

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

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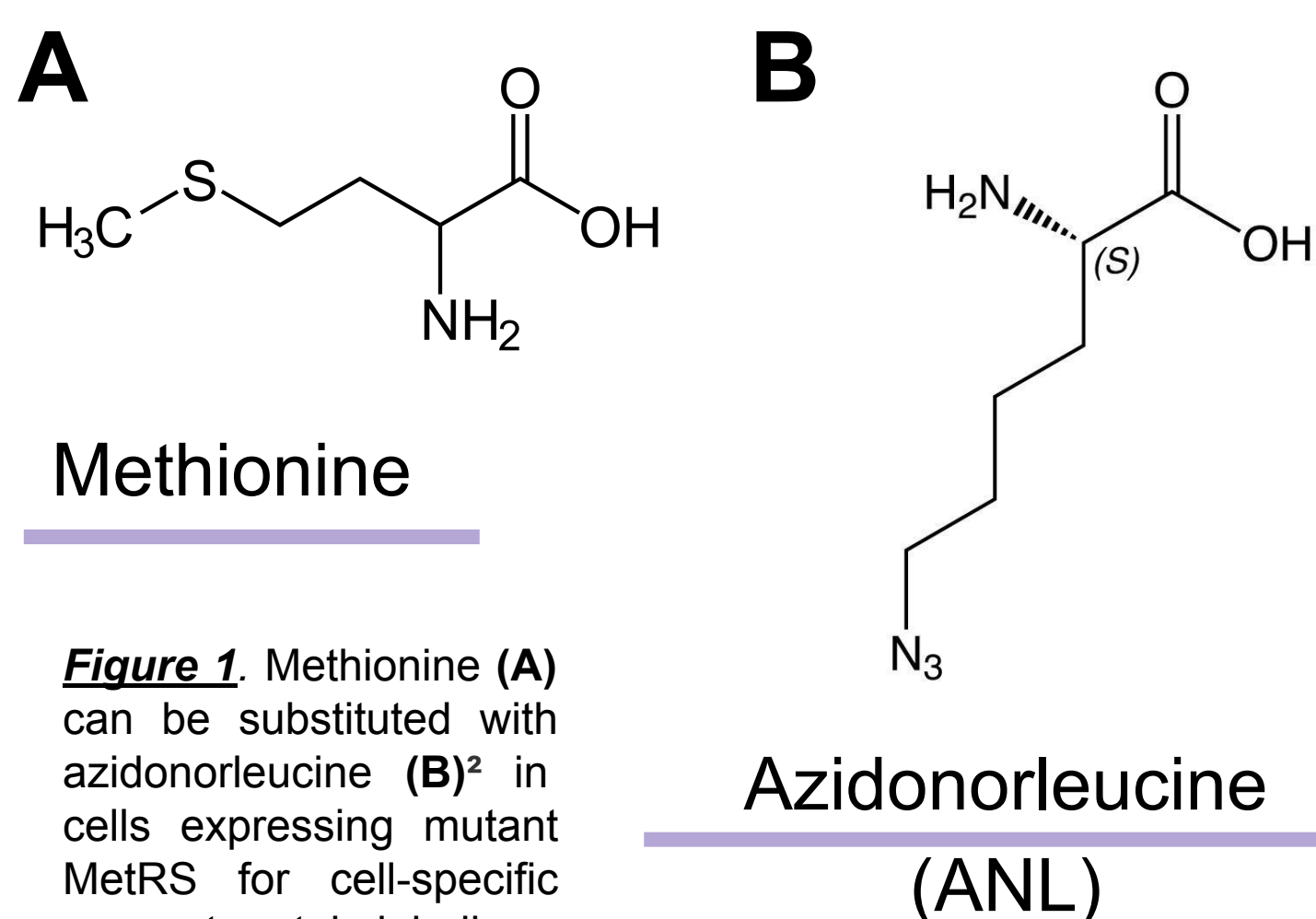
Background

Glioblastoma

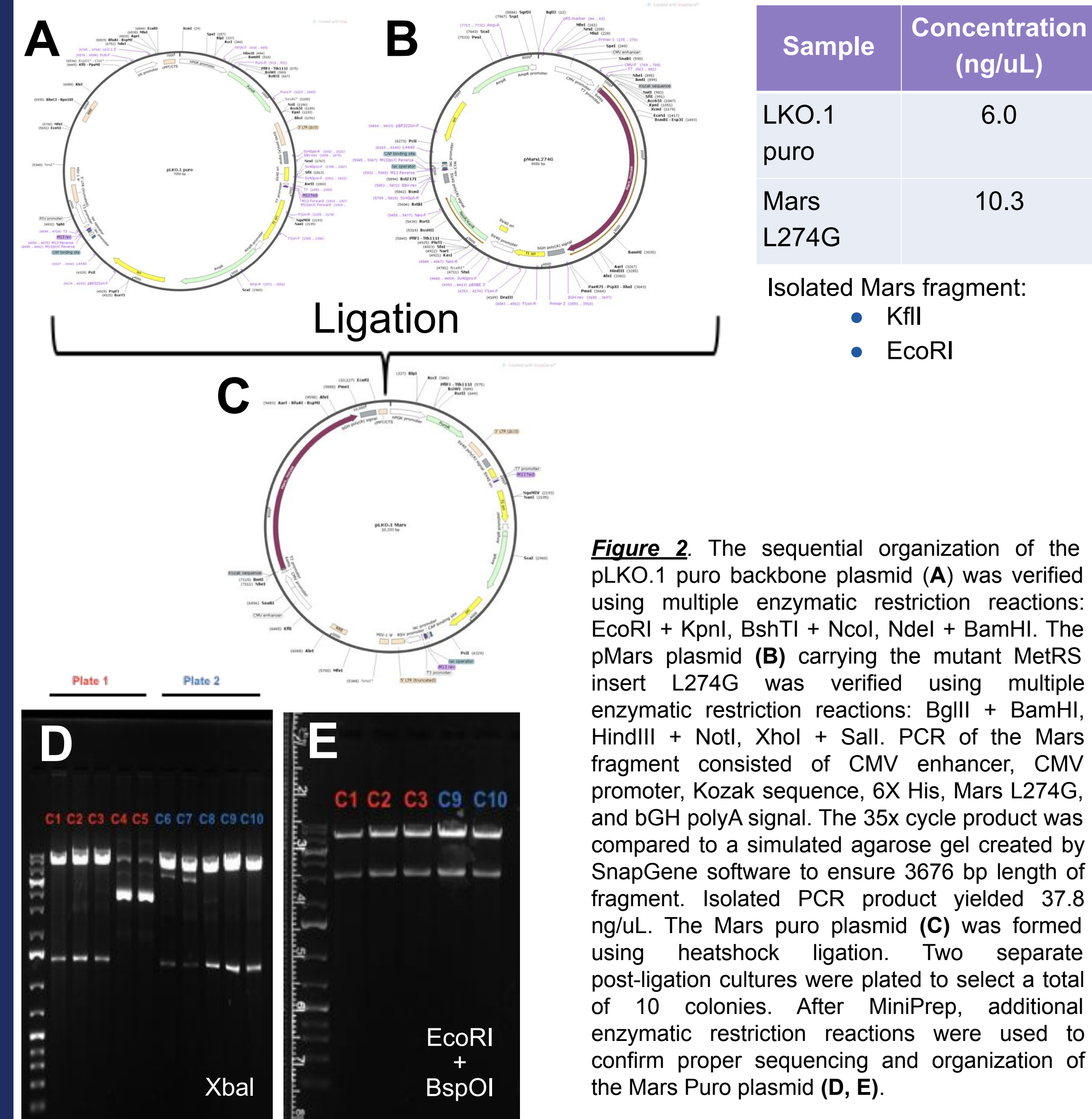
- Glioblastoma (GBM) is the most common and malignant primary tumor in adults.
- GBM tumors located near the lateral ventricle display a more aggressive recurrence pattern, negatively impacting patient survival.
 - Suggests involvement of subventricular zone neurogenic niche in GBM malignancy.

Methionyl-tRNA synthetase (MetRS)

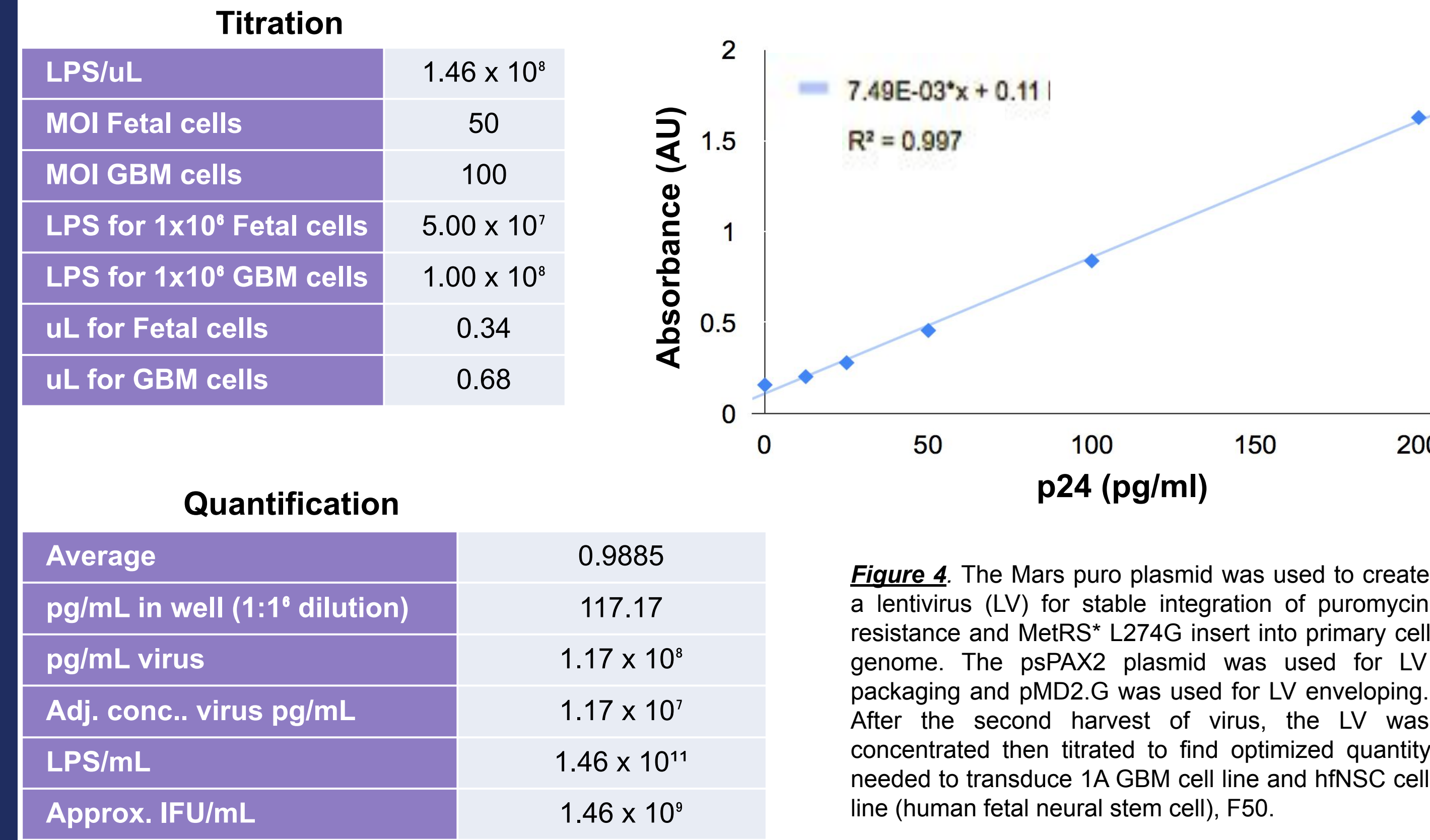
- Mutant MetRS L274G (MetRS*) allows for incorporation of azide-tagged methionine analog azidonorleucine (ANL) into newly formed proteins.



Cloning MetRS* into lentiviral plasmid



Lentivirus production



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

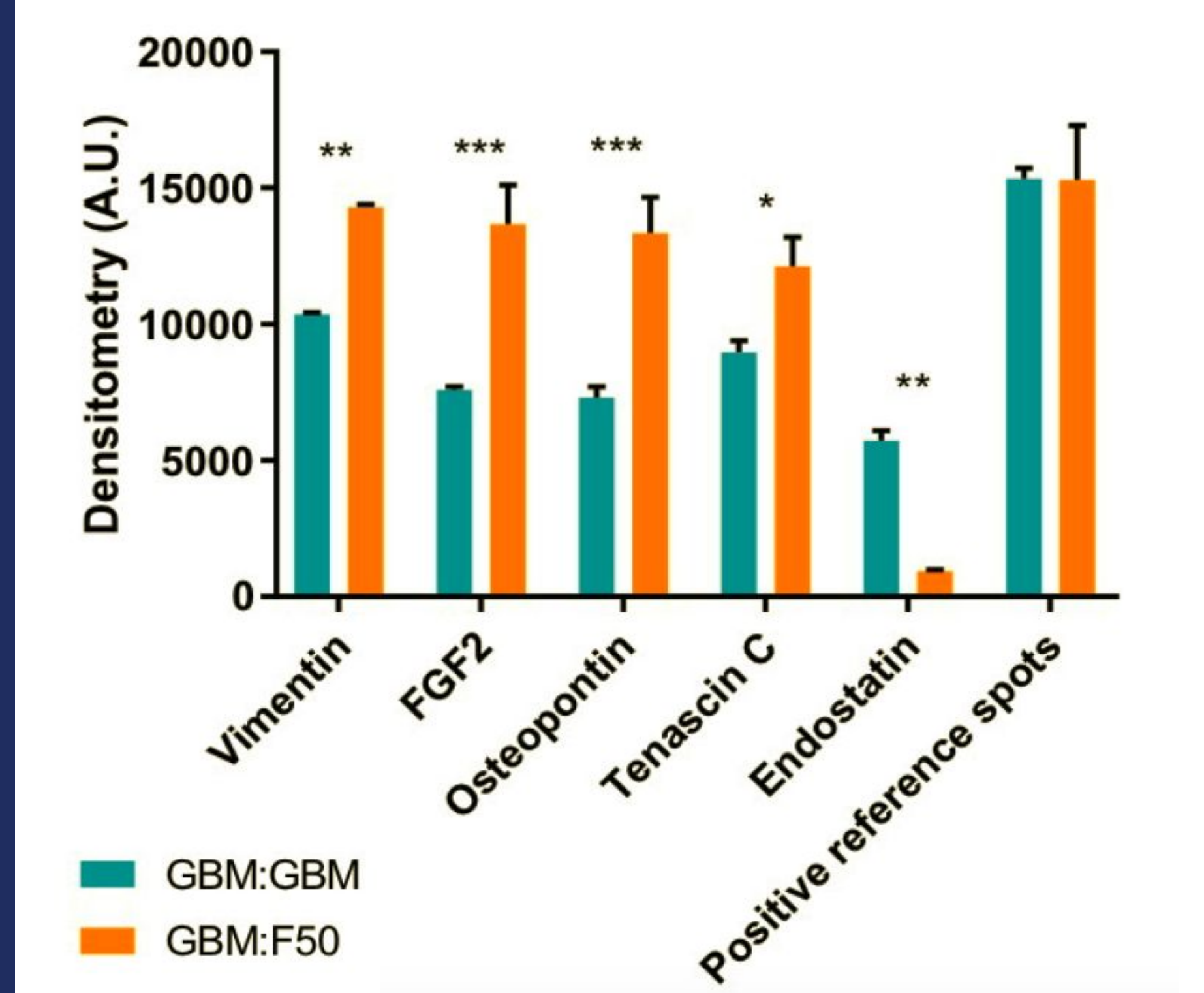


Figure 6. Co-Culture Oncology XL Proteome Profiler array results suggest an upregulation in malignancy promoting proteins. Co-cultures of F50 and 1A GBM were utilized to simulate the environment of glioblastoma neighboring neural stem cells.

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).¹

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

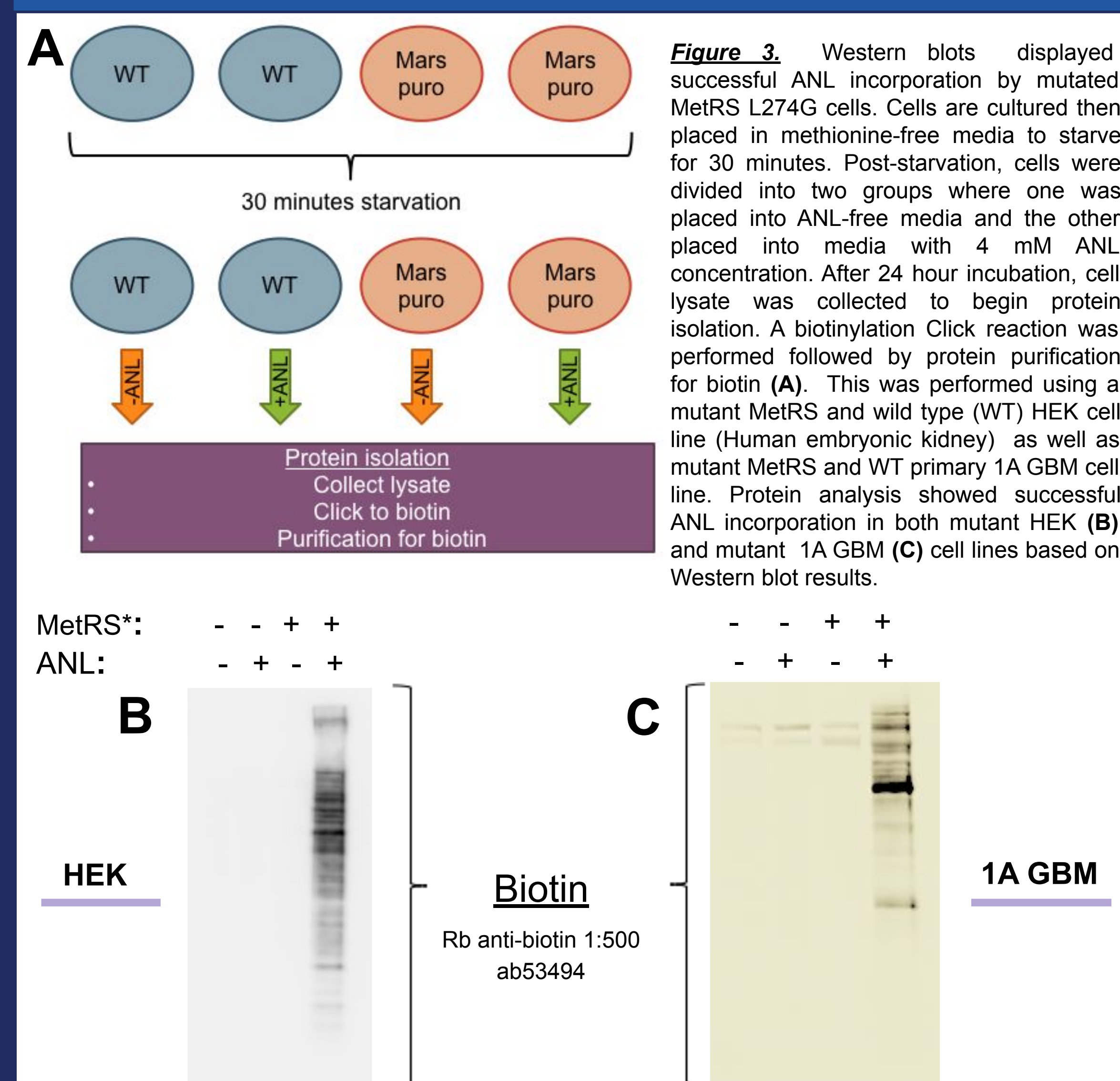
Objectives

- 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.

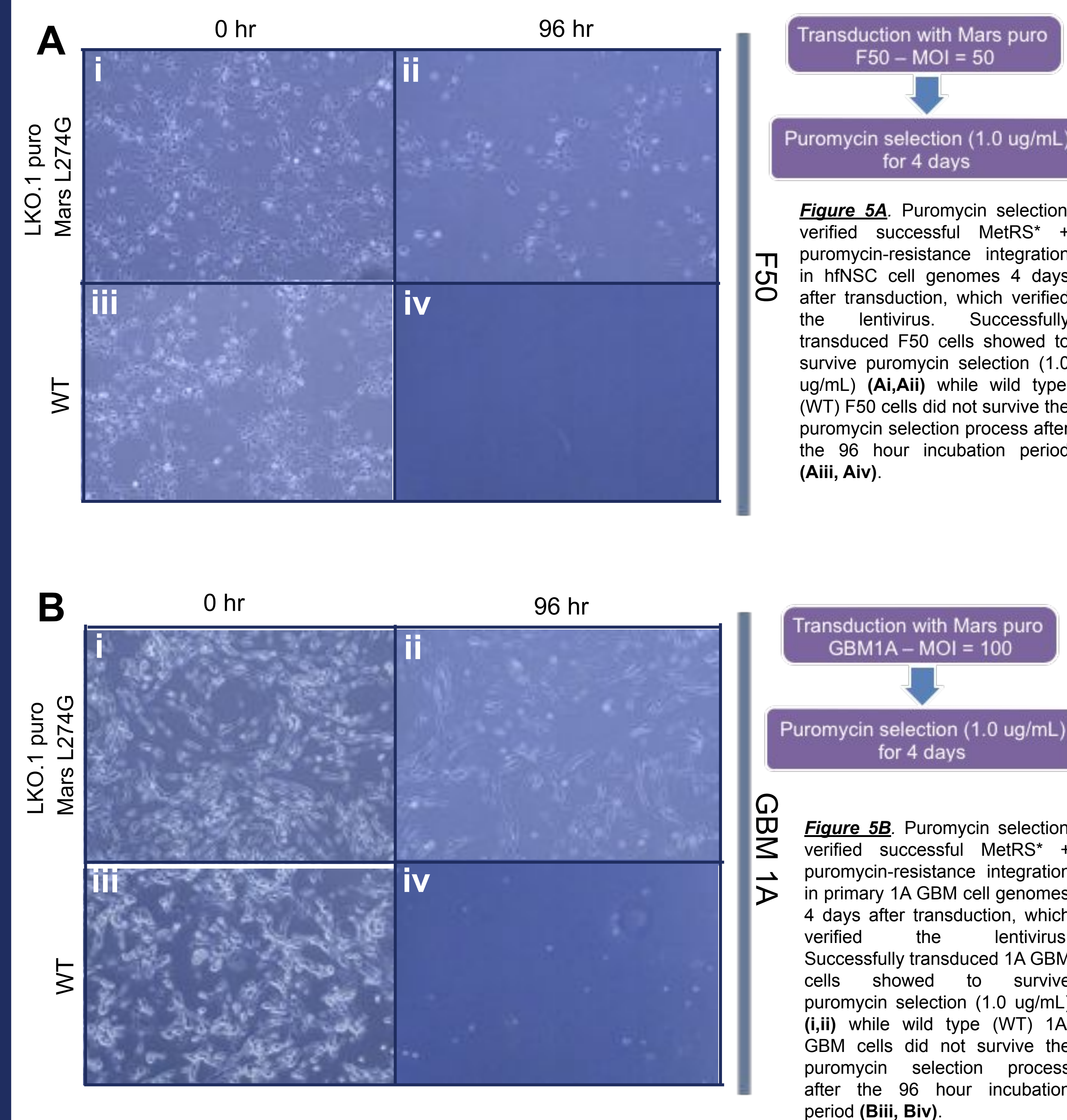
Methods

- 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

Confirmation of ANL incorporation



Puromycin selection of transduced cells



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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- Sinnaeve J, Mobley BC, Ihrie RA. Space invaders: brain tumor exploitation of the stem cell niche. *Am J Pathol.* 2018;188(1):29–38. doi: 10.1016/j.ajpath.2017.08.029.