

# Determining glioblastoma proteome changes in response to lateral ventricle neural stem cells

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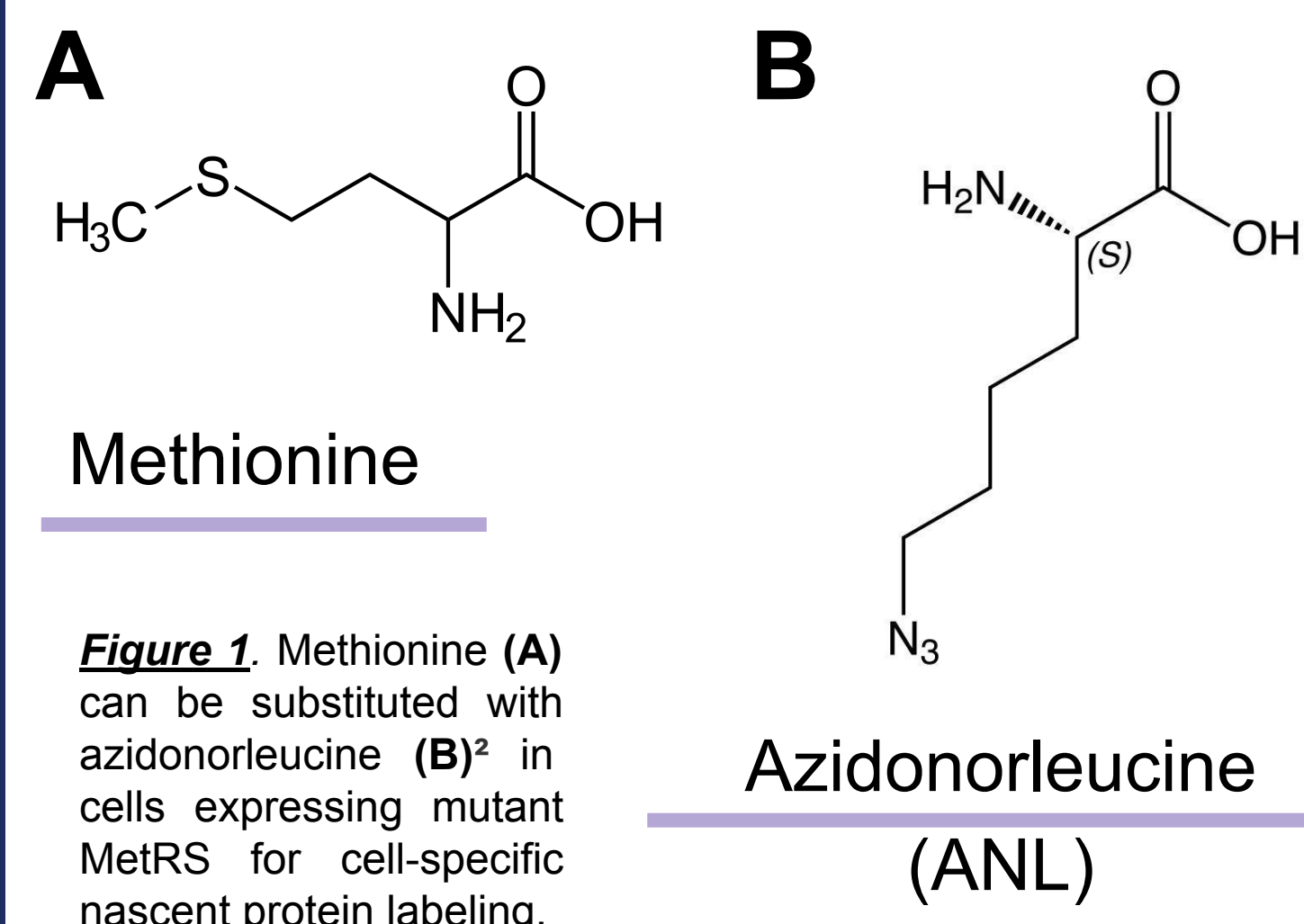
## Background

### Glioblastoma

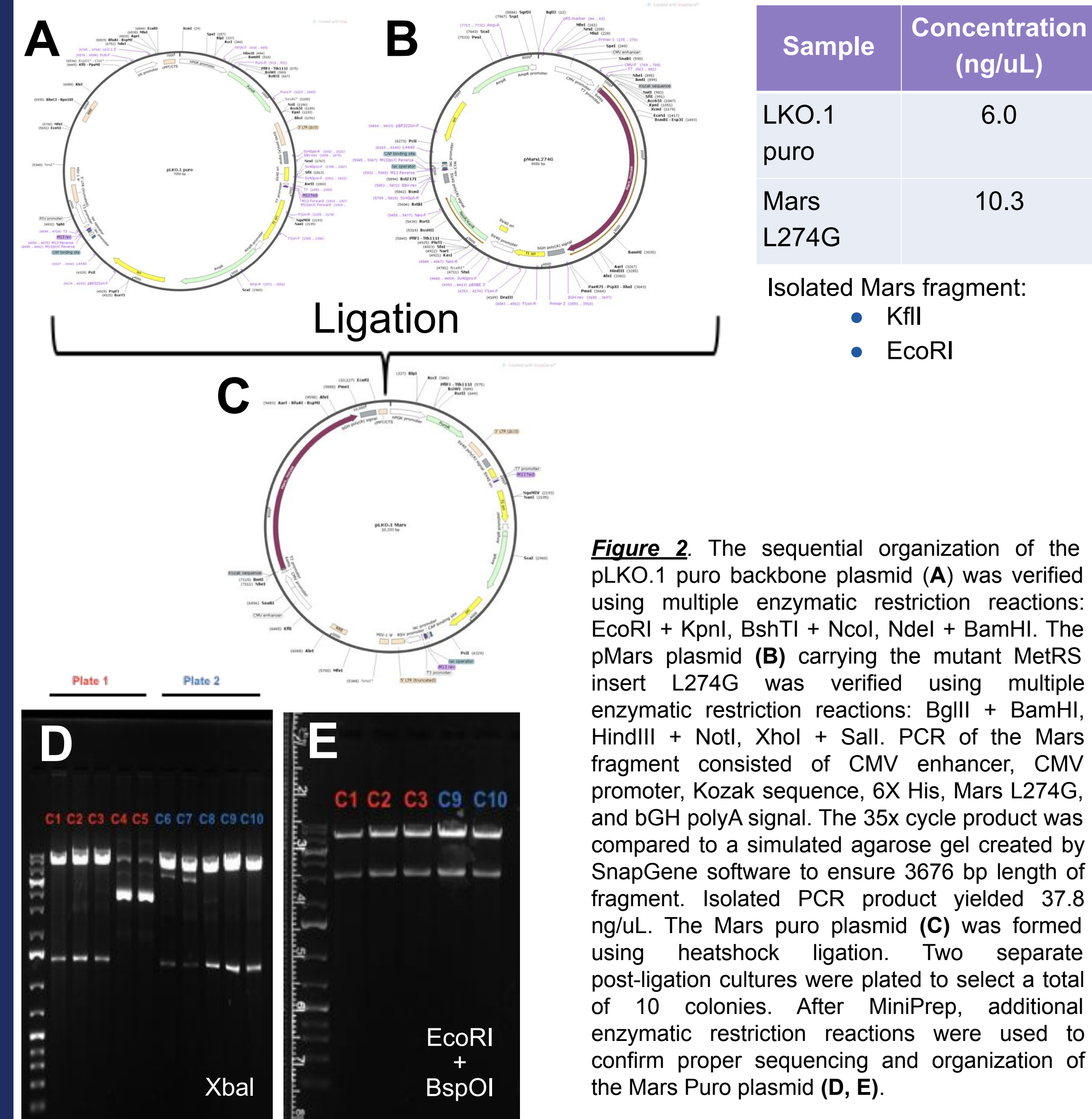
- Glioblastoma (GBM) is the most common and malignant primary tumor in adults.
- GBM tumors located near the lateral ventricle display a more aggressive recurrence pattern, negatively impacting patient survival.
  - Suggests involvement of subventricular zone neurogenic niche in GBM malignancy.

### Methionyl-tRNA synthetase (MetRS)

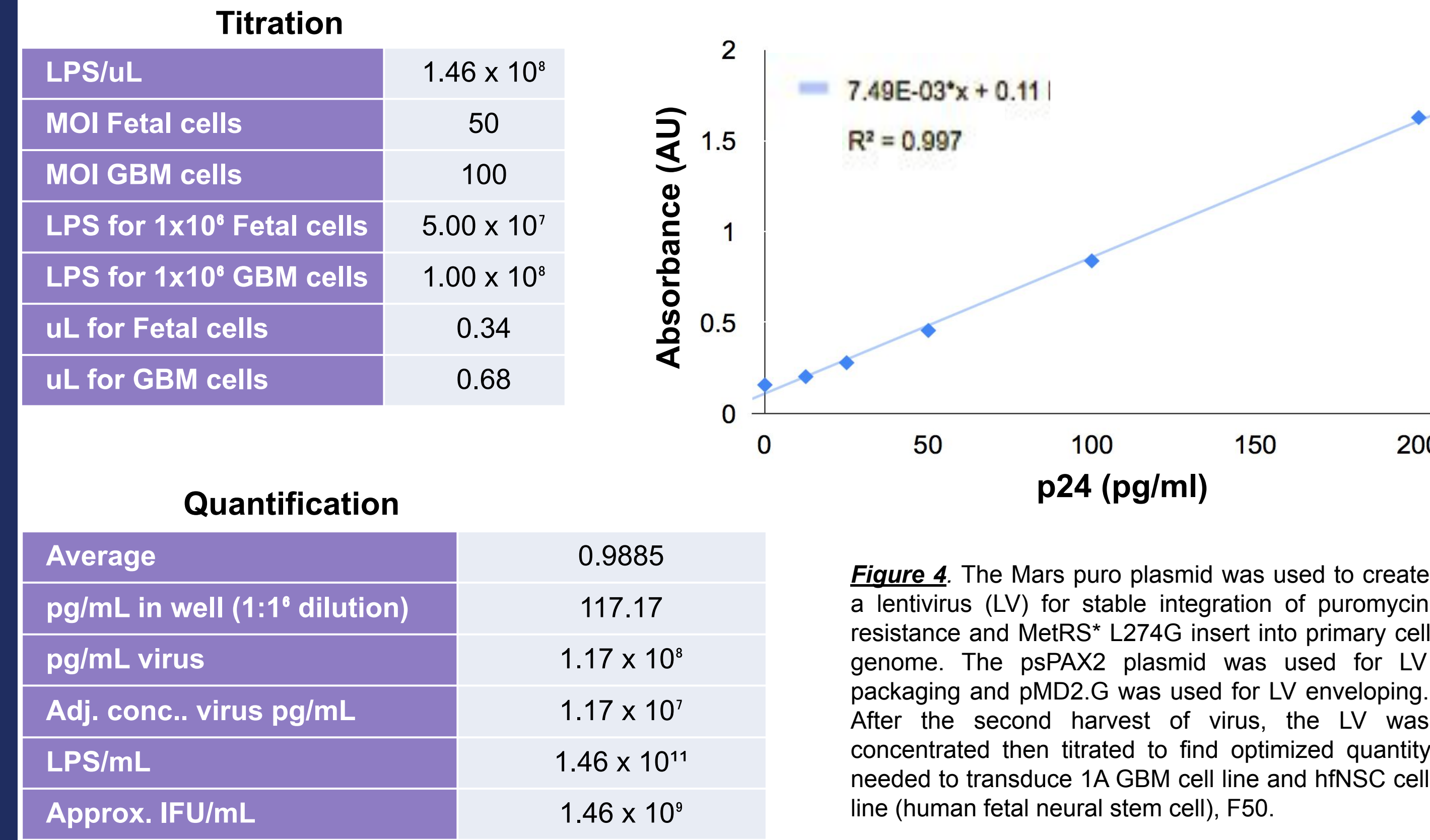
- Mutant MetRS L274G (MetRS\*) allows for incorporation of azide-tagged methionine analog azidonorleucine (ANL) into newly formed proteins.



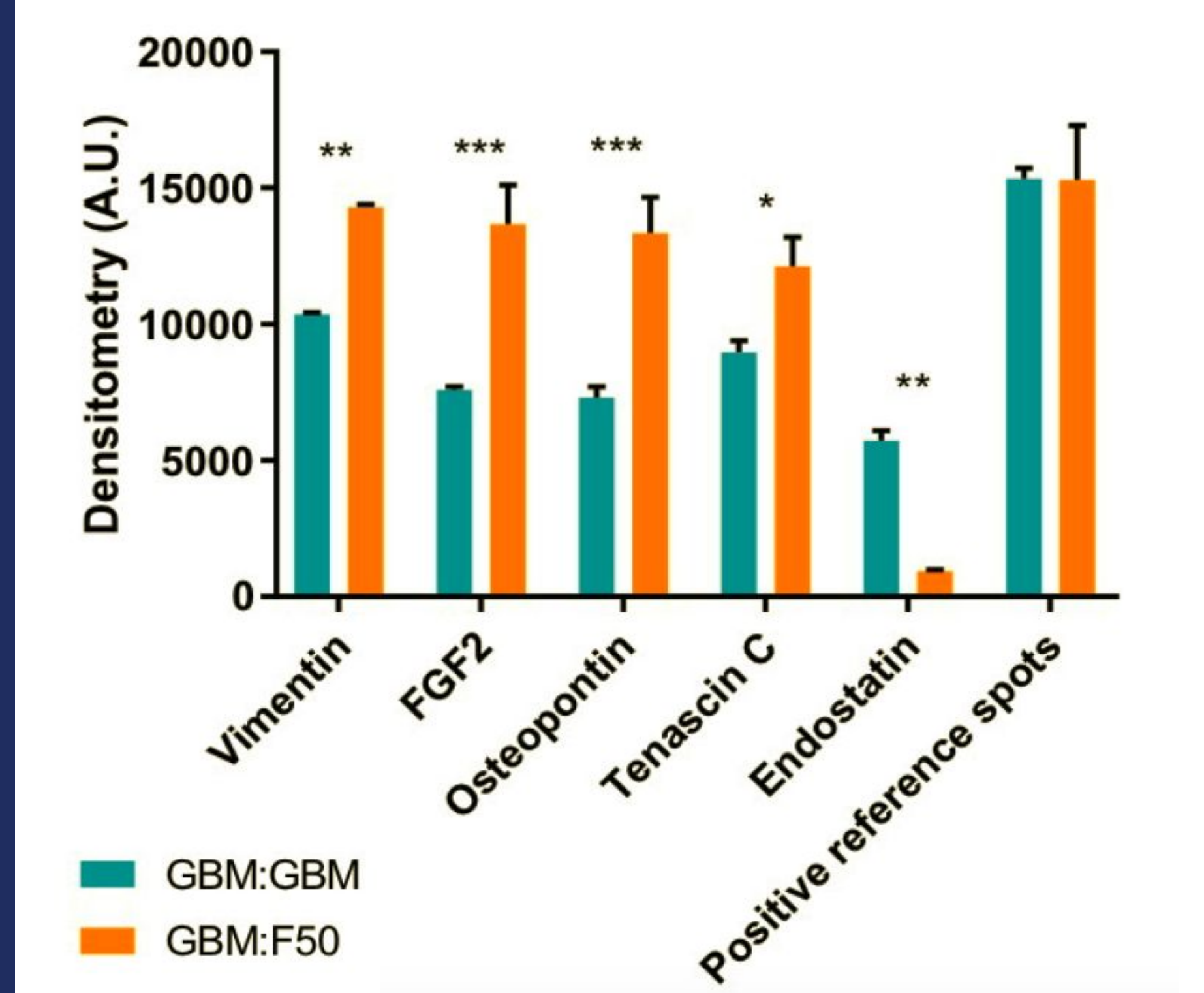
## Cloning MetRS\* into lentiviral plasmid



## Lentivirus production



## Proteomic changes in GBM cells co-cultured with neural stem cells



**Figure 6.** Co-Culture Oncology XL Proteome Profiler array results suggest an upregulation in malignancy promoting proteins. Co-cultures of F50 and 1A GBM were utilized to simulate the environment of glioblastoma neighboring neural stem cells.

**Vimentin (upregulation):** Component of intermediate filament cytoskeleton. Contributes towards processes including migration, metastasis, and cholesterol signaling. Usually overexpressed in cancers (cell invasion, metastatic tumor spread) and best known in cancers as a marker of cellular epithelial mesenchymal transition (EMT).

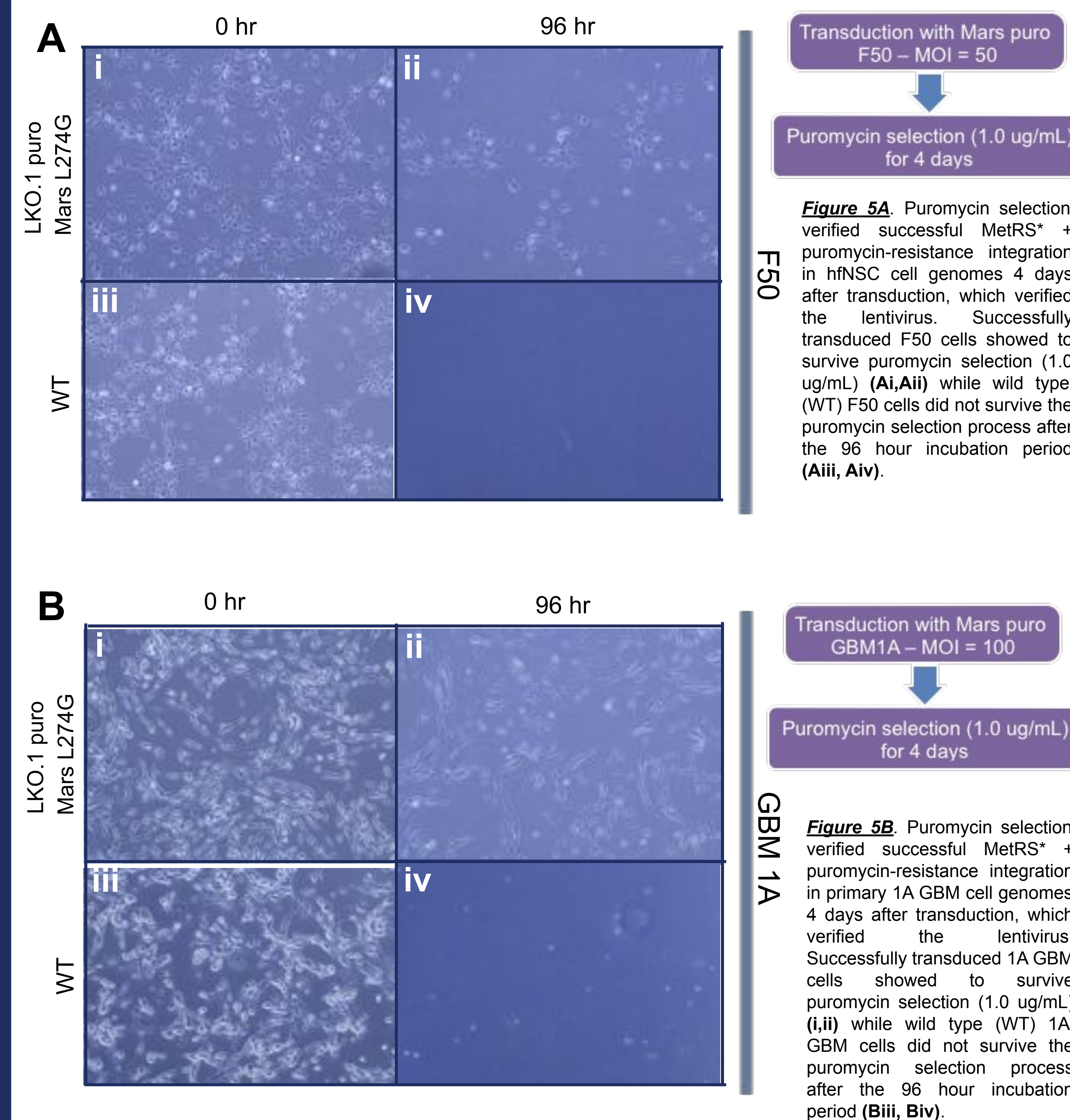
**FGF2 (upregulation):** Commonly expressed in malignant tumors. This protein can promote cell motility, proliferation, increase tumor angiogenesis and inhibit apoptosis.

**Osteopontin (upregulation):** Typically mediates normal physiological function including cell adhesion, migration, and tissue repair. Specifically in glioblastoma, this protein can act as a chemokine by recruiting macrophages to the GBM tumor site. Also mediates crosstalk between GBM cells.

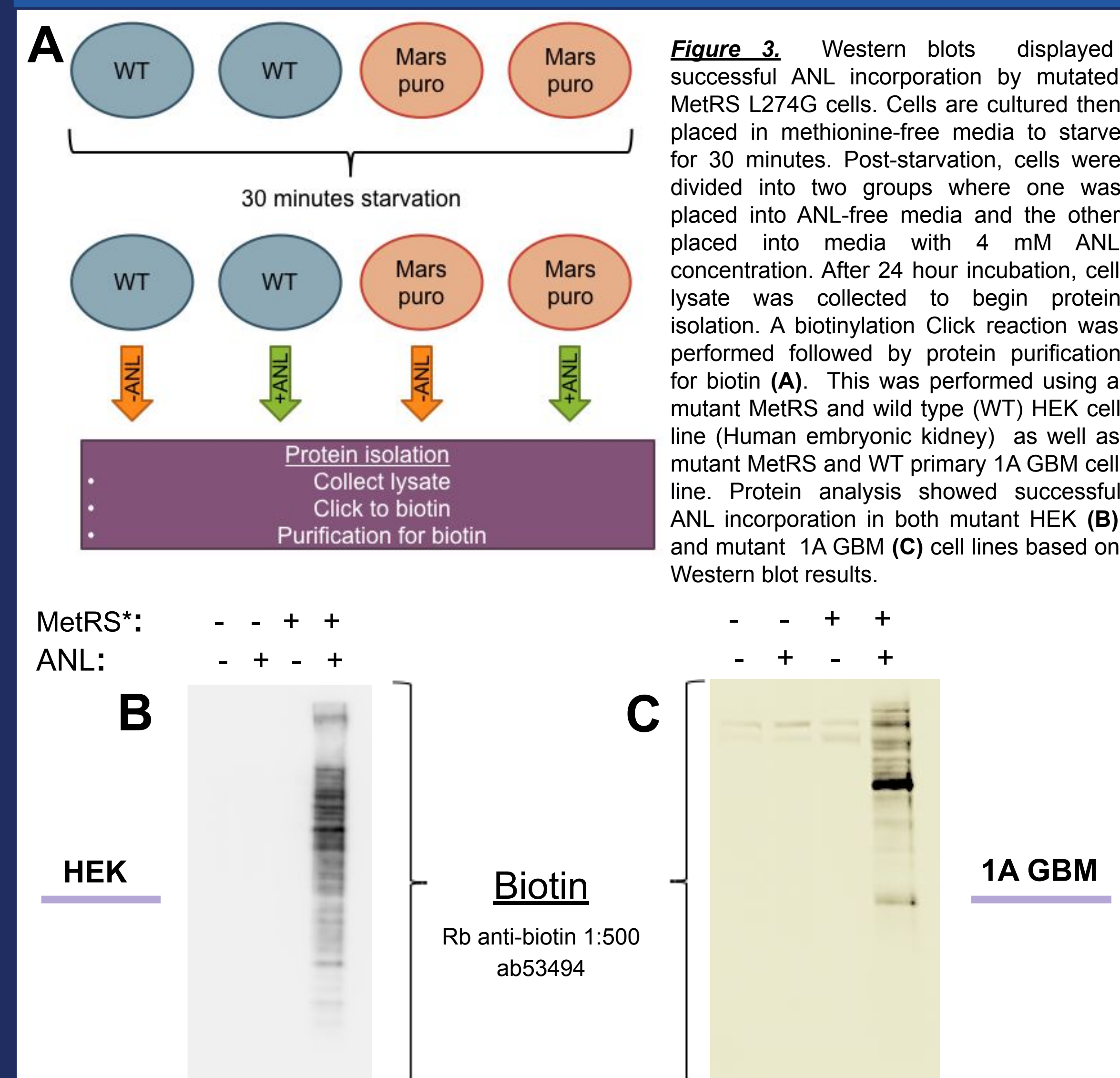
**Tenascin C (upregulation):** Extracellular matrix glycoprotein. An upregulation of this protein has been seen as a biomarker for a high grade glioma, Diffuse intrinsic pontine glioma(DIPG).<sup>1</sup>

**Endostatin (downregulation):** Specifically inhibits endothelial proliferation while significantly inhibits angiogenesis and tumor growth.

## Puromycin selection of transduced cells



## Confirmation of ANL incorporation



## Objectives

- To optimize a tool to determine cell-specific proteomic changes of GBM cells in response to neural stem cell proximity.
- To define the intercellular communication between neural stem cells and GBM cells.

## Methods

- Molecular cloning of MetRS\* into lentiviral backbone with puromycin selection
- Confirmation of ANL incorporation into multiple MetRS mutant cell lines using western blot and silver stain
  - HEK
  - 1A GBM (patient-derived primary GBM line)
- Lentivirus production from cloned plasmid
  - Quantification and concentration
  - Titration
- Lentivirus validation using puromycin selection
- Co-culture proteomic analysis

## Conclusions

- MetRS\* metabolic labeling can be successfully cloned into a lentivirus and utilized as a tool for cell-specific proteomics with the use of ANL.
- GBM cells within close proximity of neural stem cells show an increase of proteins representative of malignant cancer spread.

## References

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