent 2º antibody

Final Product ready for imaging

Signal from 2º antibody is now strengthened
Easy to see under scope

Provides info on tumor heterogeneity

Final Product ready for imaging

TSA-IHC   Dapi

GFP   Ptprz 1

Dapi   GFP
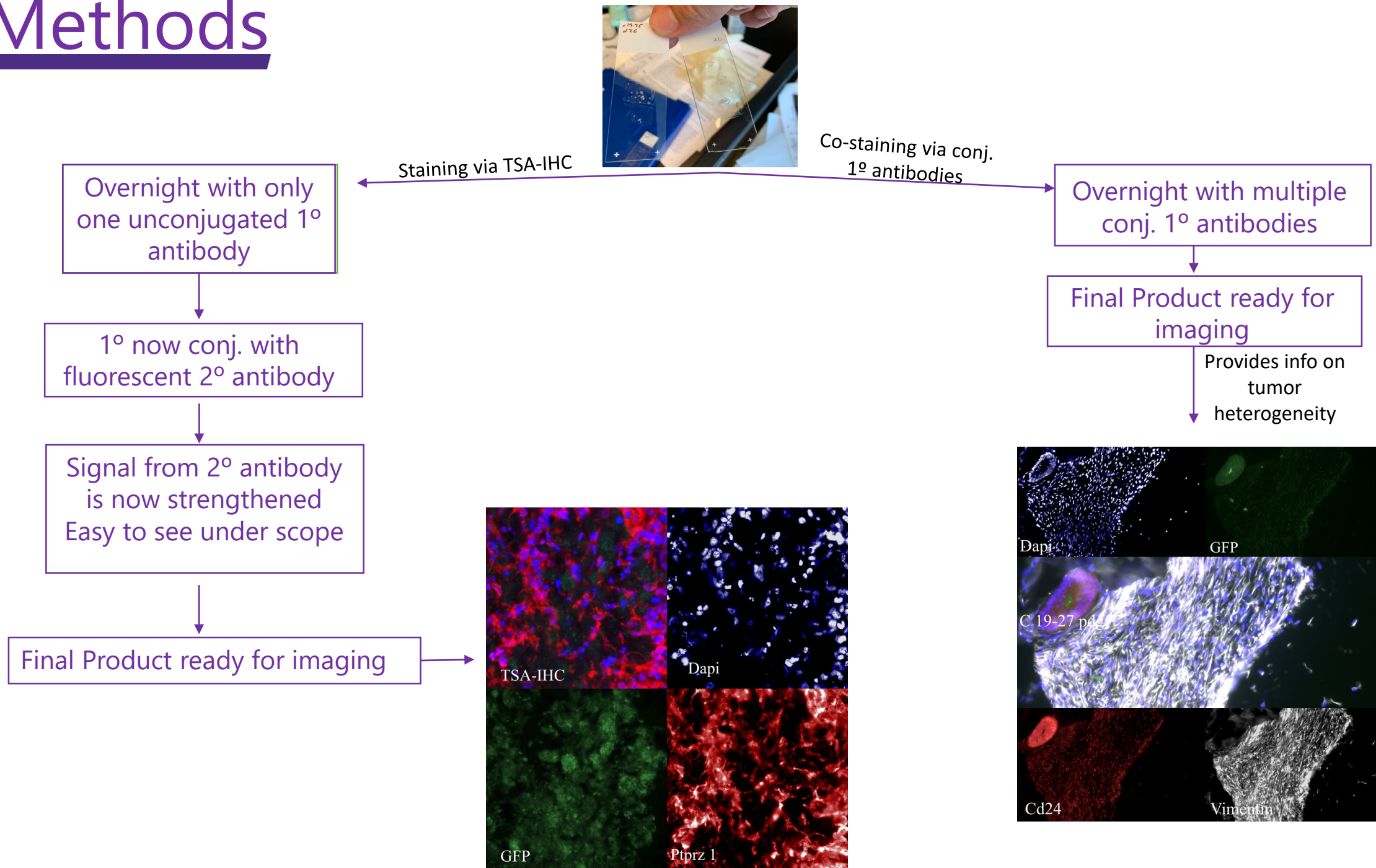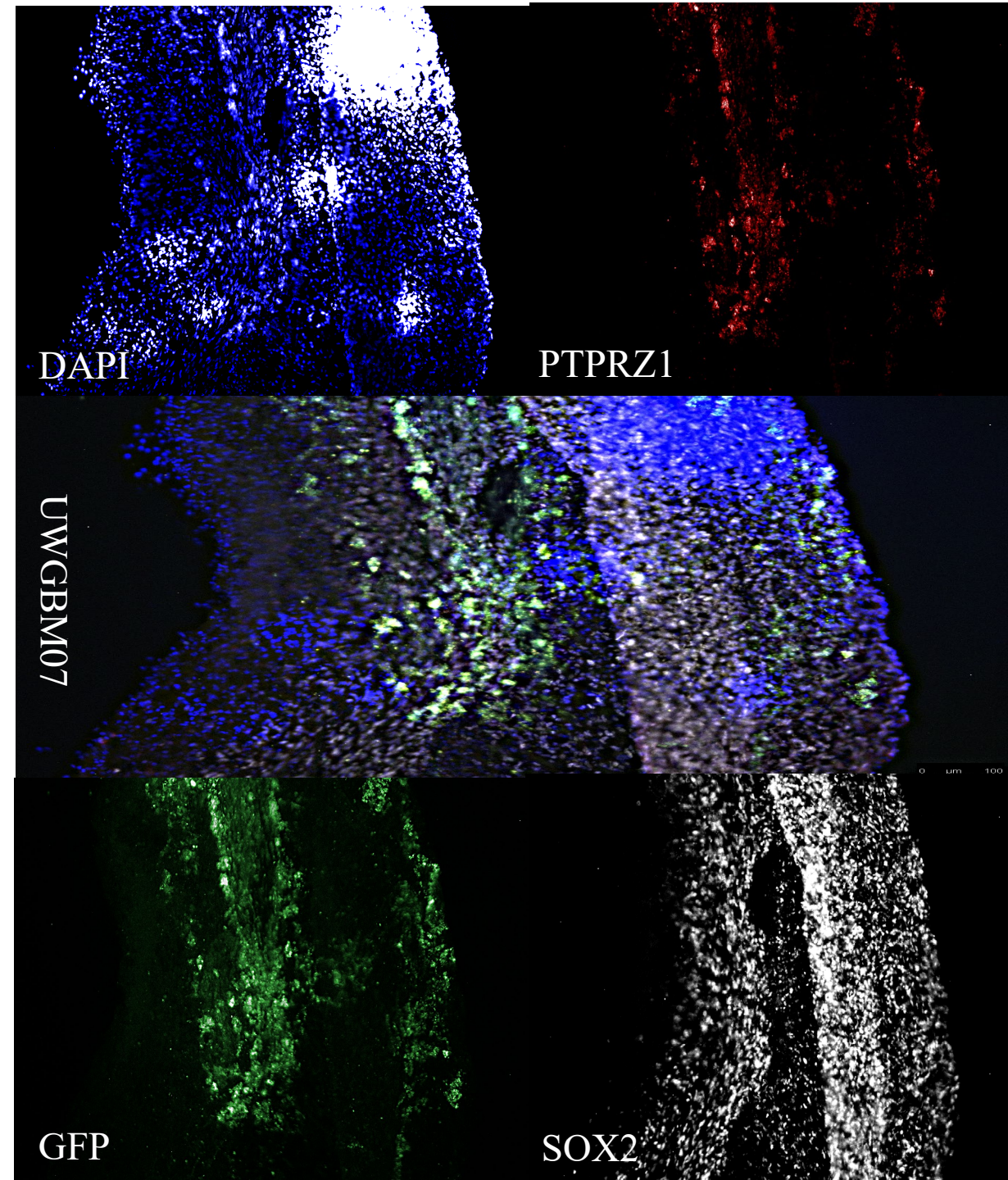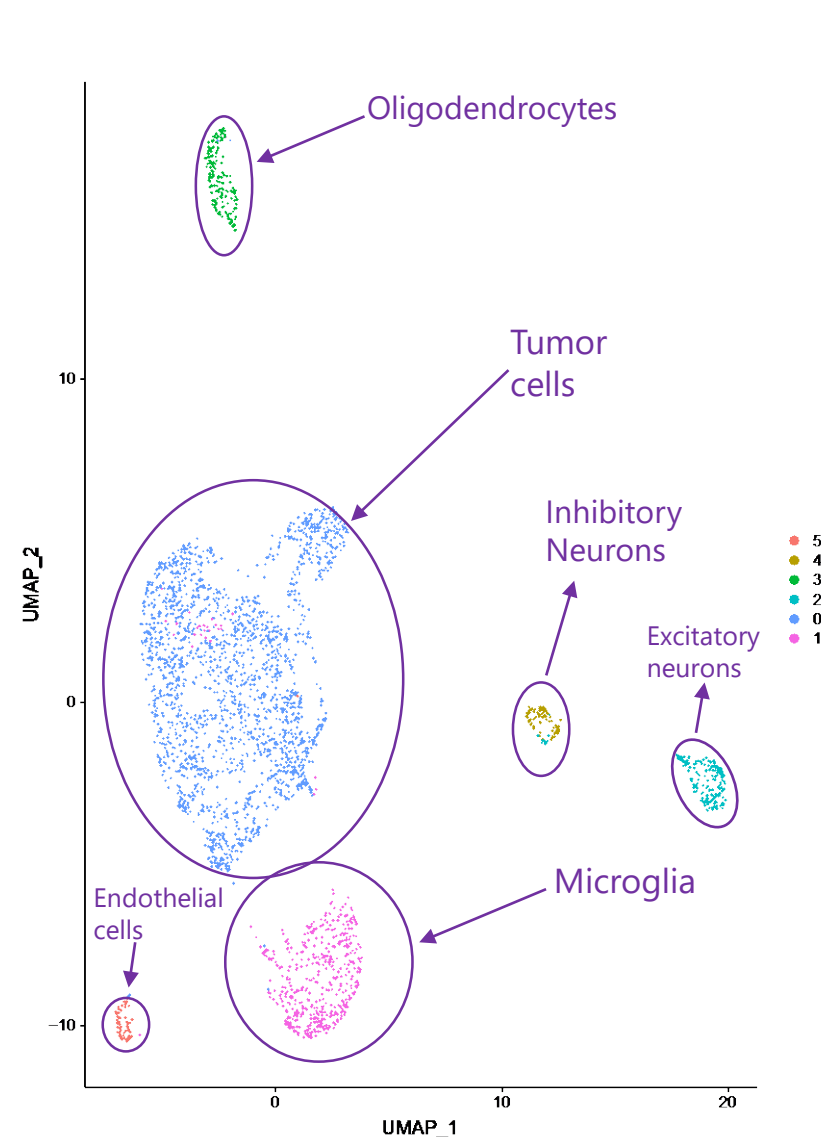
C 19-27 pdg

Cd24   Vimentin
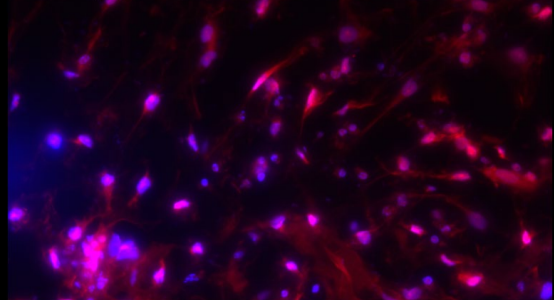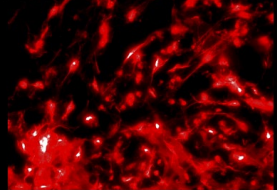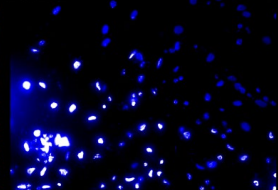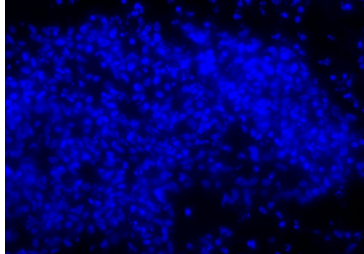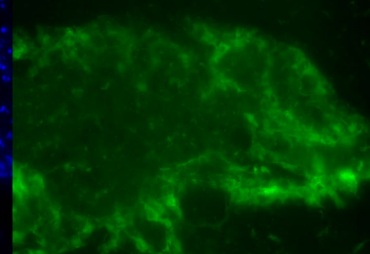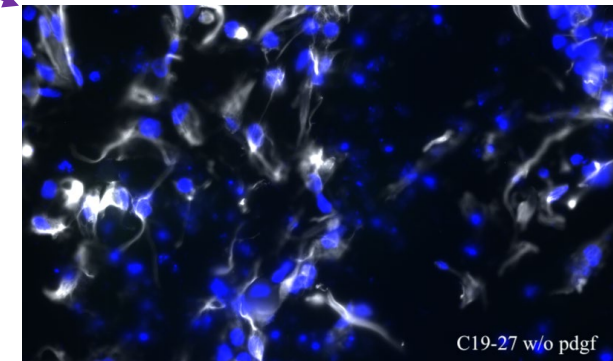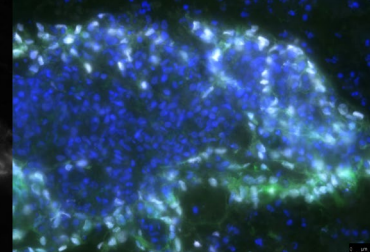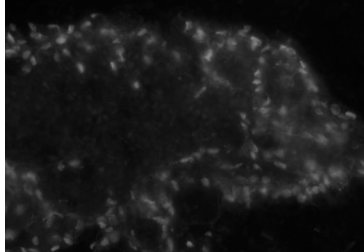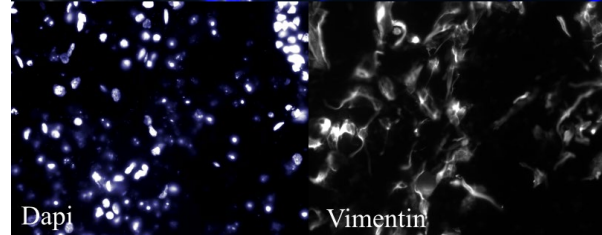
Proving Tumor Cells are in Organoids

- Validated staining protocol

- Stained for all tumor cells (SOX2)

- Would expect organoids to be sox 2 and ptprz1 , but not GFP

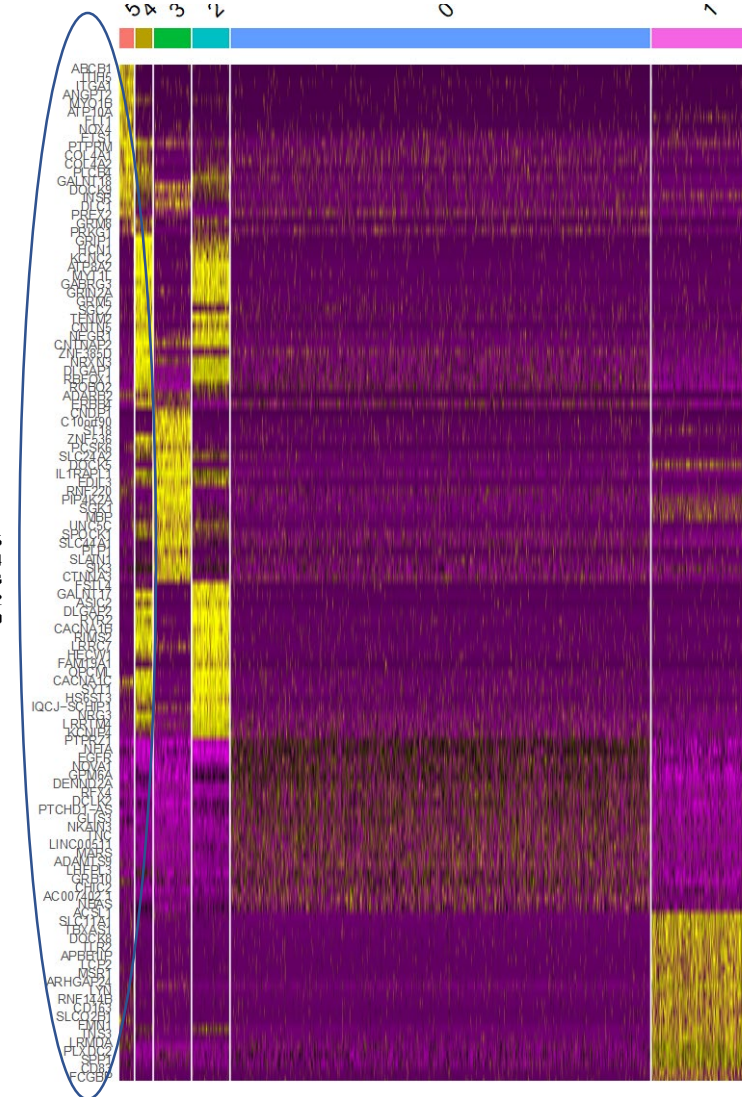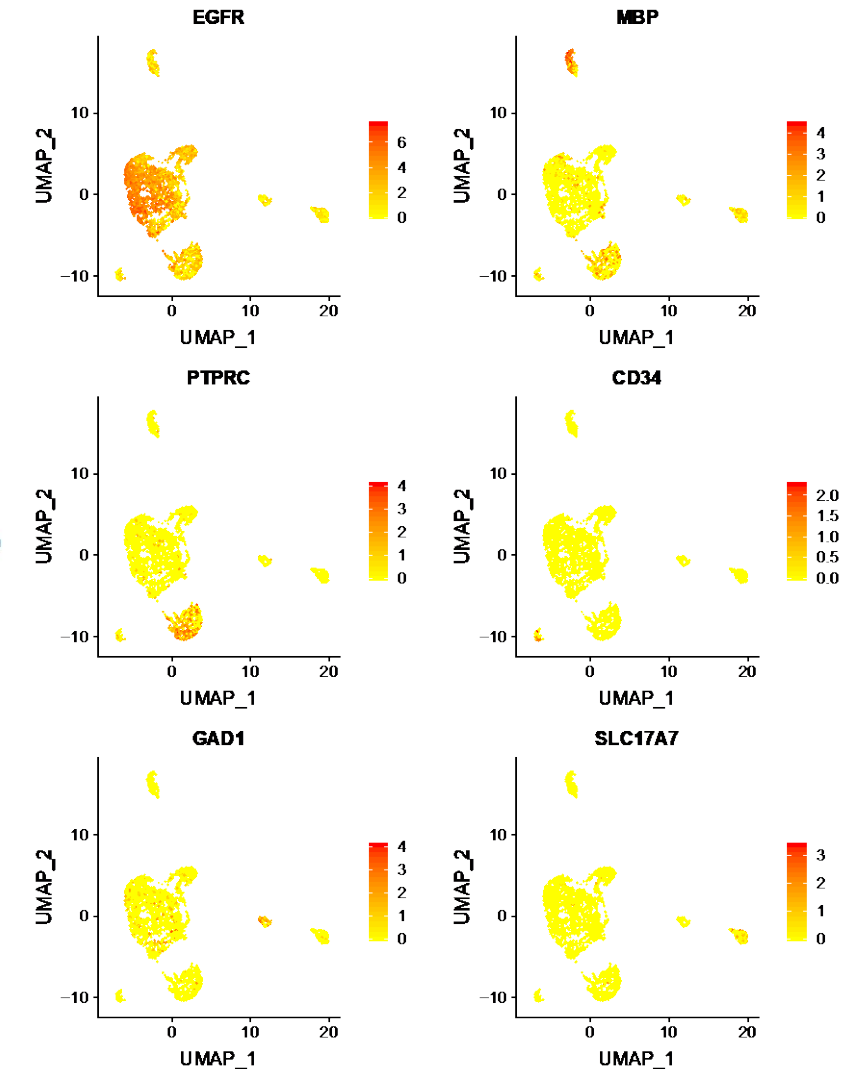- Next step: Compare heterogeneity patient tumor to organoid via scRNA-seq
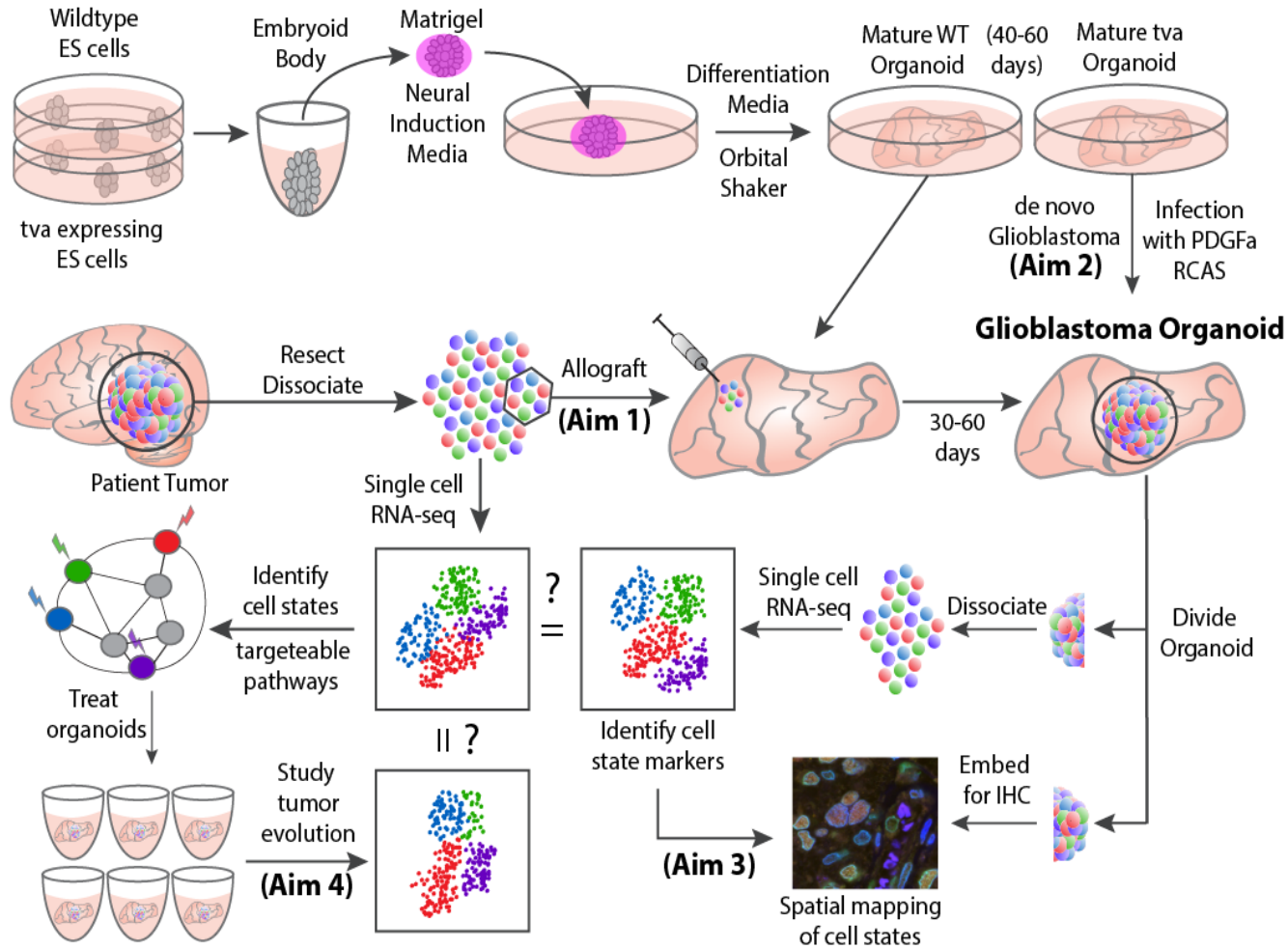
Comparing sc-RNAseq to Stains

Umap graph shows clustering of similar cells.

Heat map showing groups of cells that express similar genes

Future Ventures

Figure by Anoop Patel

Goals:

1. Improve allograft injection method of organoid with human GBM cells
2. Use FISH to ultimately identify human tissue in organoids
3. Use scRNA-Seq to sequence organoids to prove model viability
4. Continue to use staining method to visualize heterogeneity of GBMs embedded in organoids

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