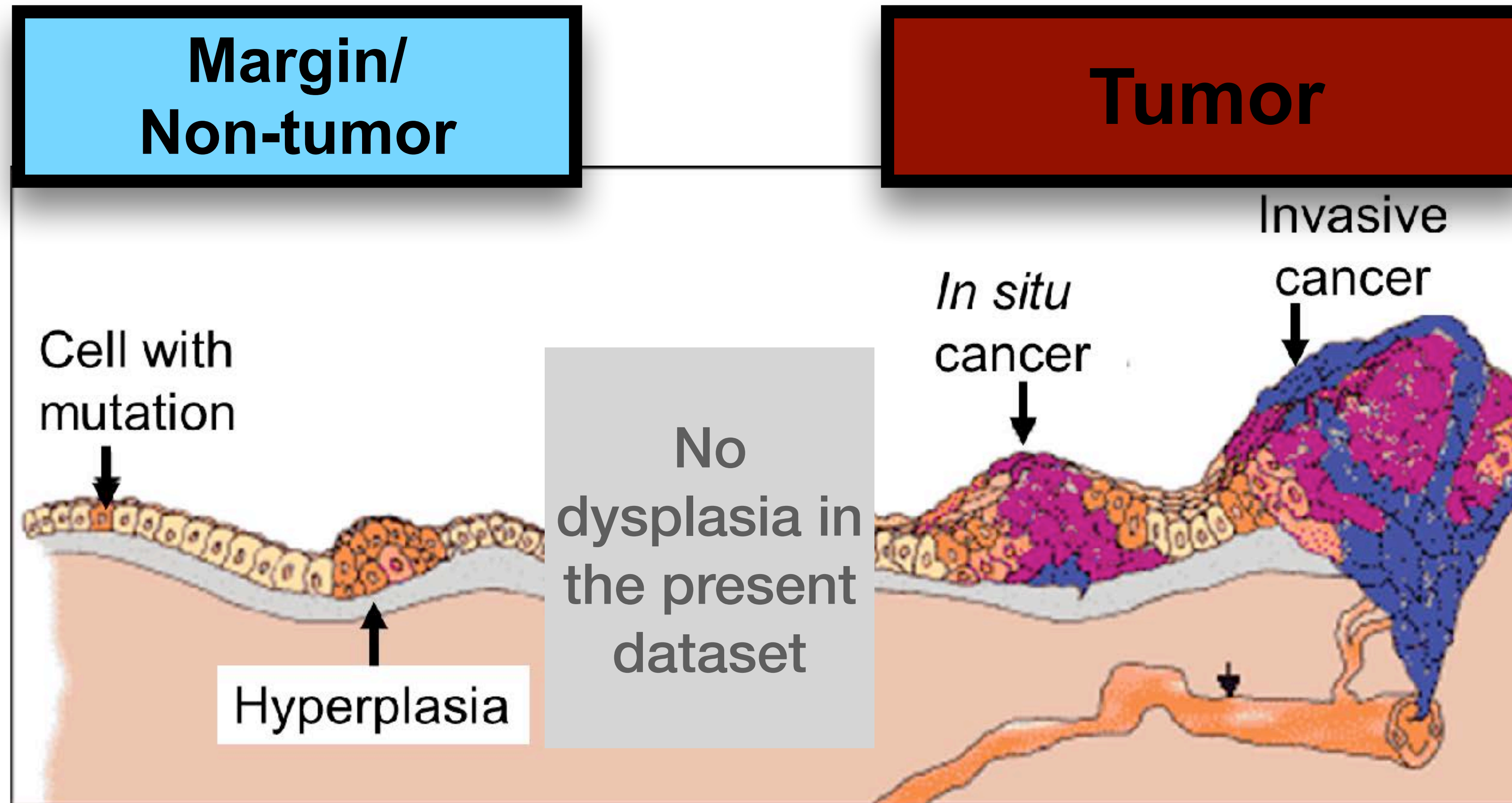


PARPi-FL staining of fresh biopsy tissues for identification of tumor and margin tissue

Blinded study

Training set

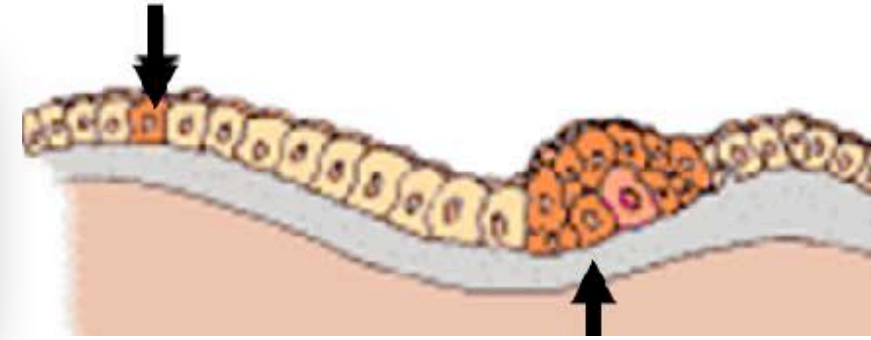
Oral cancer - basic histology



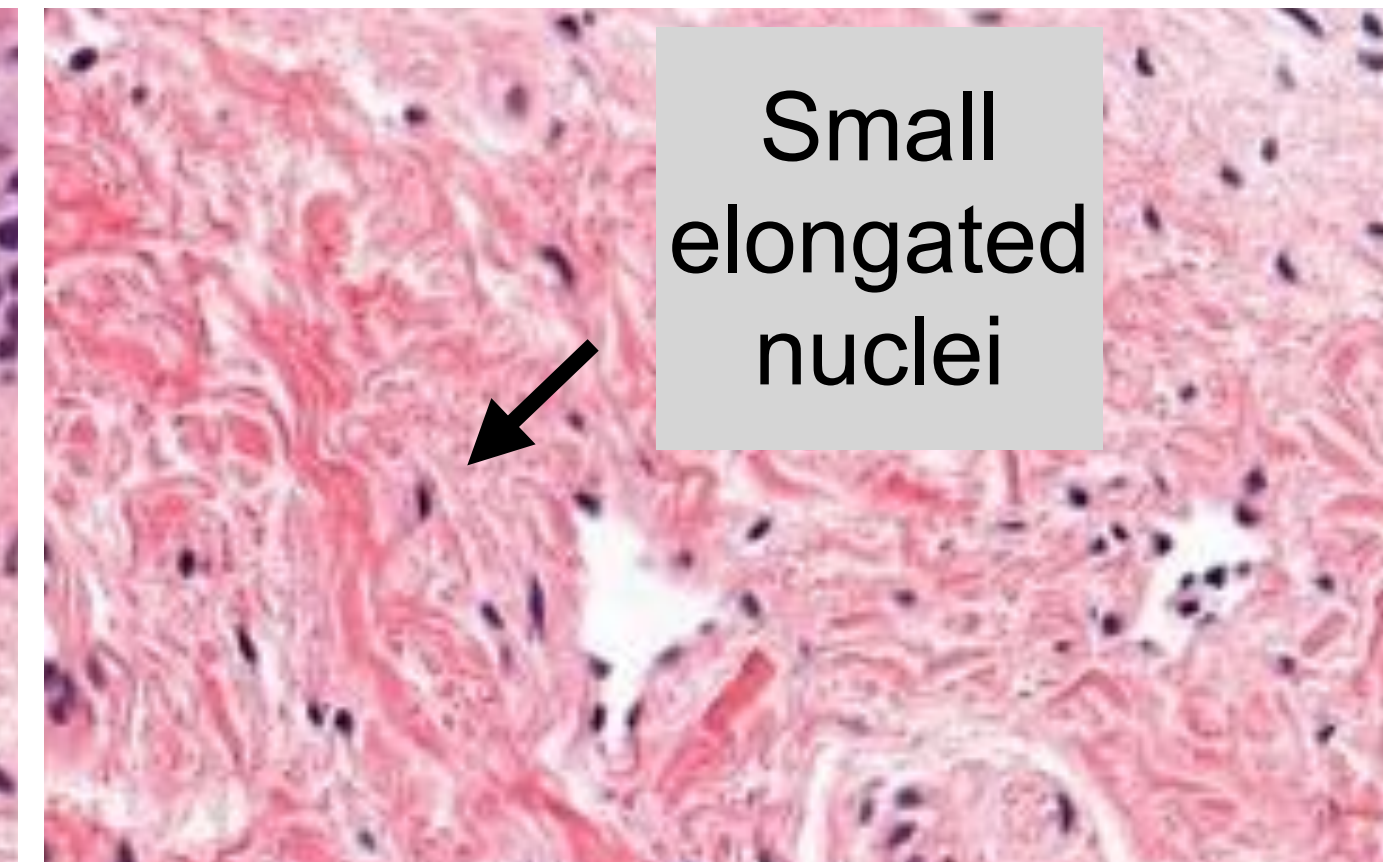
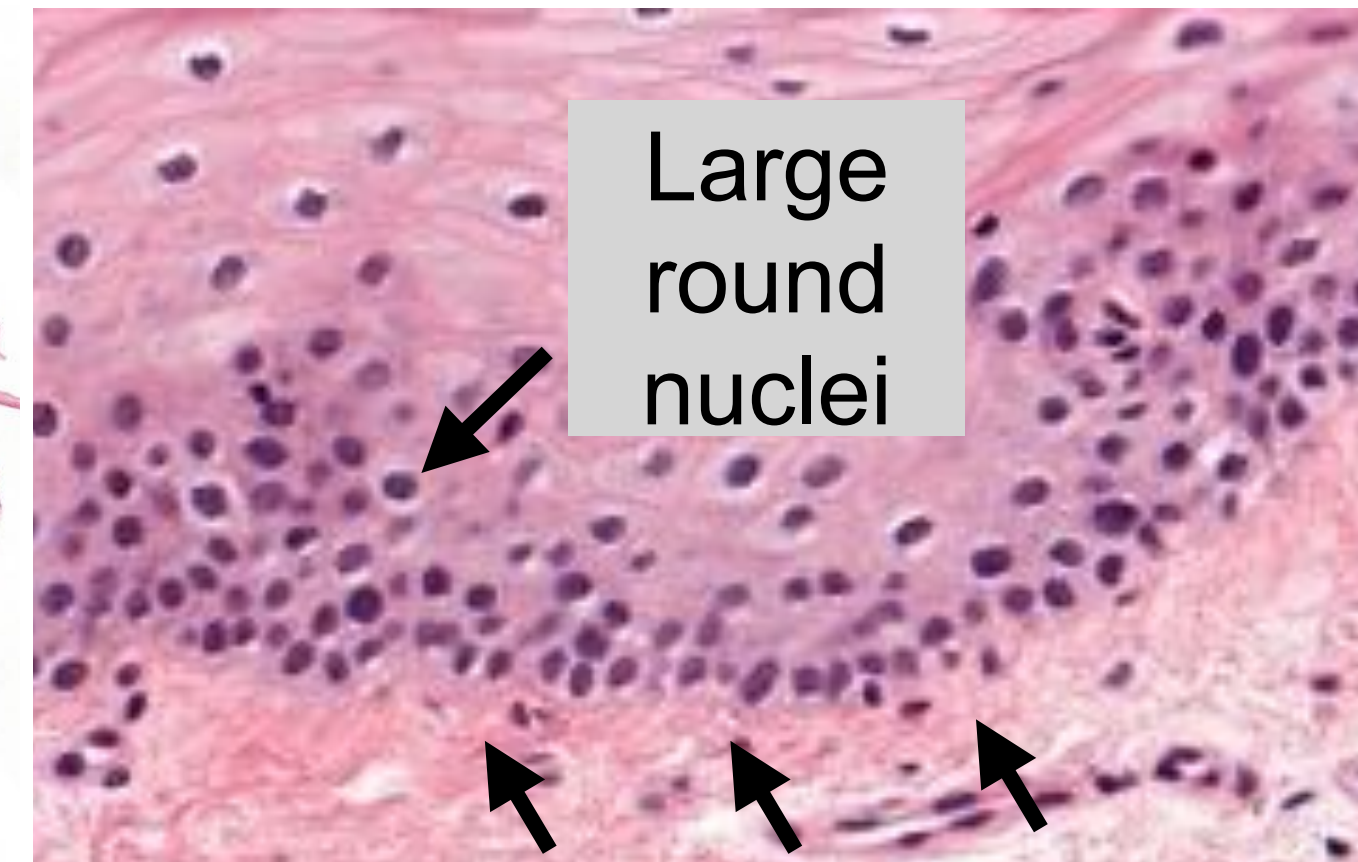
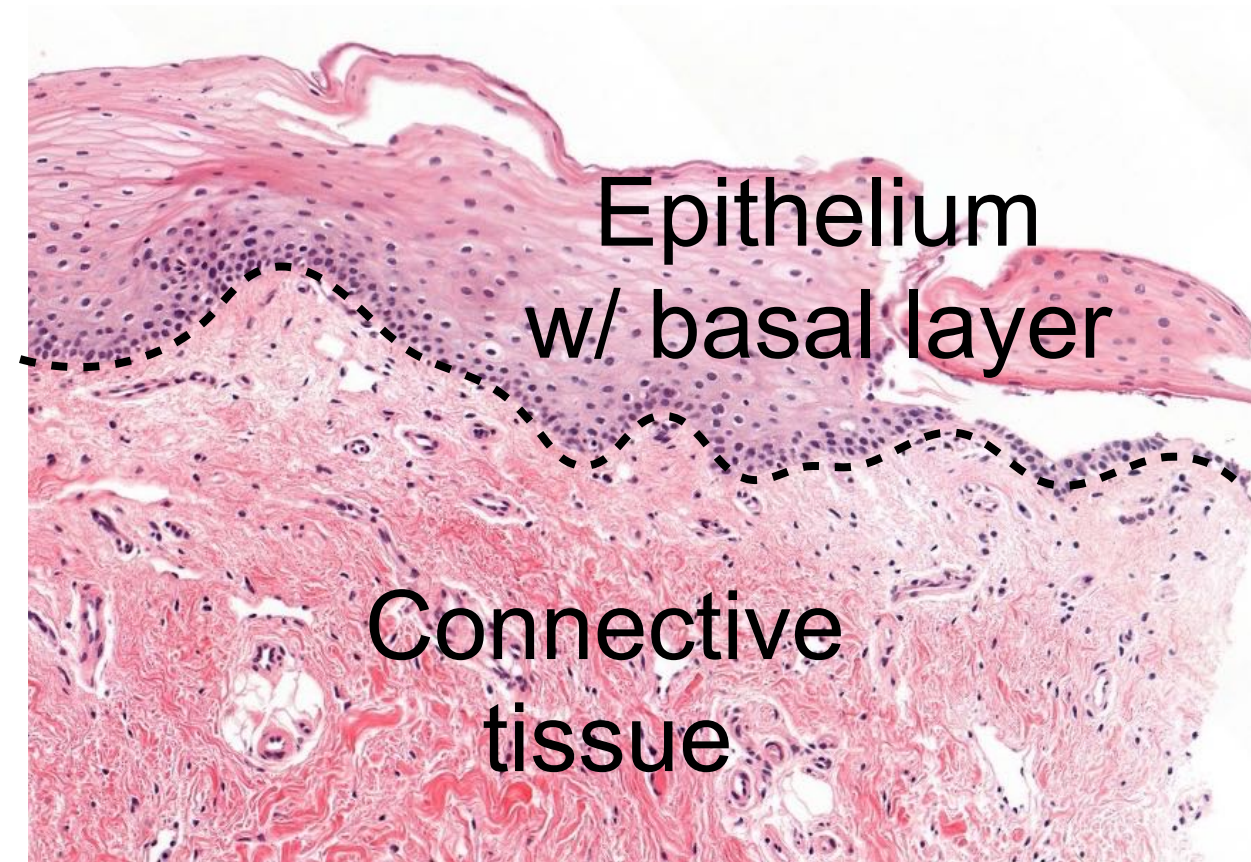
Goal: Identify tissues as tumor or margin based on confocal microscopy images (after staining with our fluorescent marker PARPi-FL)

➡ what are the features that define tumor vs. margin tissues?

Margin [1]

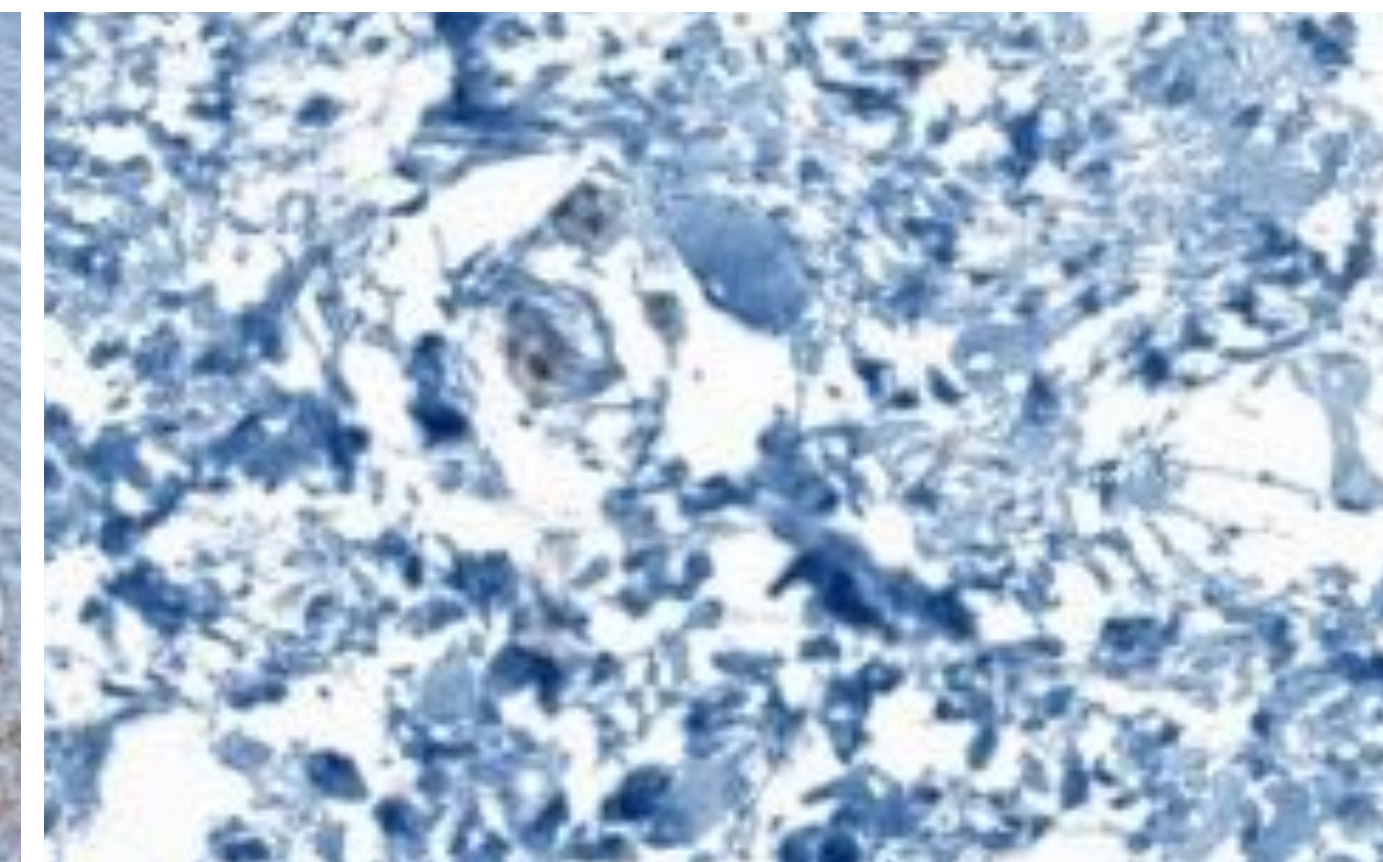
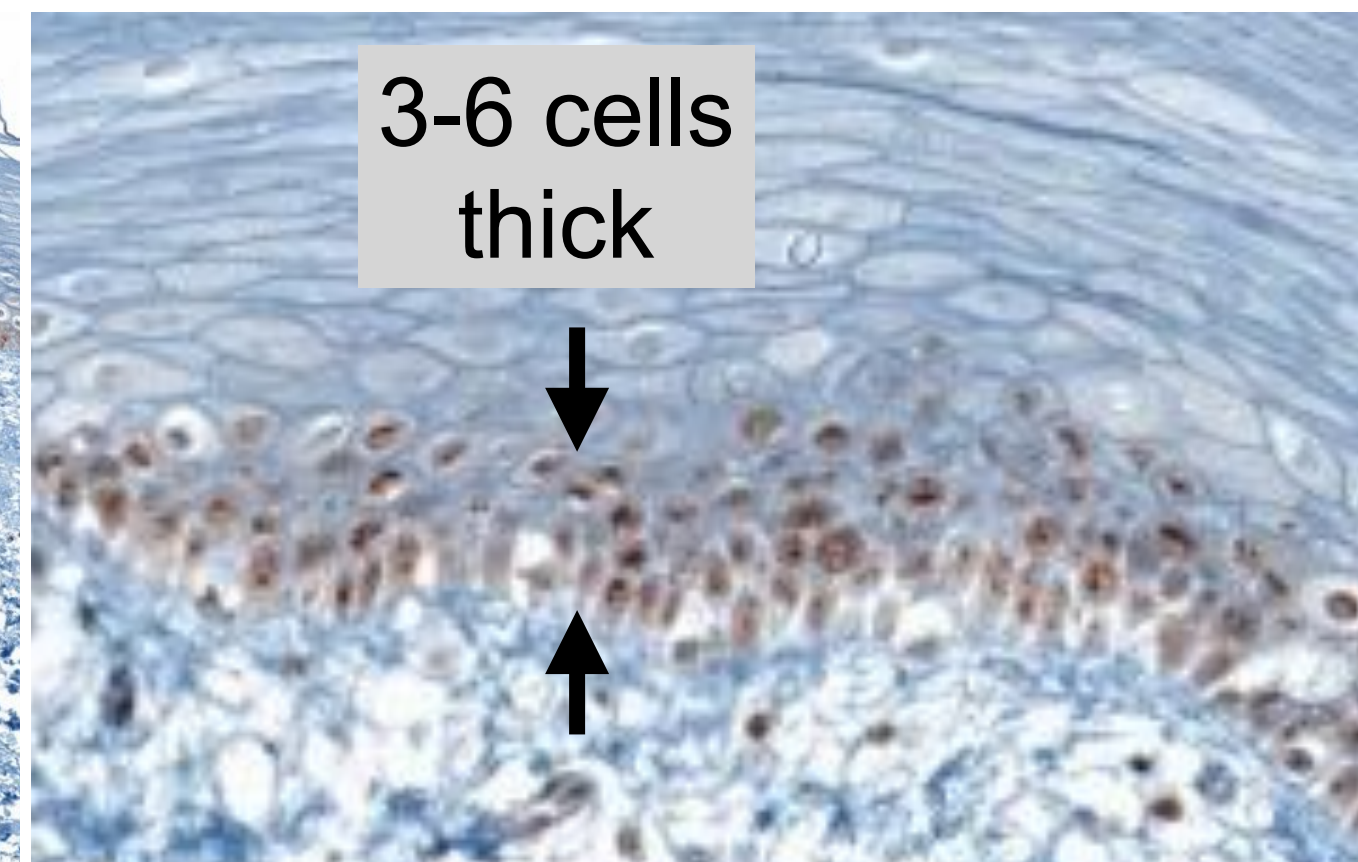
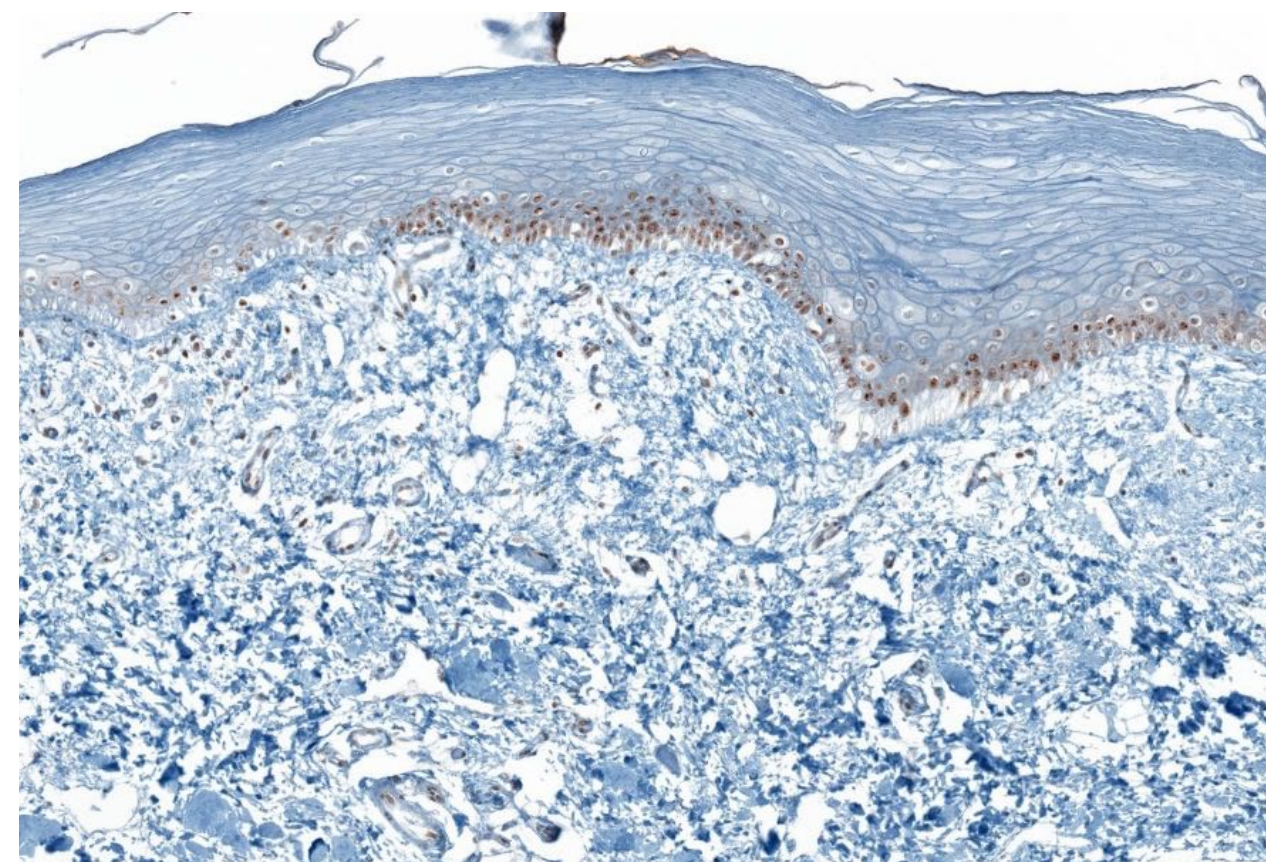


Morphological
stain (H&E)



PARP1 stain

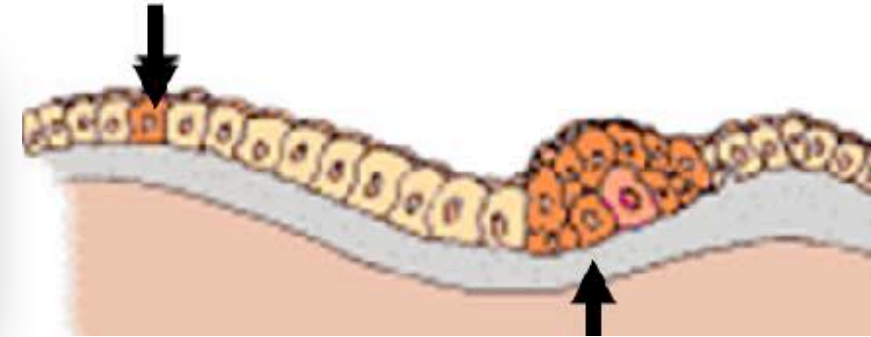
[PARP1 = brown
staining]



- PARP1 in basal layer
- PARP1 in large, round nuclei
- Thin layer of cells (3-6 cells)
- straight or arched line

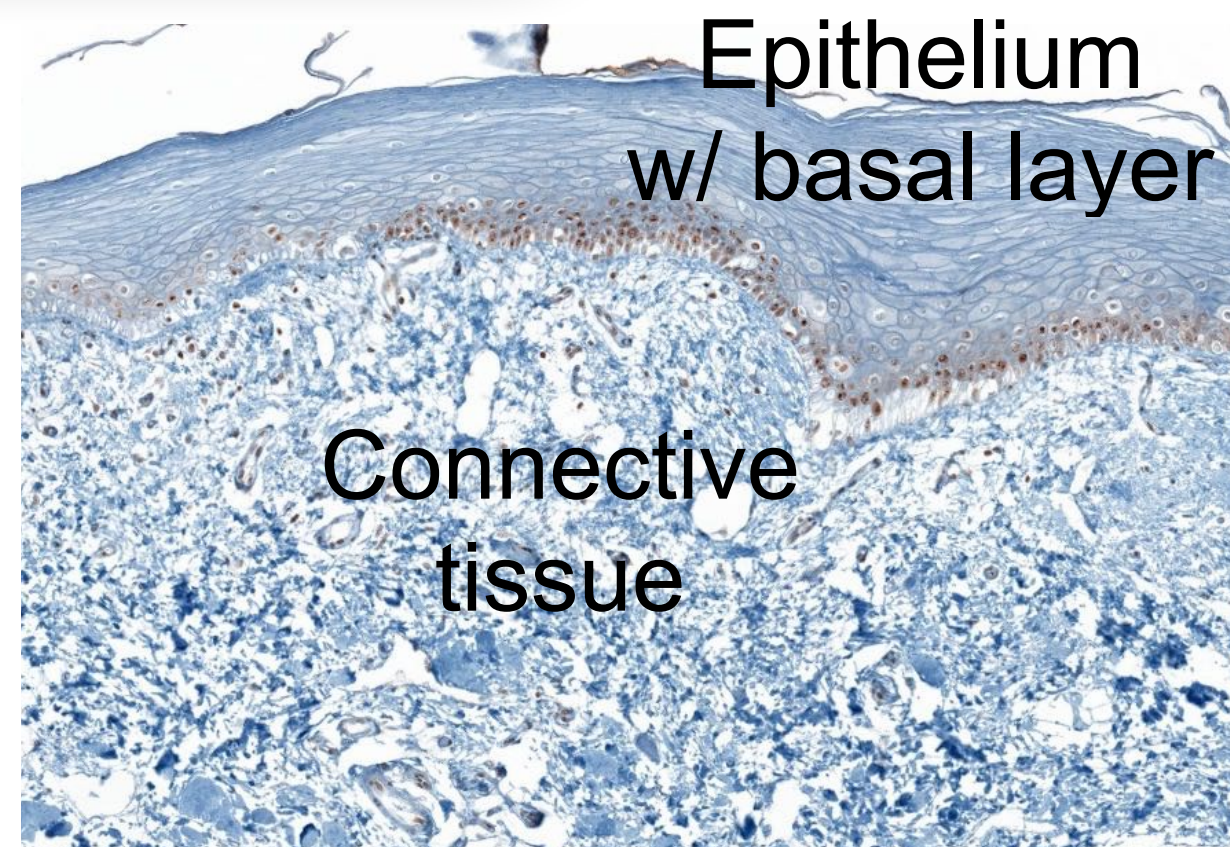
- Nuclei smaller and elongated compared to epidermis

Margin [2]



PARP1 stain

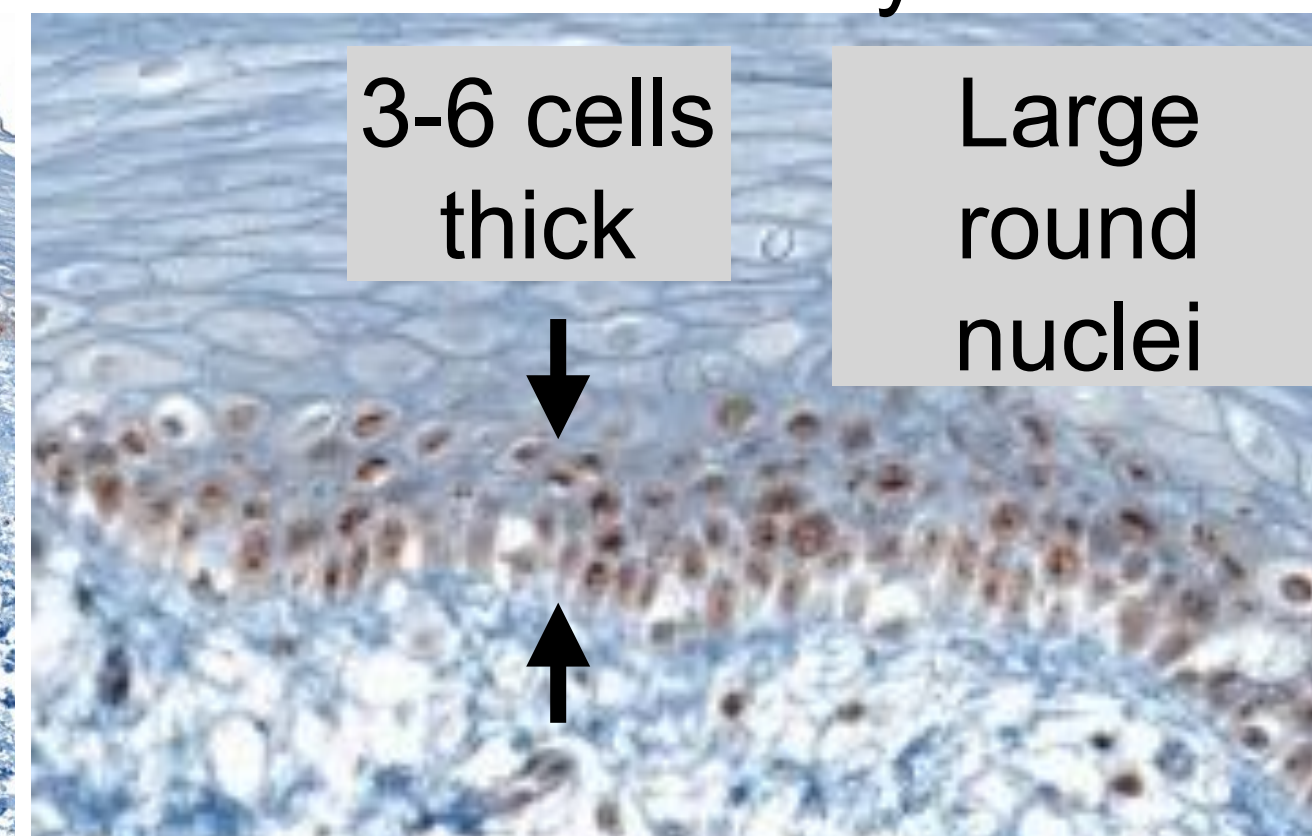
[PARP1 = brown staining]



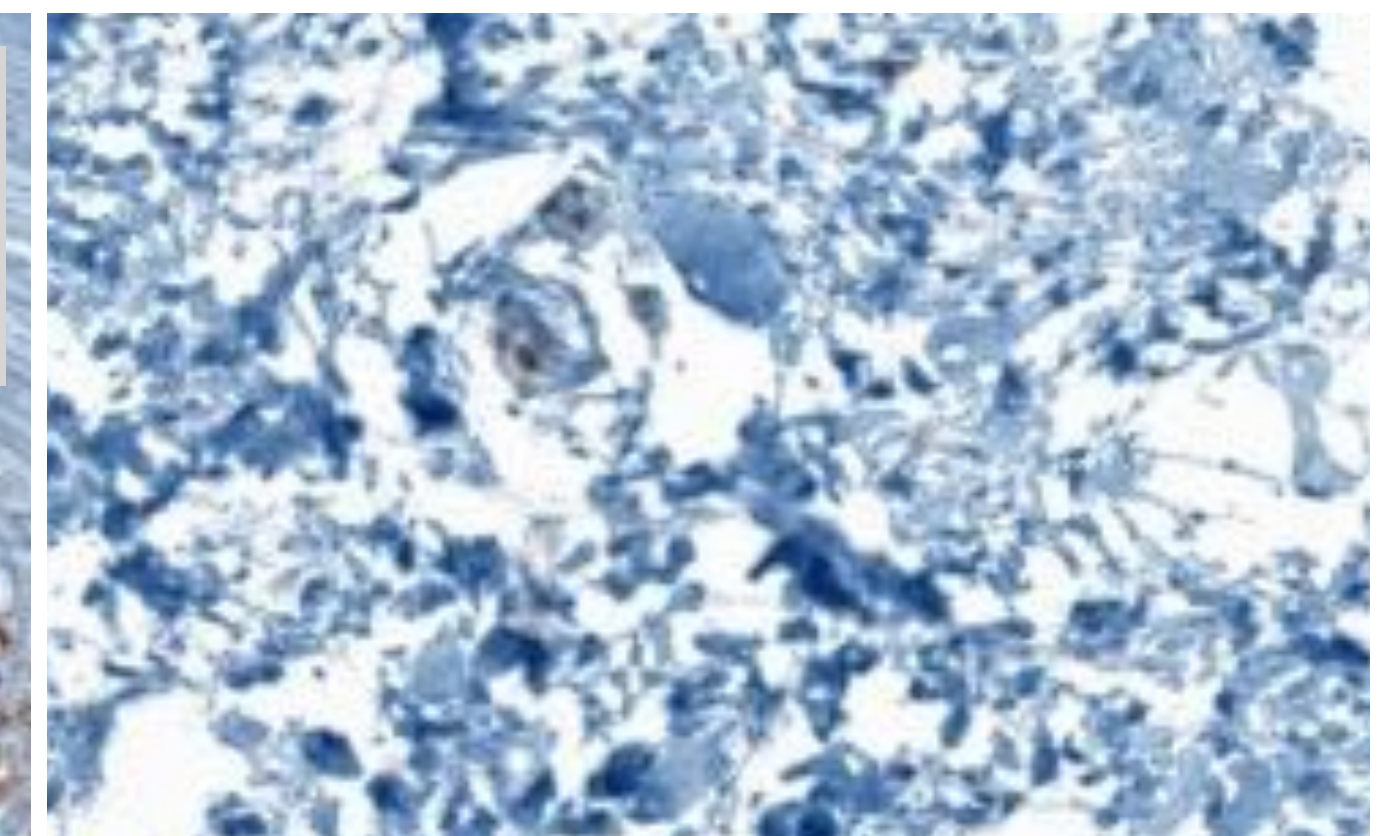
Basal layer

3-6 cells thick

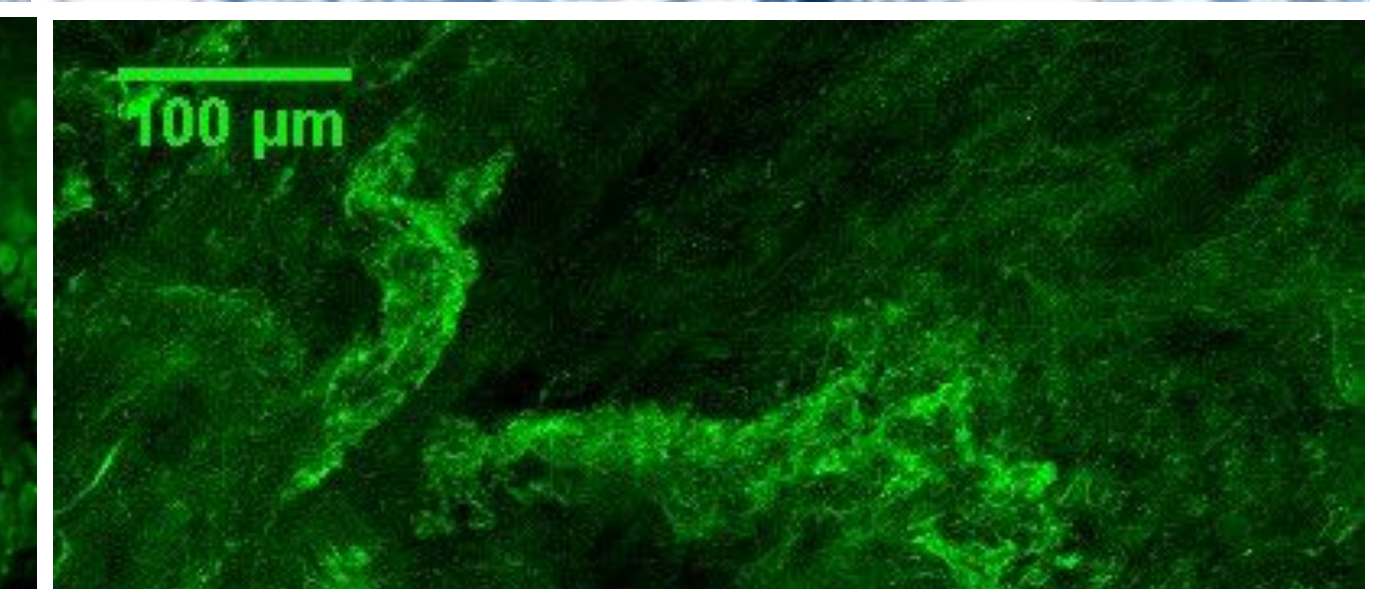
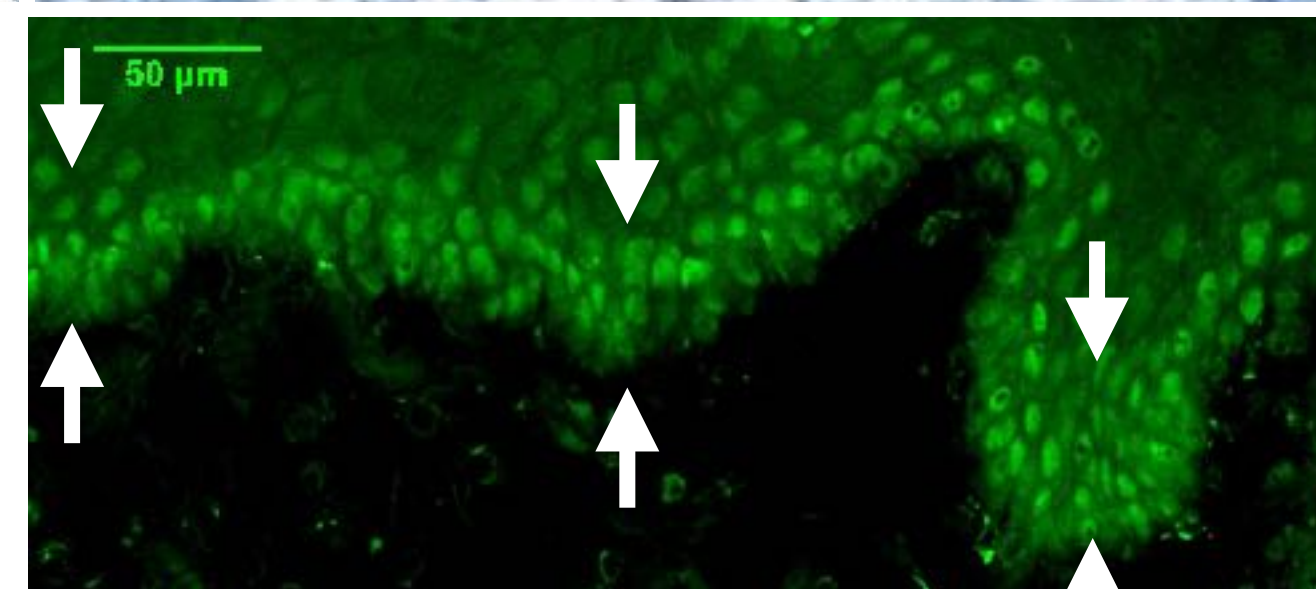
Large round nuclei



Connective tissue



PARPi-FL



- ▶ PARP-FL related fluorescence confined to basal layer
- ▶ Fluorescence in large, round cell nuclei
- ▶ Straight or arched basal layer

- ▶ Connective tissue can have quite high autofluorescence (AF)
- ▶ AF is flat, uniform fluorescence with sometime very bright streaks
- ▶ No round shapes/cell nuclei!
- ▶ Not indicative if tissue is tumor or margin!

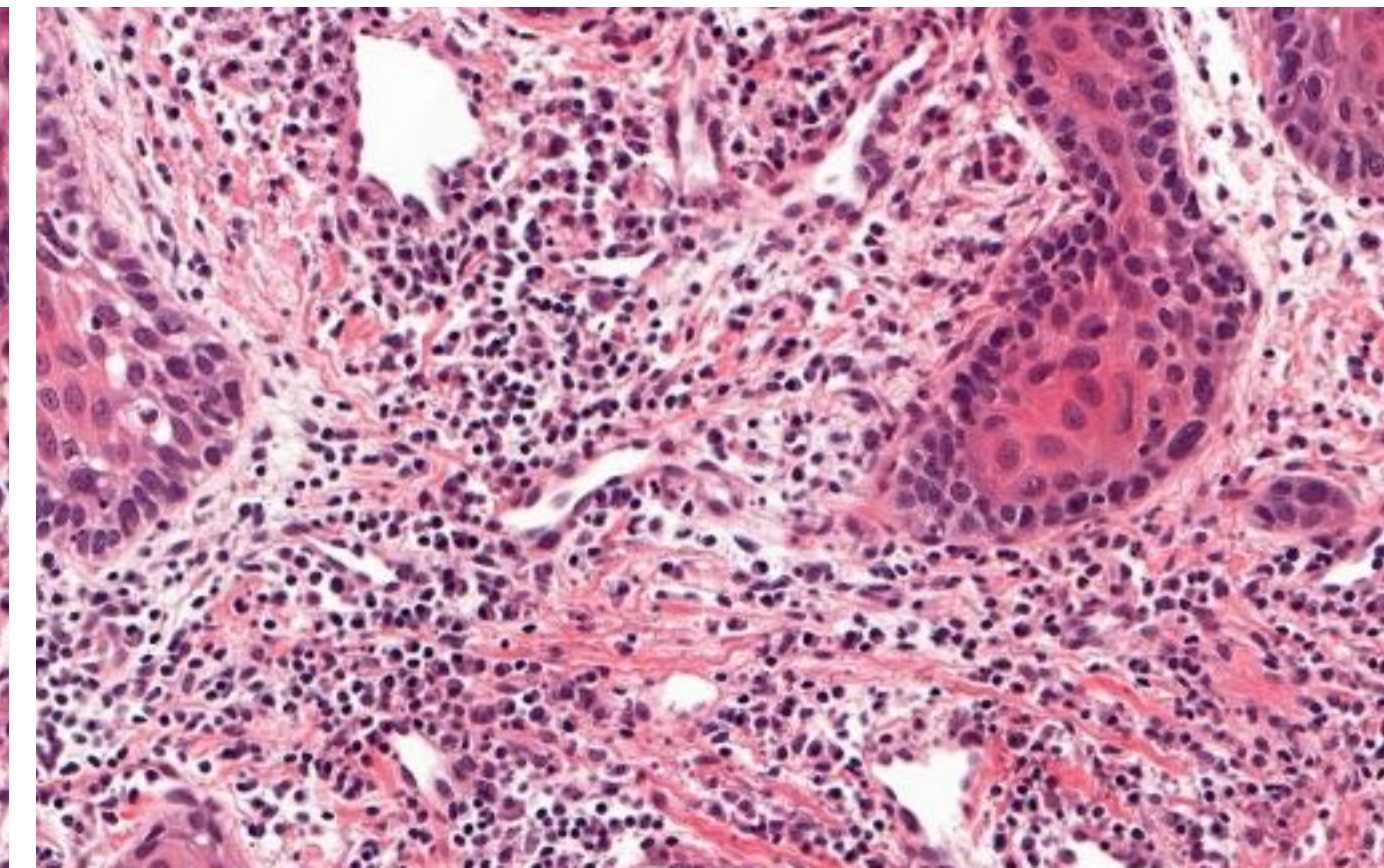
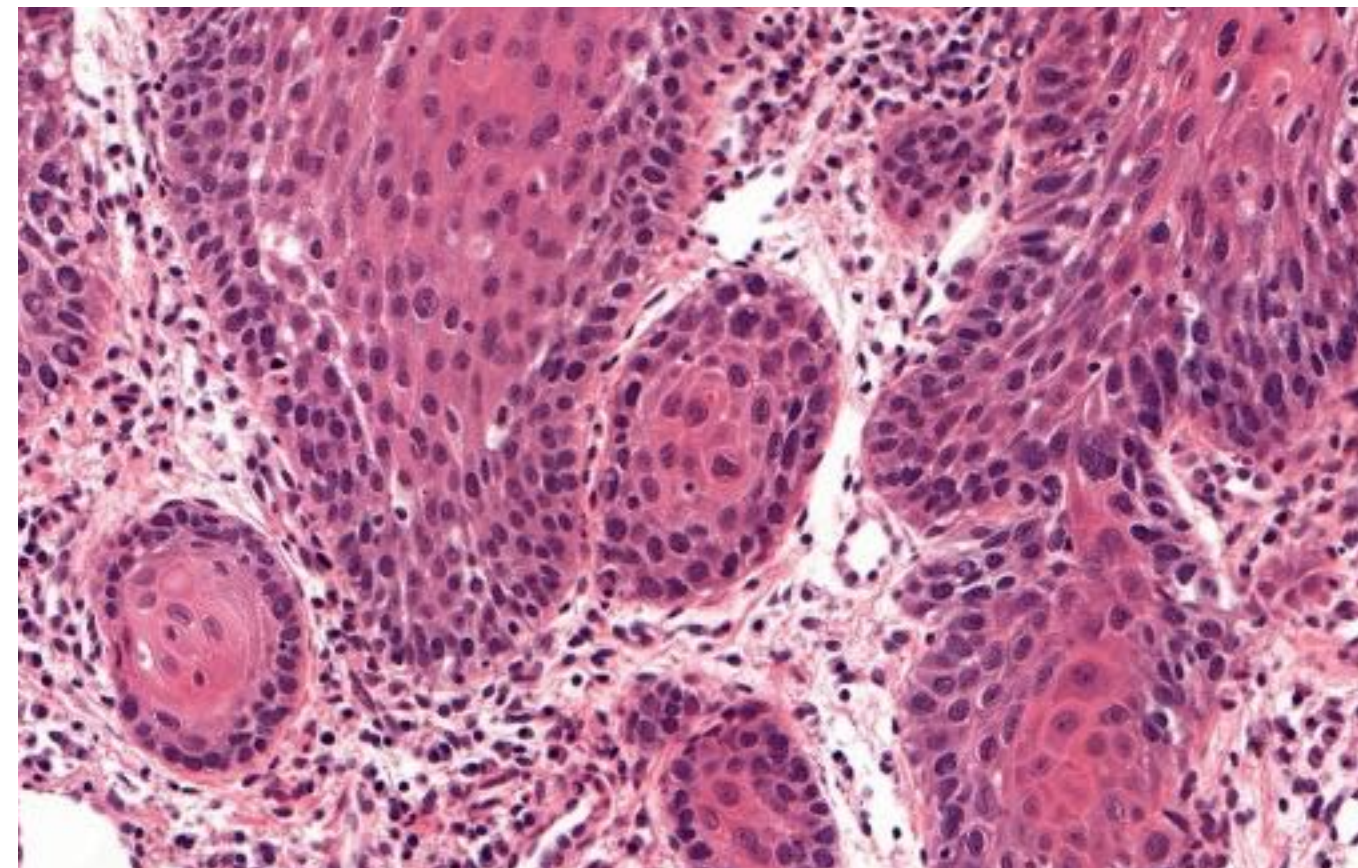
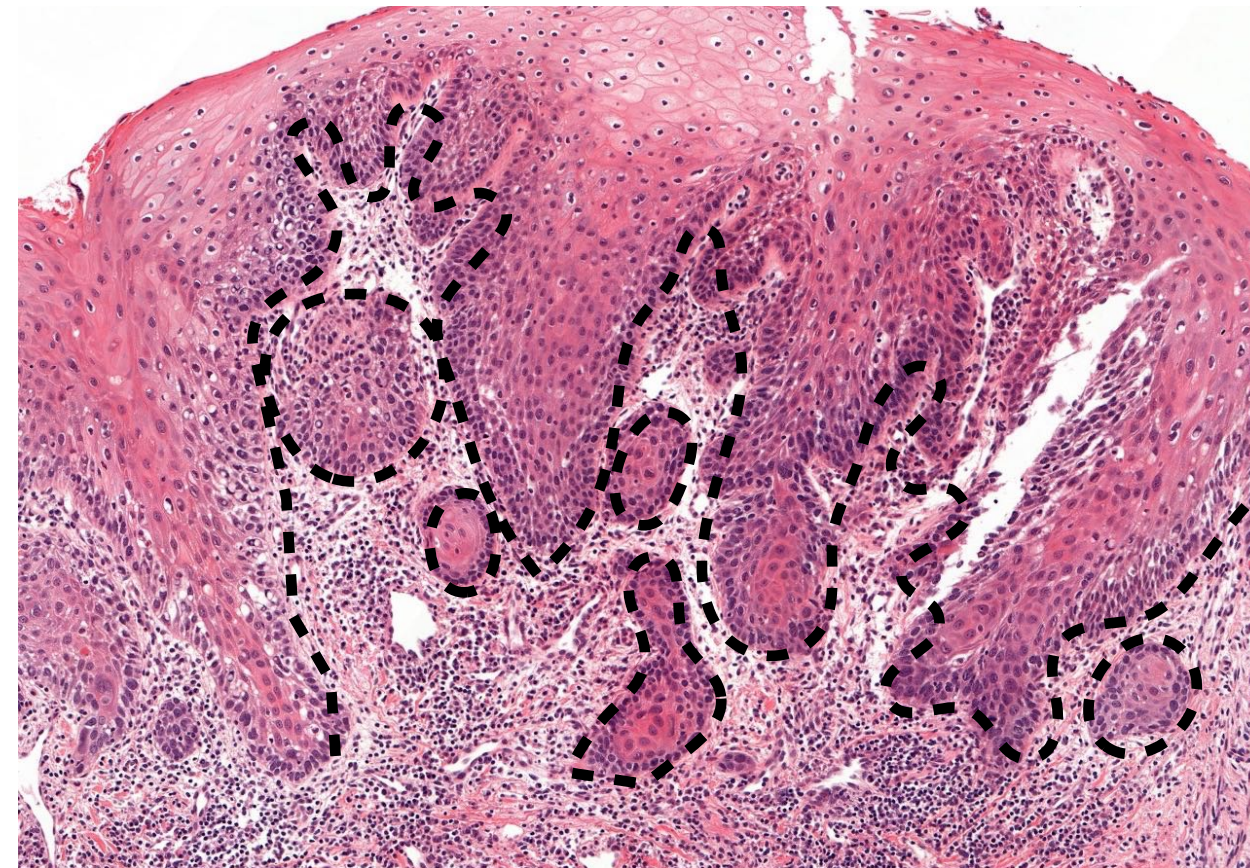
Tumor



Basal layer

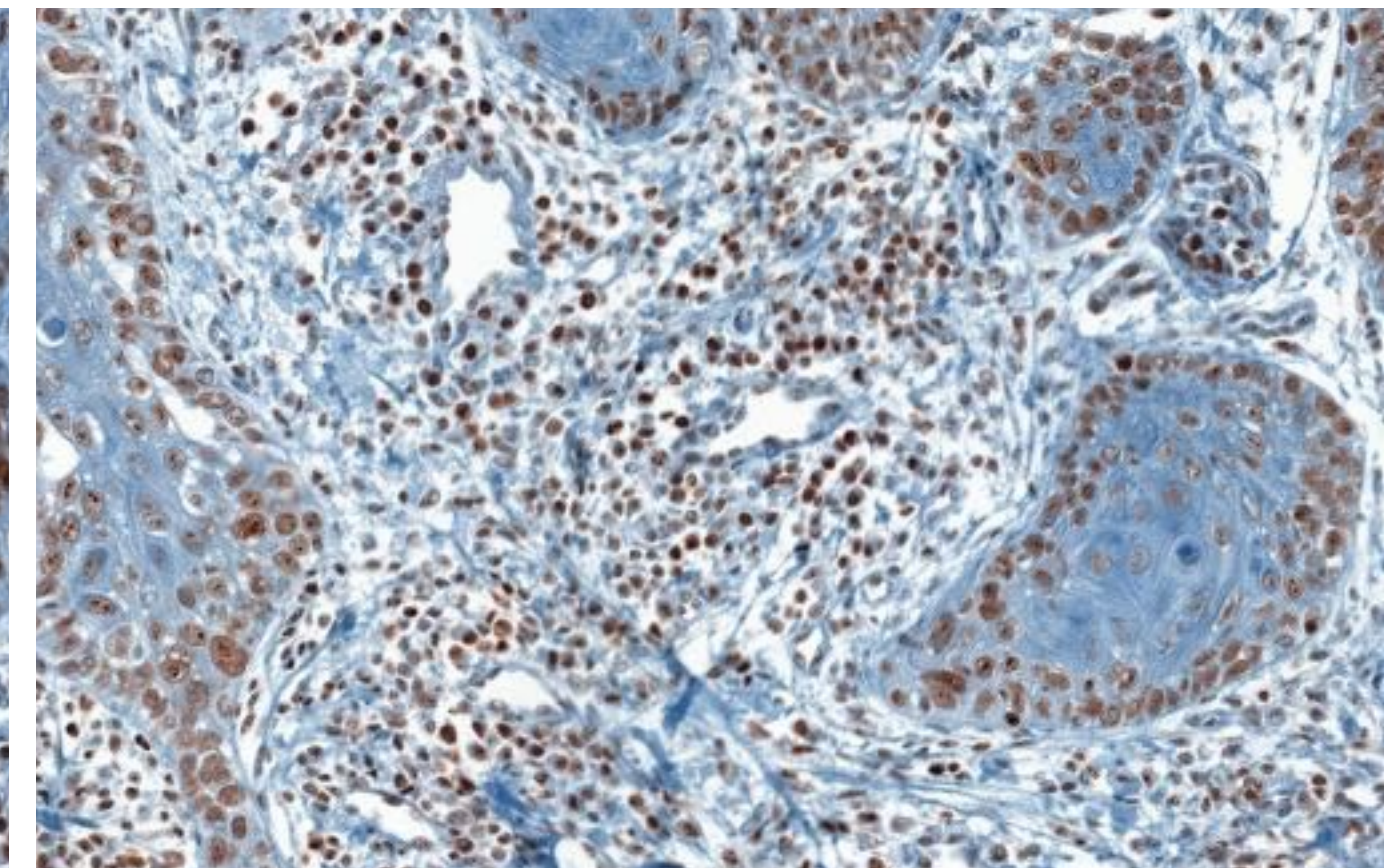
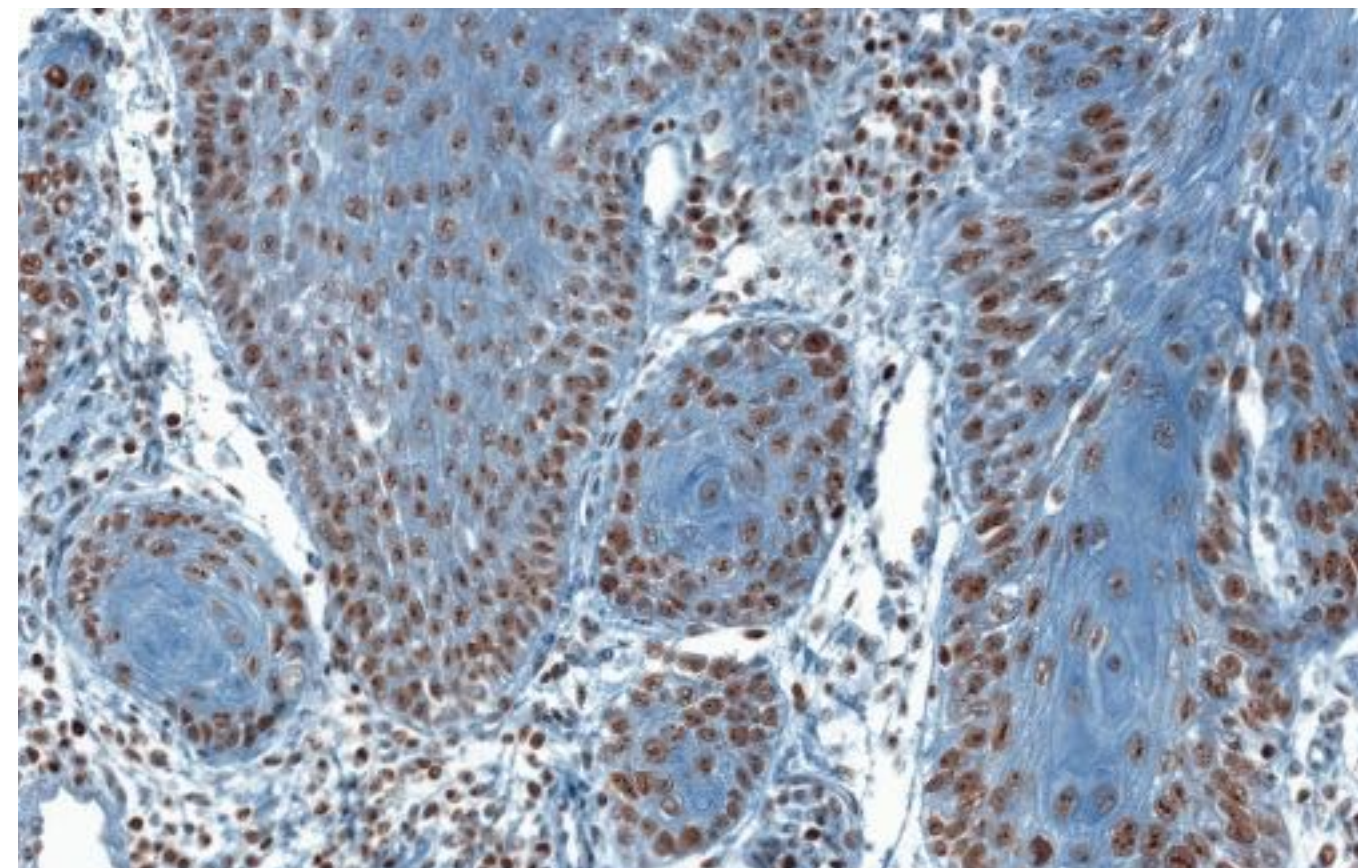
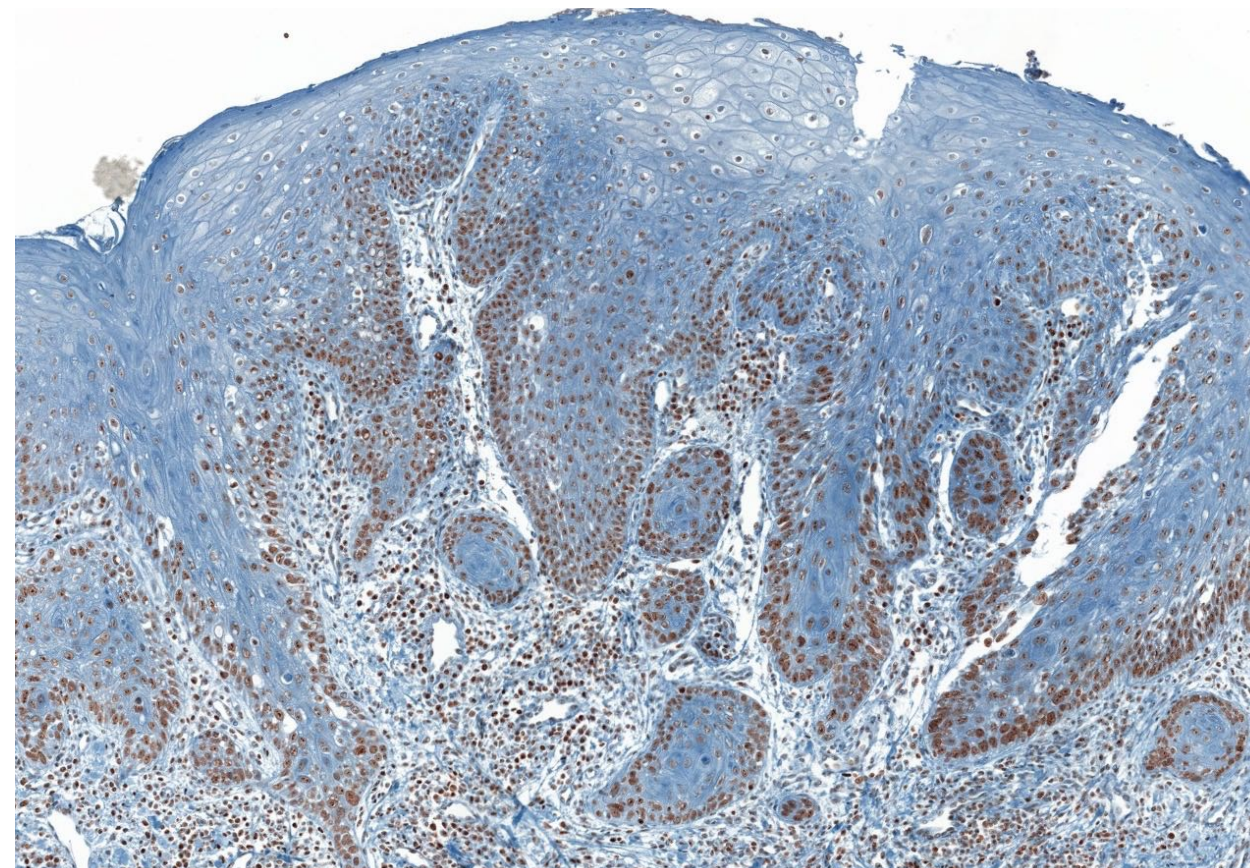
Connective tissue

Morphological
stain (H&E)



PARP1 stain

[PARP1 = brown
staining]



- ▶ Basal layer disorganized (extends into deeper layers)
- ▶ PARP1 in large, round nuclei outside of a basal layer like structure

- ▶ Tumor cells (round nuclei) can extend deep into the connective tissue

Tumor

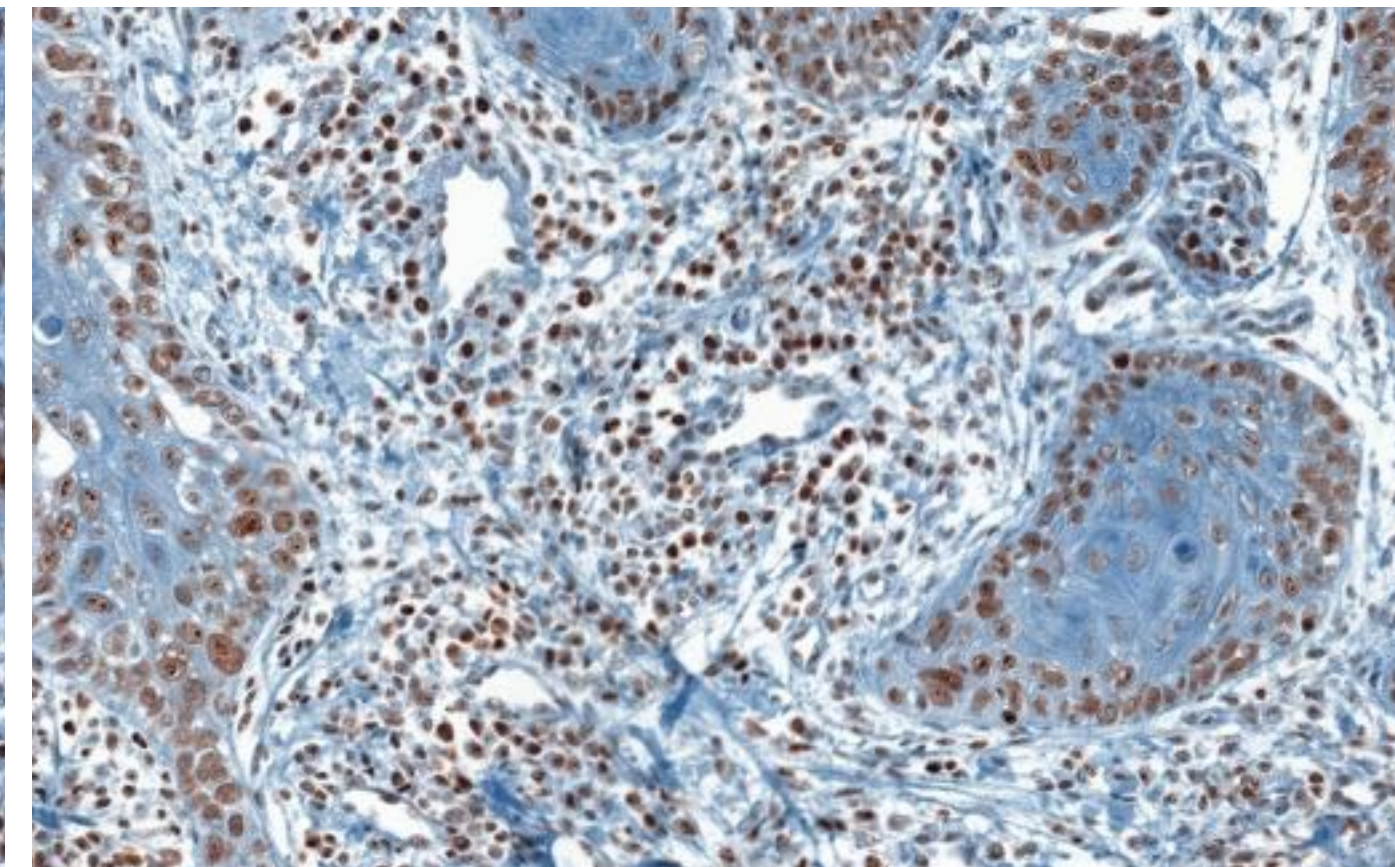
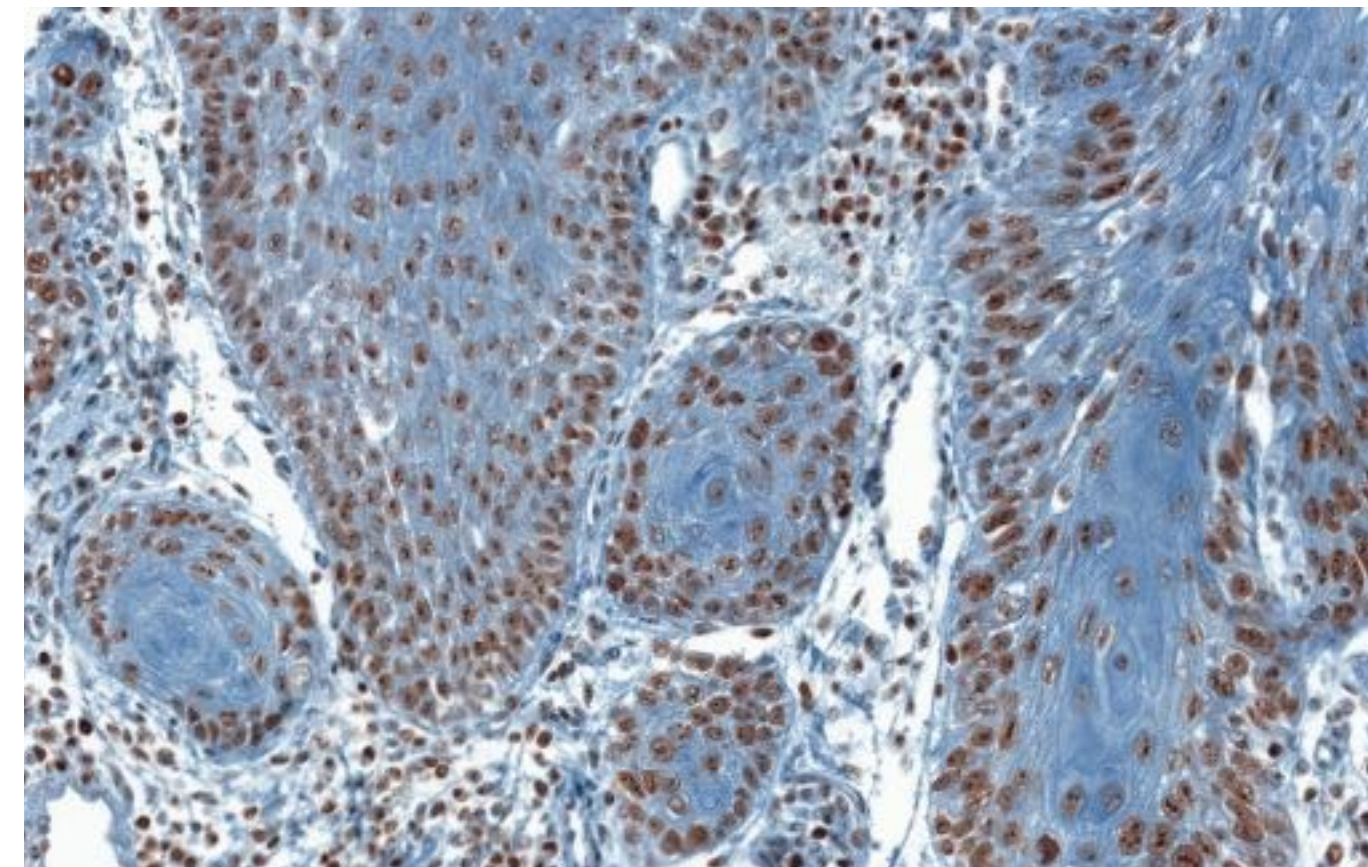
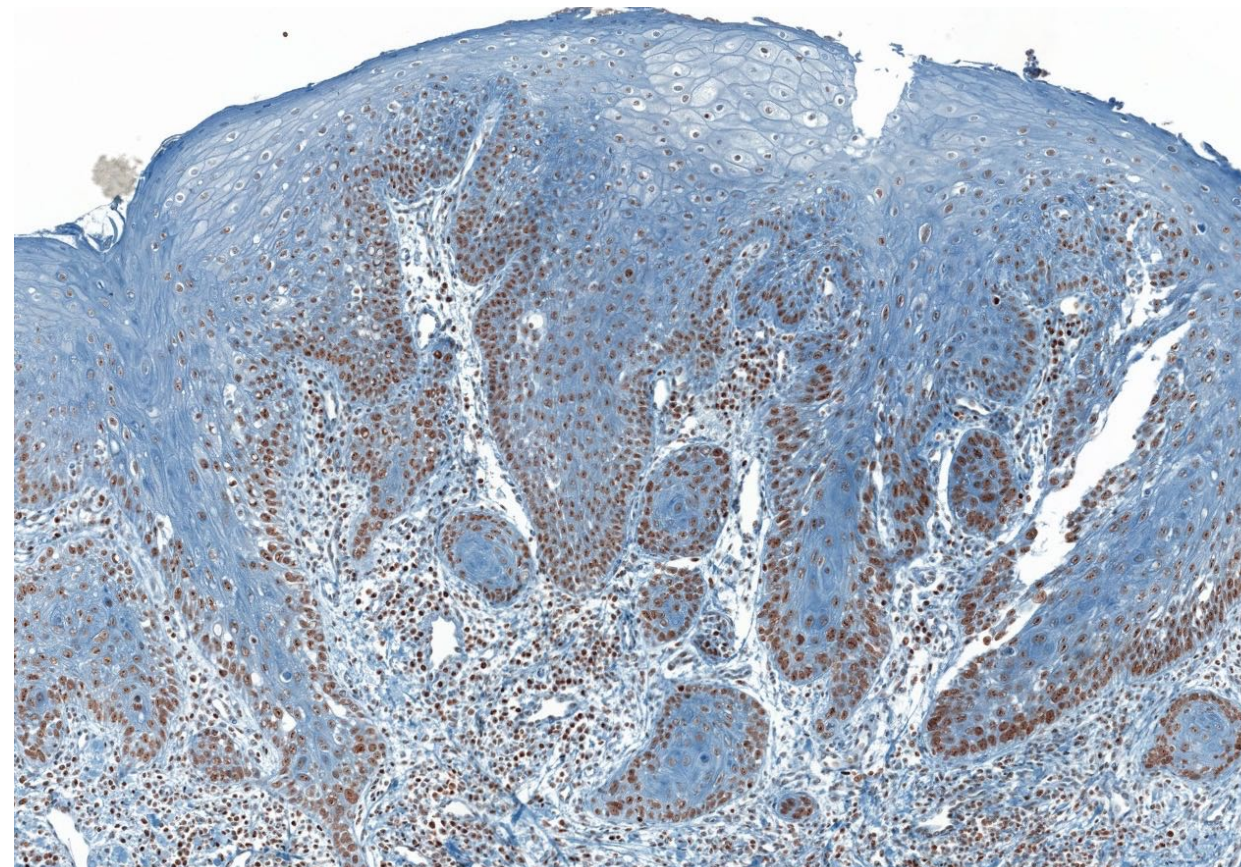


Basal layer

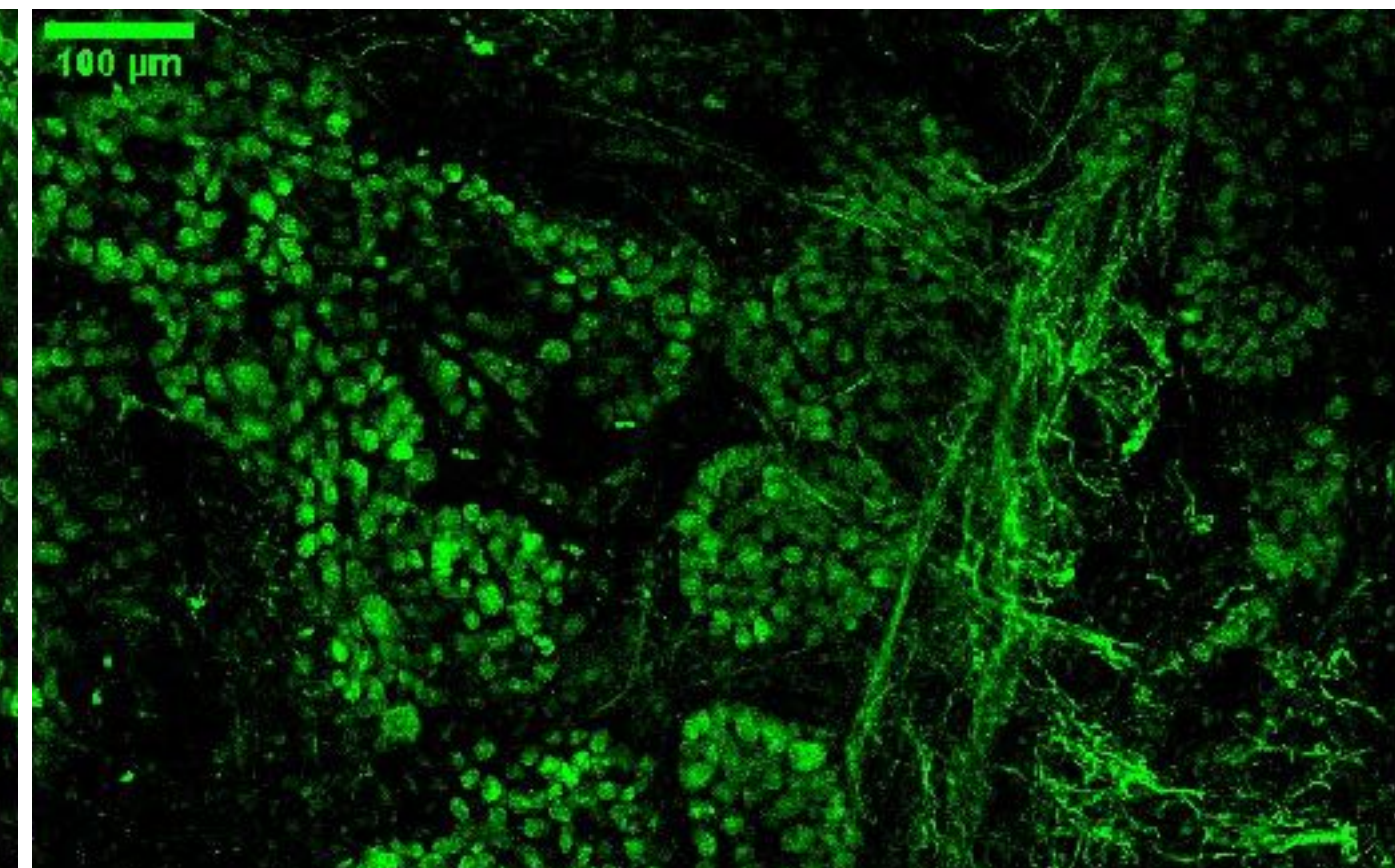
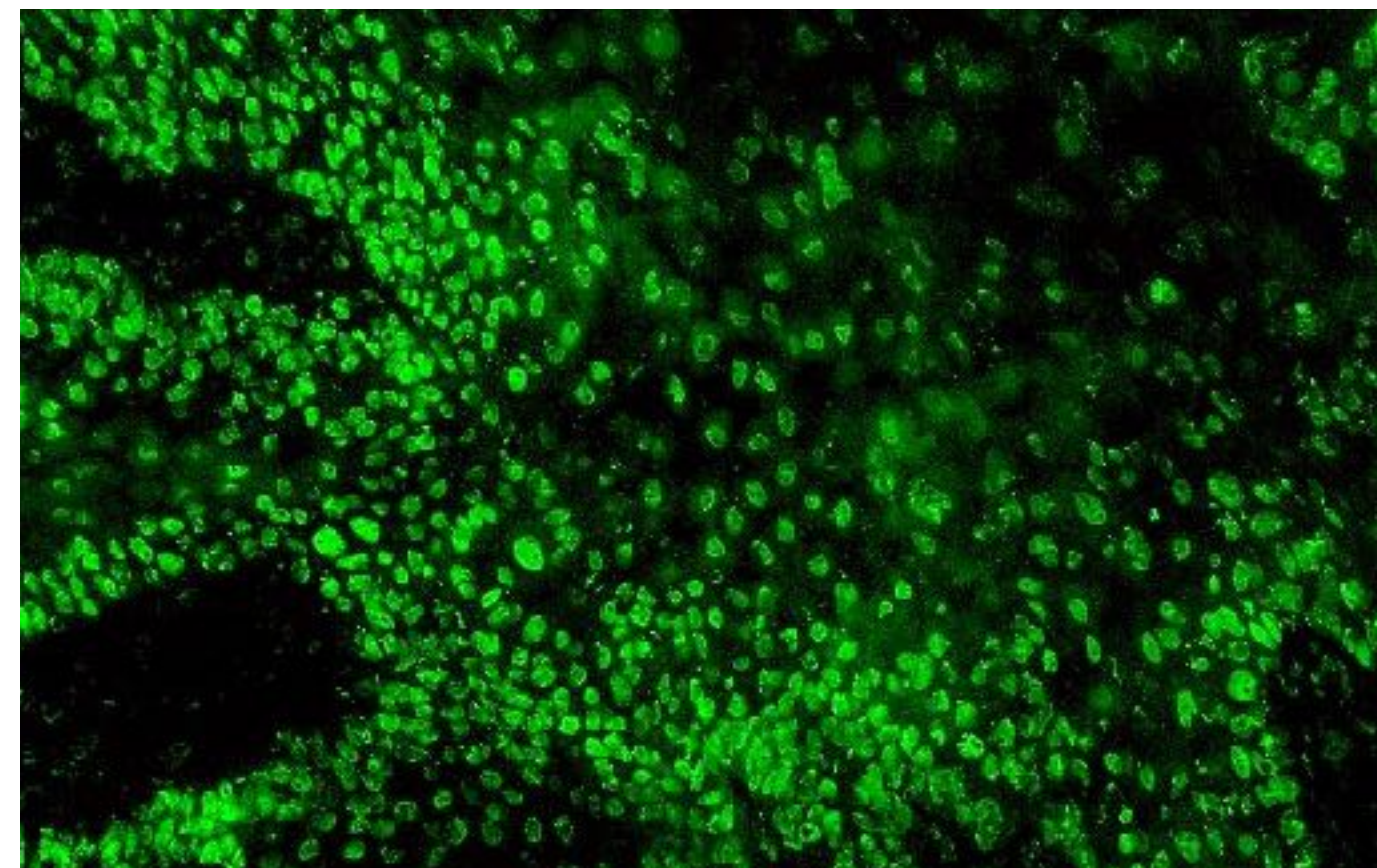
Connective tissue

PARP1 stain

[PARP1 = brown staining]



PARPi-FL



- PARPi-FL in round cells in more or less organized fashion
- Extension of green fluorescent cells beyond a thin basal layer indicates tumor

- Tumor cells (round nuclei) can extend deep into the connective tissue
- Structures with strong AF present (areal, flat fluorescence or bright streaks)

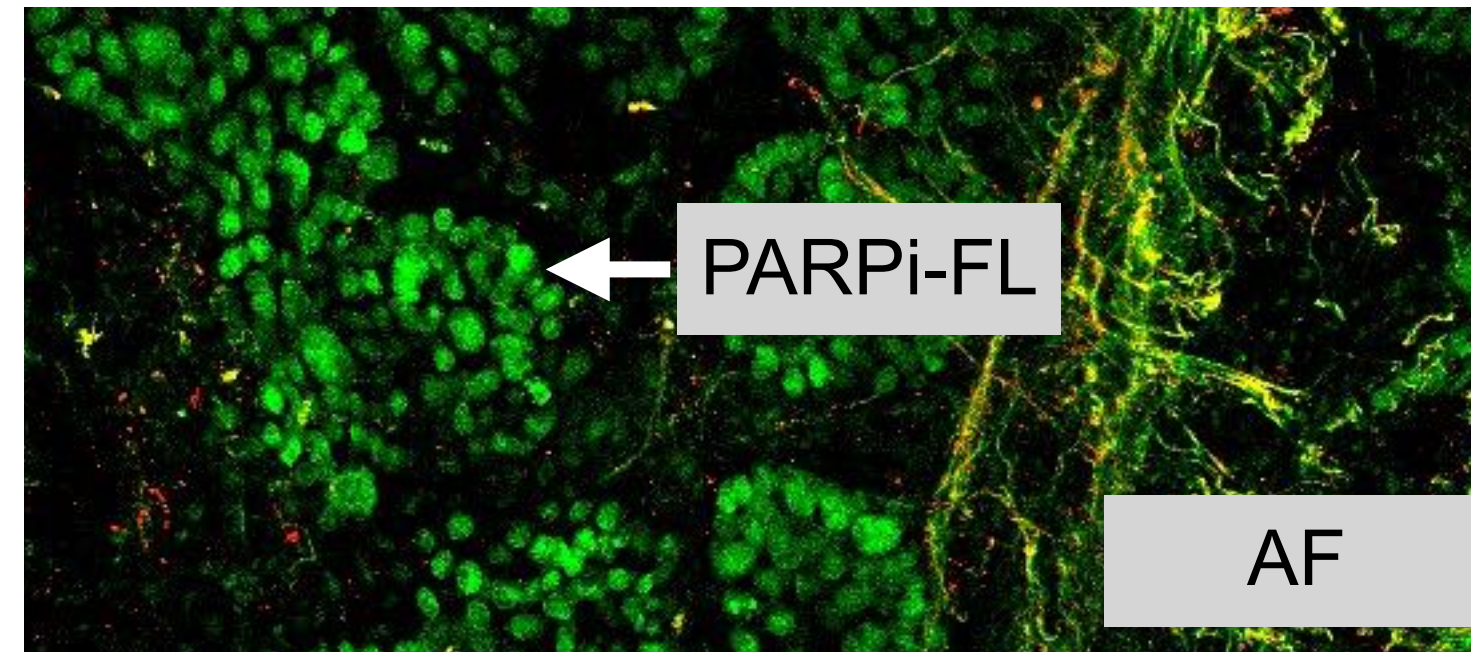
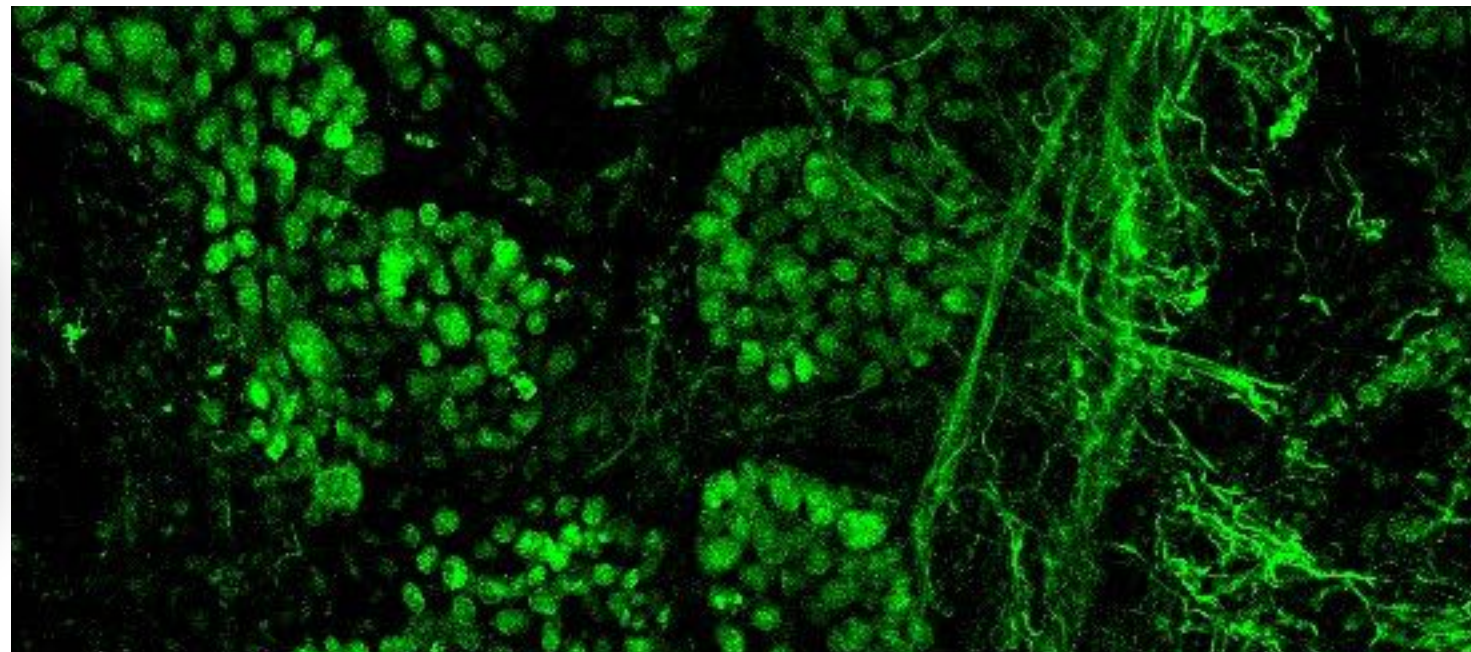
Autofluorescence

Green only:
PARPi-FL: green
AF: green

Overlay:
PARPi-FL: green
AF: red/yellow

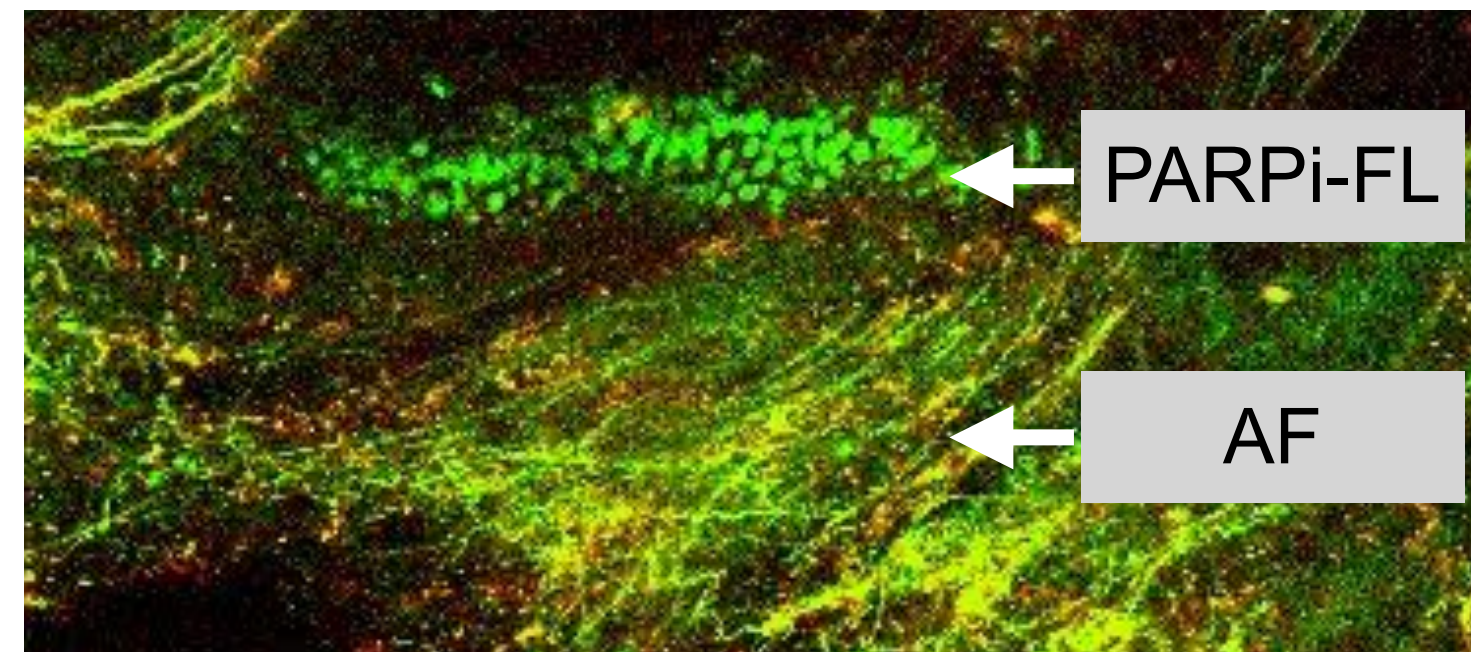
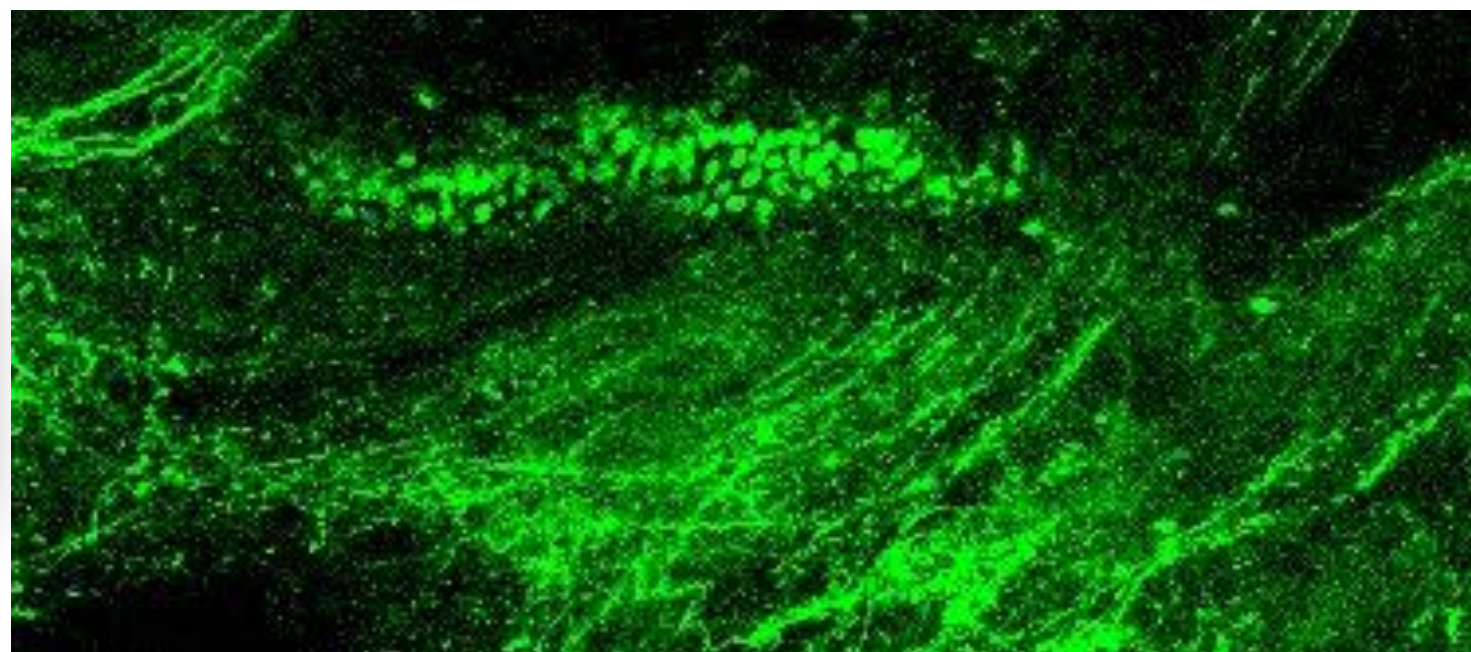
Most important characteristics

Tumor



- PARPi-FL stained large, round cells beyond basal layer (**MUST** be present)
- AF **CAN** be present

Margin

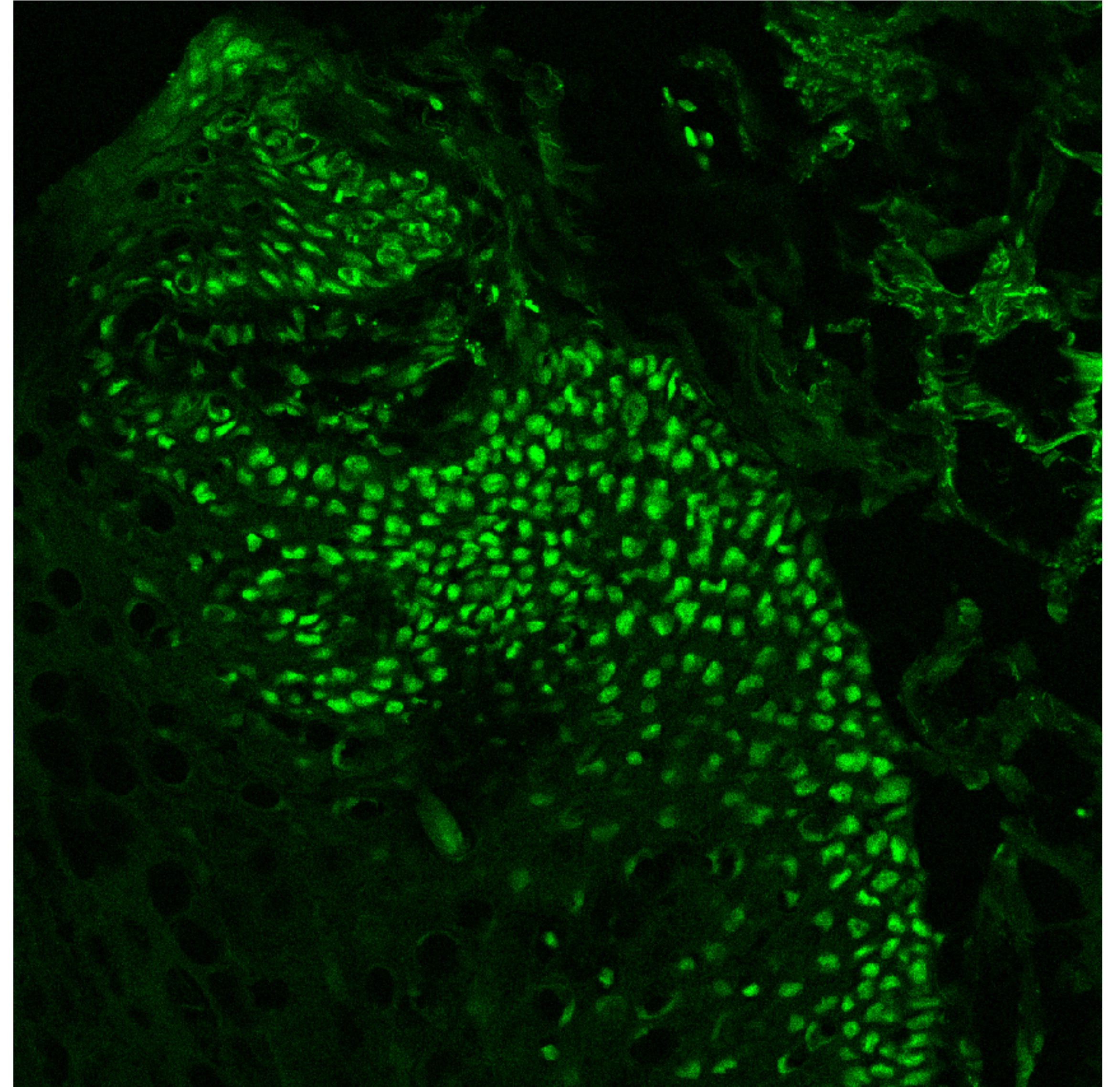
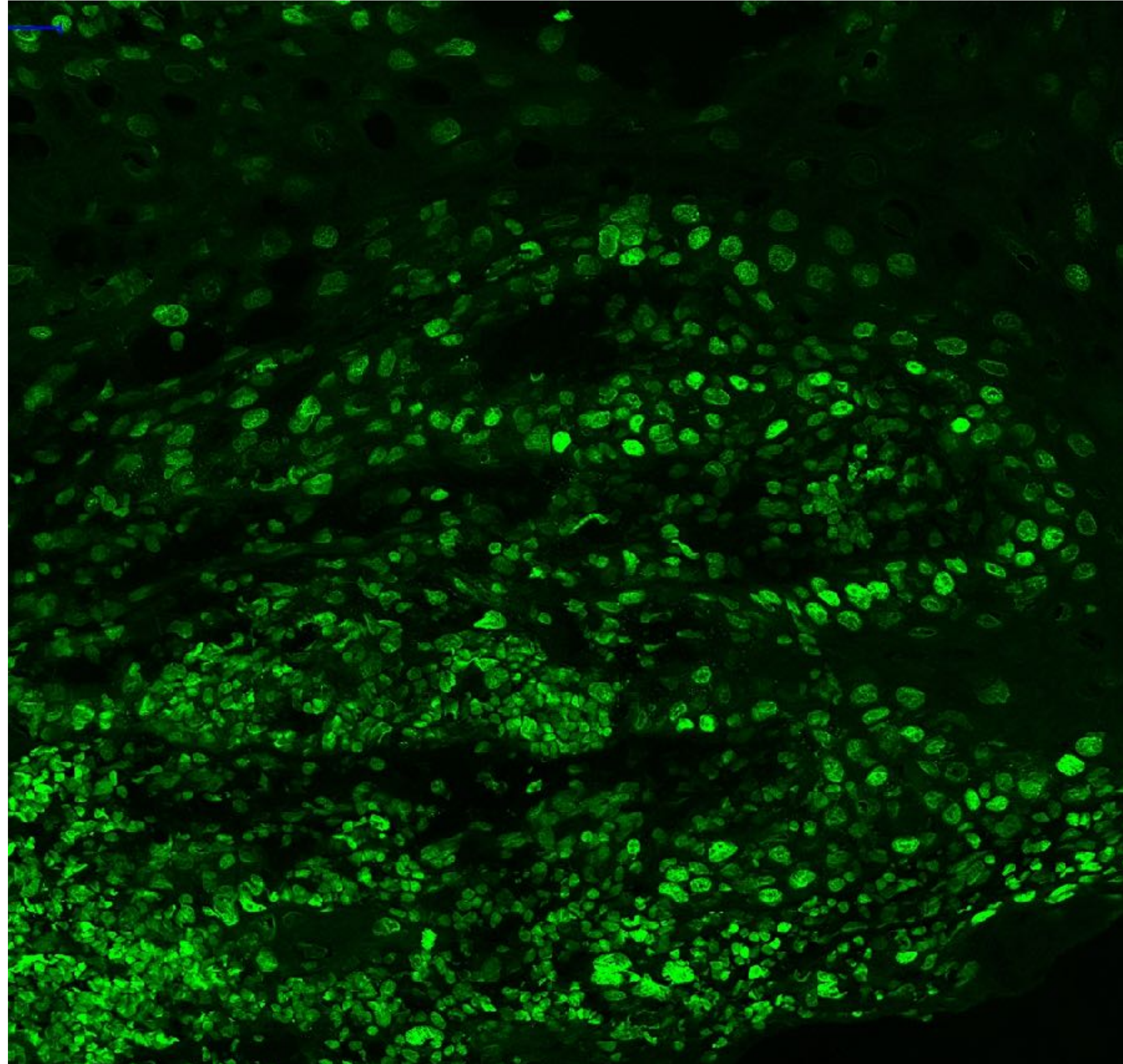


- Thin, regular basal layer with PARPi-FL stained cells (<6 cell layers) **CAN** be present
- AF **CAN** be present

- ➡ Round cells: PARPi-FL
- ➡ flat/area/streak stained: Autofluorescence

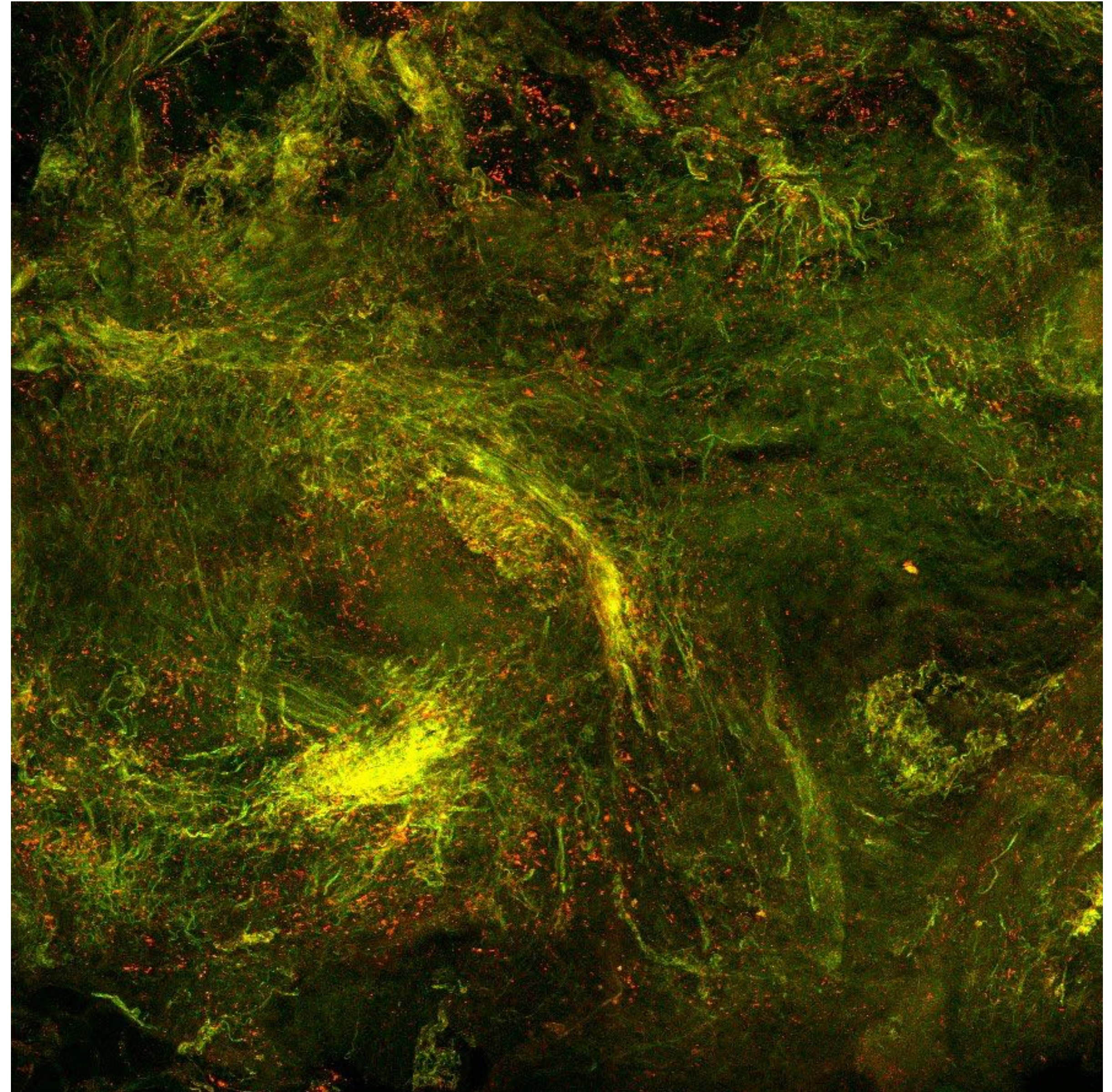
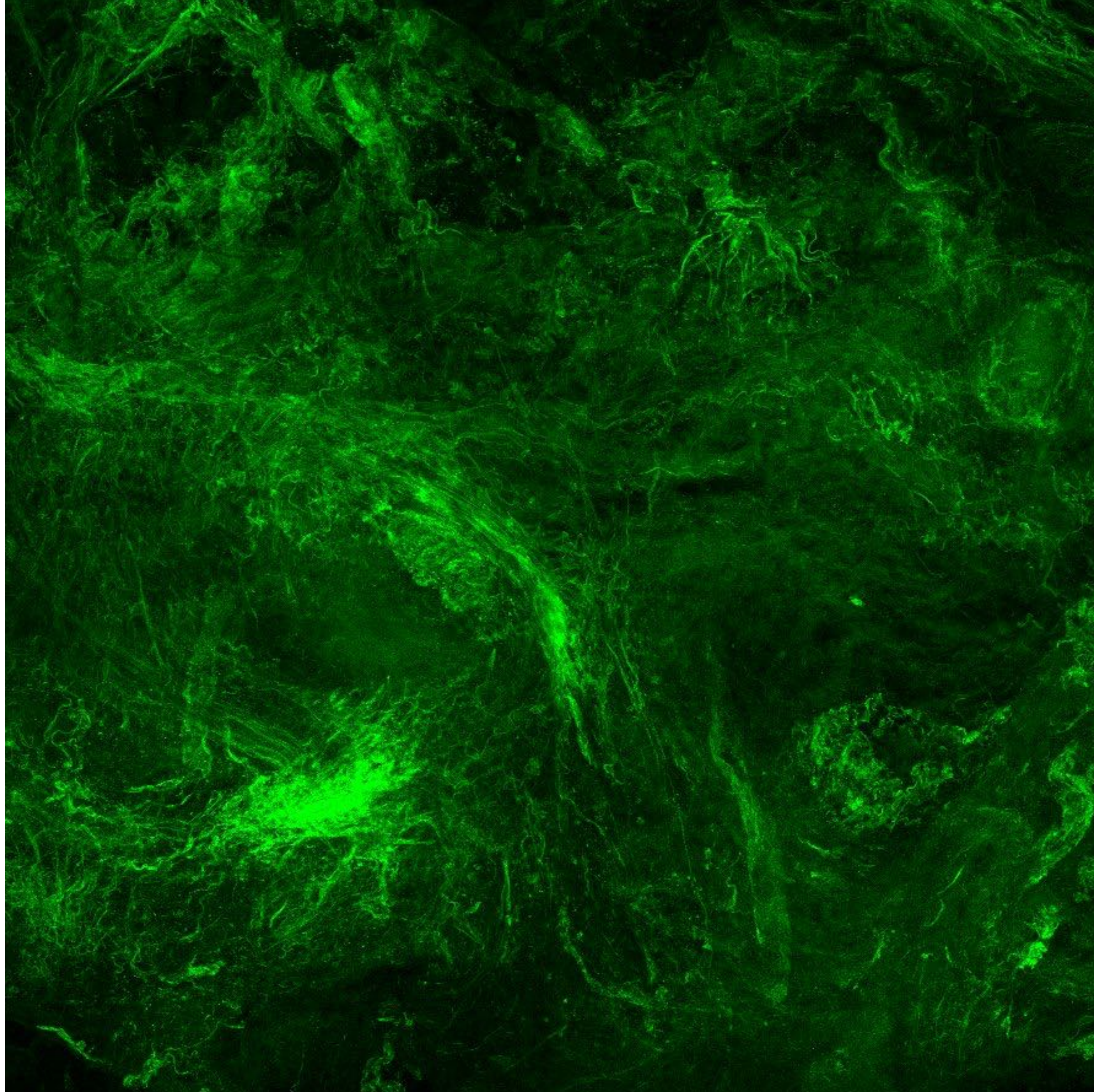
- ➡ Green round cells: PARPi-FL
- ➡ red/yellow signals: Autofluorescence (not diagnostic/ does not help to decide if a tissue is tumor or margin)

Tumor

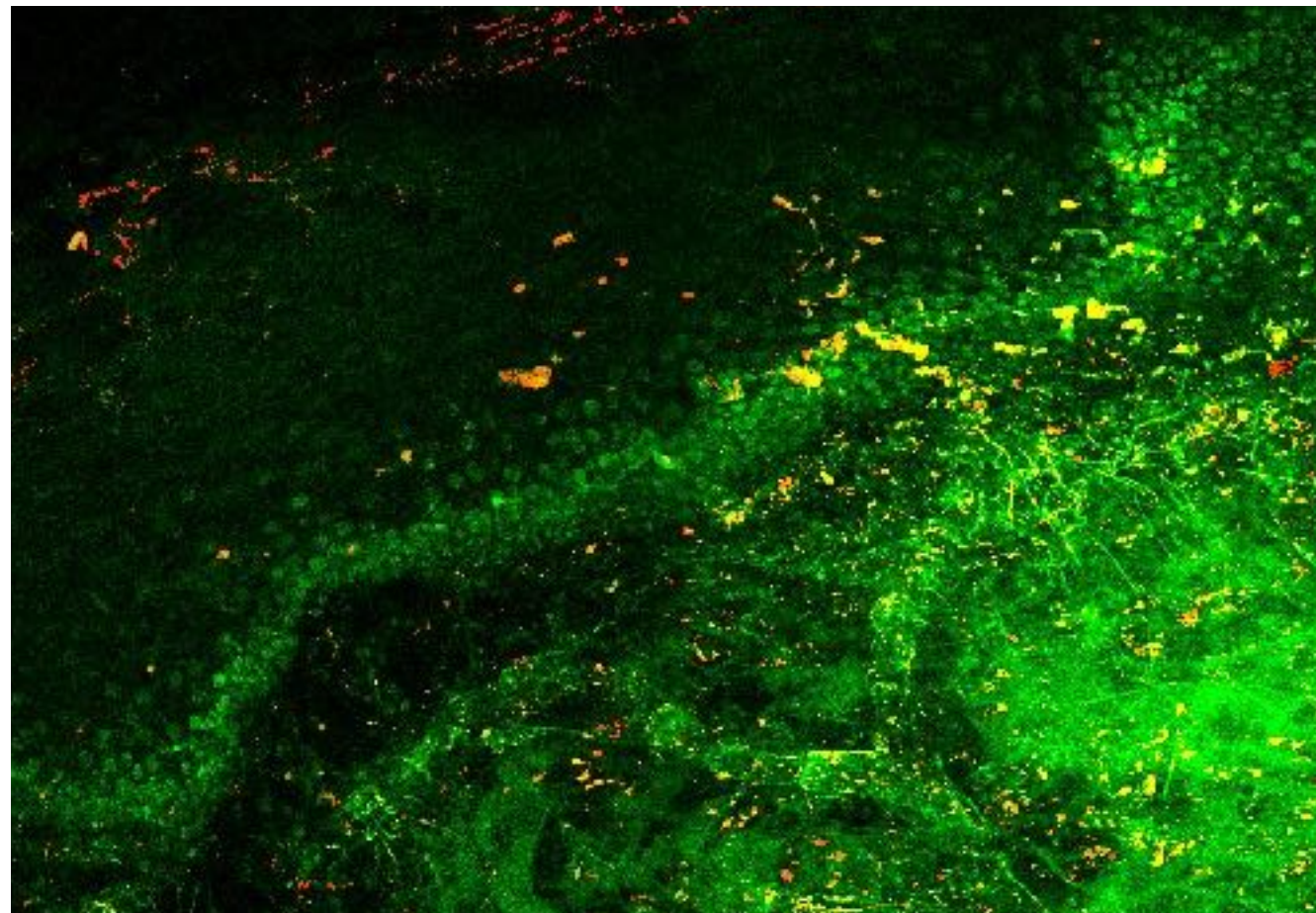
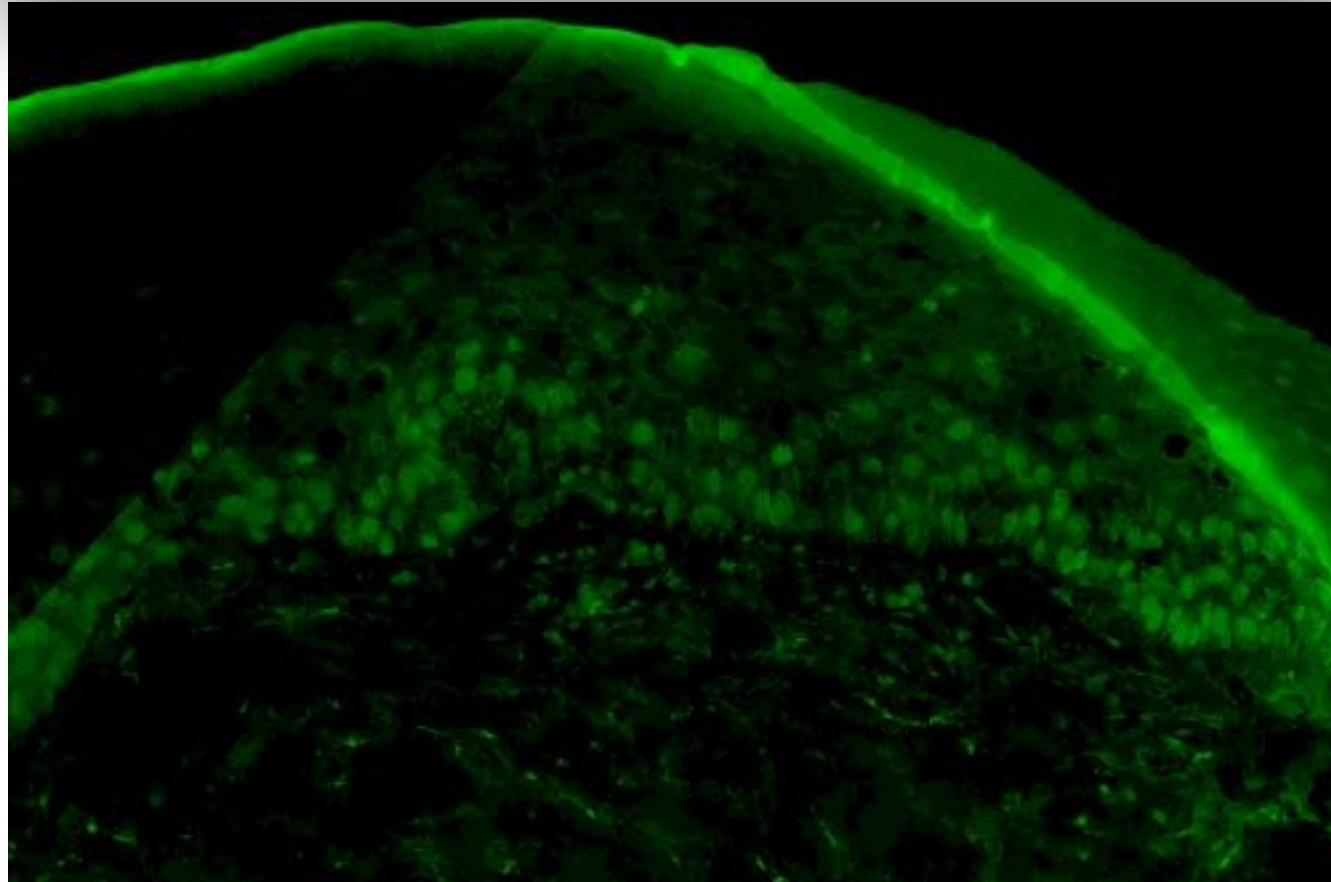


PARPi-FL only (green)

Margin

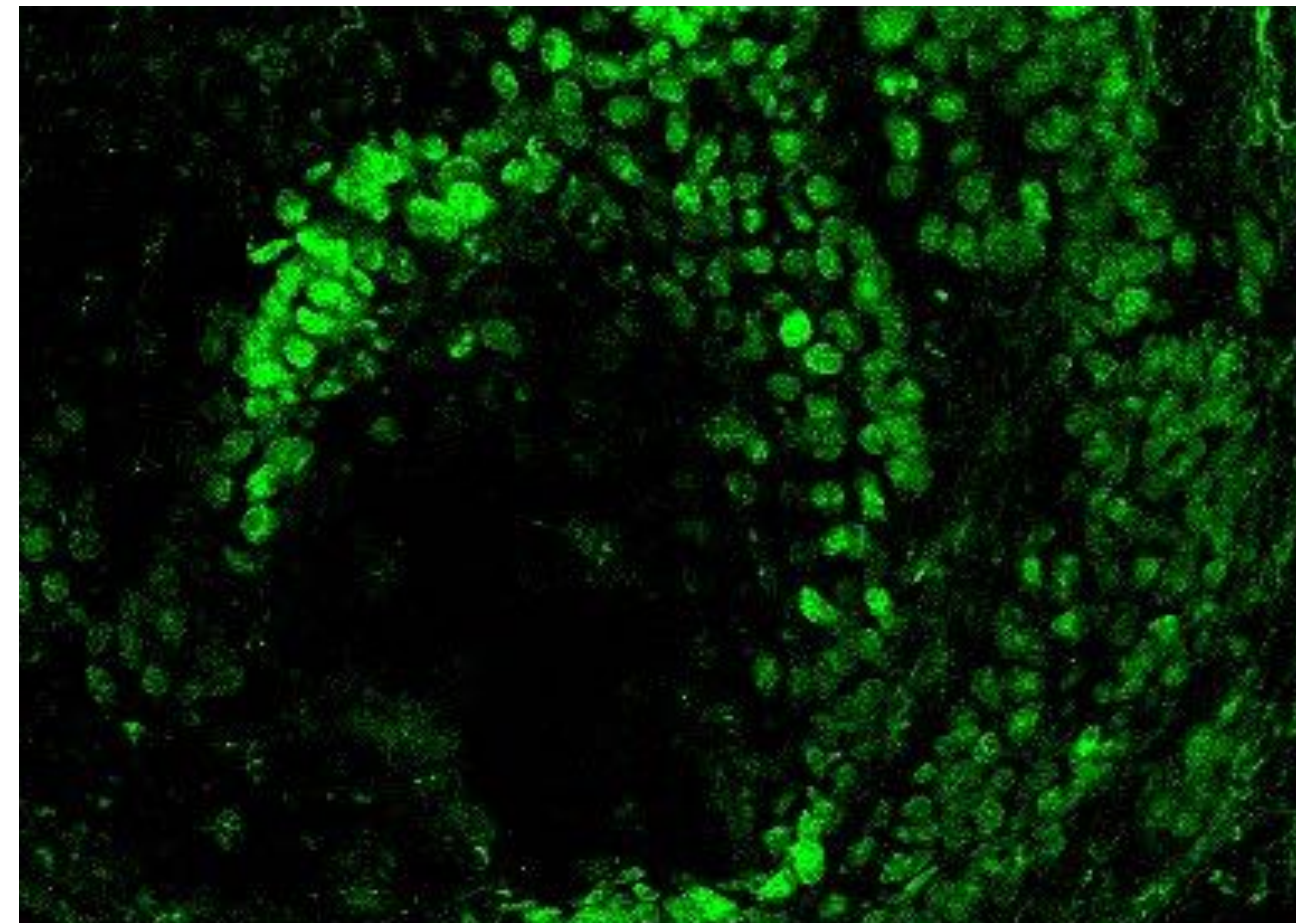
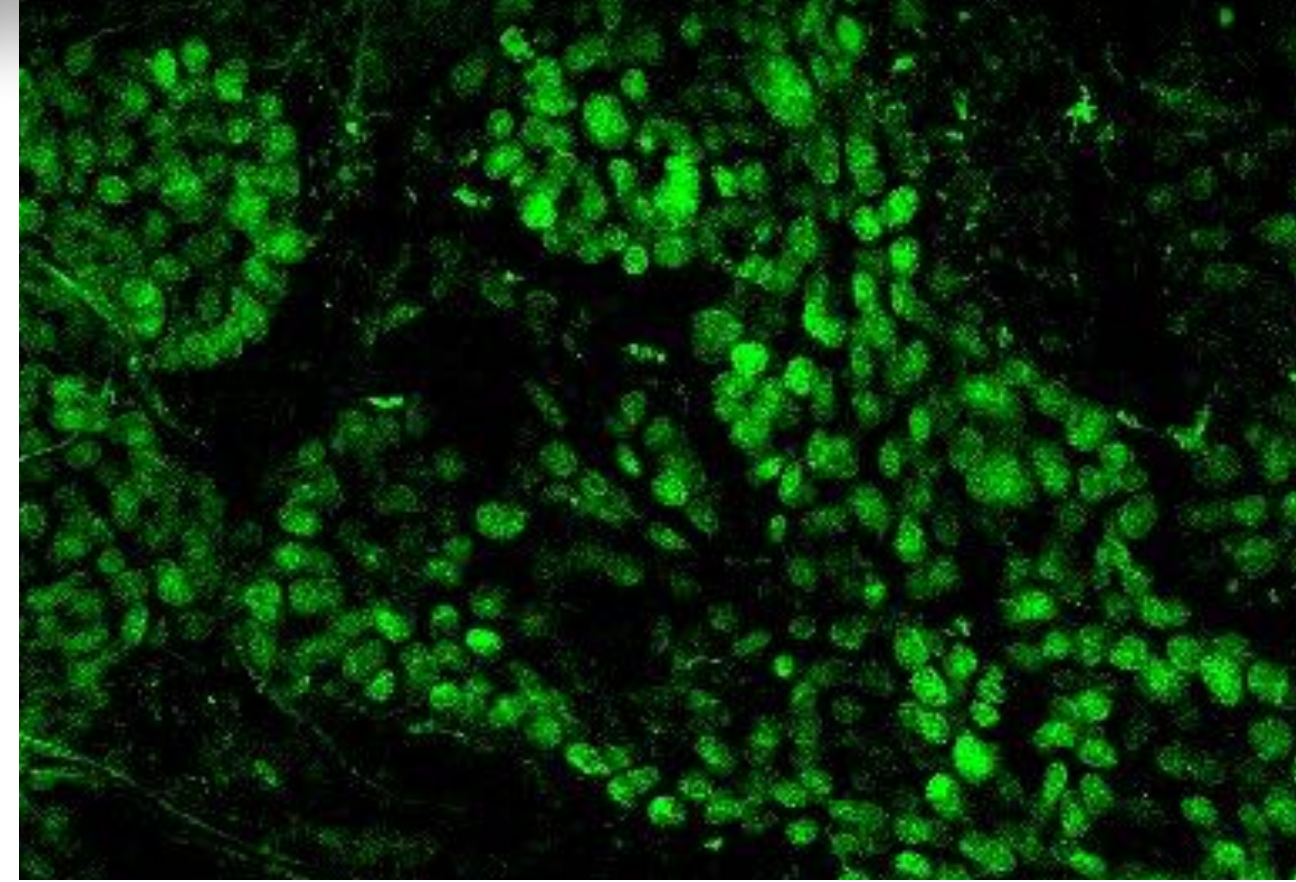


Margin



- ▶ PARP-FL related fluorescence confined to thin, regular basal layer (<6 cell layers)
- ▶ PARPi-FL signal in large, round cell nuclei **CAN** be present
- ▶ Autofluorescence **CAN** be present
- ▶ basal layer not always visible

Tumor



- ▶ PARPi-FL stained large, round cells in structures other than thin, regular basal layer **MUST** be present
- ▶ PARPi-FL signal in large, round cell nuclei
- ▶ AF **CAN** be present (check on overlay image)

How to decide

Round, green cells present in any of the images?

No

Margin

Yes

Are they confined to a thin basal layer (regular or with arches/crypts)?

Yes

Margin

No

Tumor