MOL231

Regulation of mRNA processing by lipid signaling in endometrial cancer cells

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Background

mRNA processing, including alternative splicing, is targeted by cancer cells, generating tumour-promoting cancer mRNA variants. Dysregulation of this process is not well understood but recent studies have shown a potential implication of the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway (1). PI3K generates phosphatidylinositol(3,4,5)triphosphate(PtdIns(3,4,5)P_3), a signaling lipid that recruits Akt to the plasma membrane via its PH domain leading to its full activation via phosphorylation on Ser473 (Figure 1). This promotes various downstream processes such as cell survival and proliferation (2).

In cancer cells, the PI3K/Akt pathway is hyperactive due to loss of PTEN and/or SHIP2 causing persistent PtdIns(3,4,5)P_3 accumulation (Figure 1), which prevents cell apoptosis and promotes tumor growth.

AIM: To create molecular tools to manipulate the levels of PtdIns(3,4,5)P_3 in cancer cells that are PTEN and SHIP2 negative, and use the tools to determine mRNA processing of a known cancer-promoting mRNA target (*NUMB*).

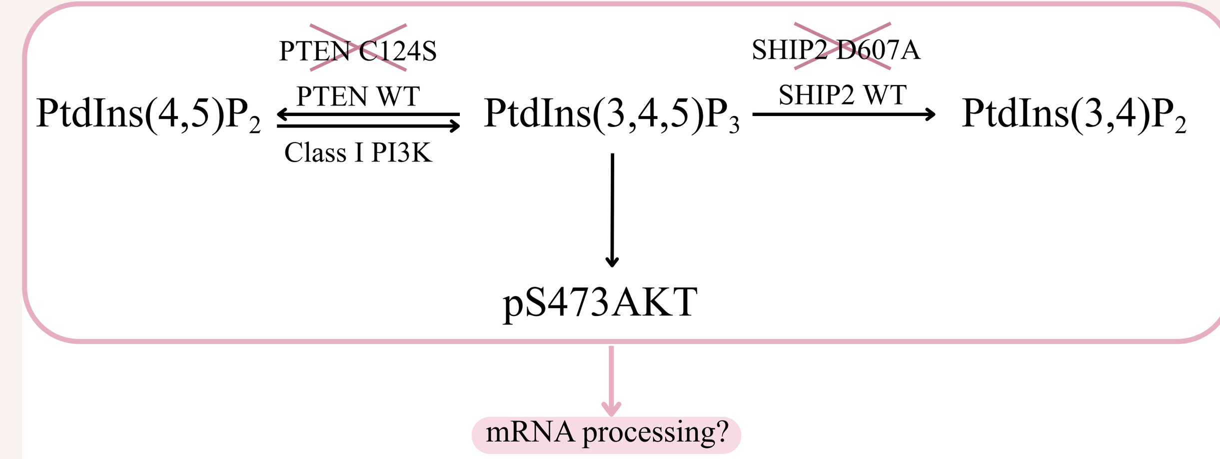


Figure 1: PtdIns(3,4,5)P_3 metabolism and potential regulation of mRNA processing. PtdIns(3,4,5)P_3 is generated from PtdIns(4,5)P_2 by class I PI3K, and signals for the phosphorylation of AKT. PTEN and SHIP2 are enzymes responsible for the dephosphorylation of PtdIns(3,4,5)P_3, and the mutations used to make the enzymes phosphatase-dead.

Methods

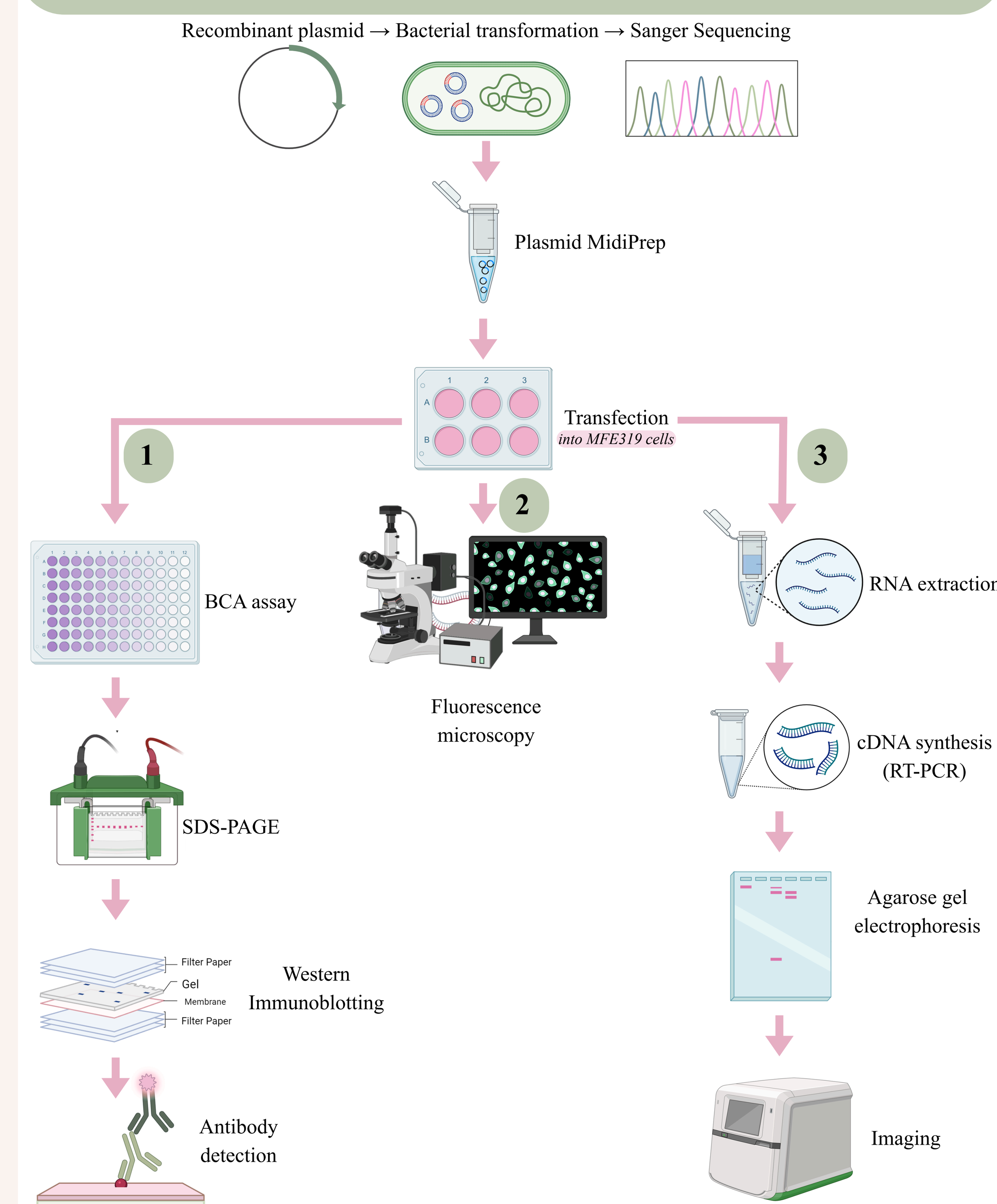
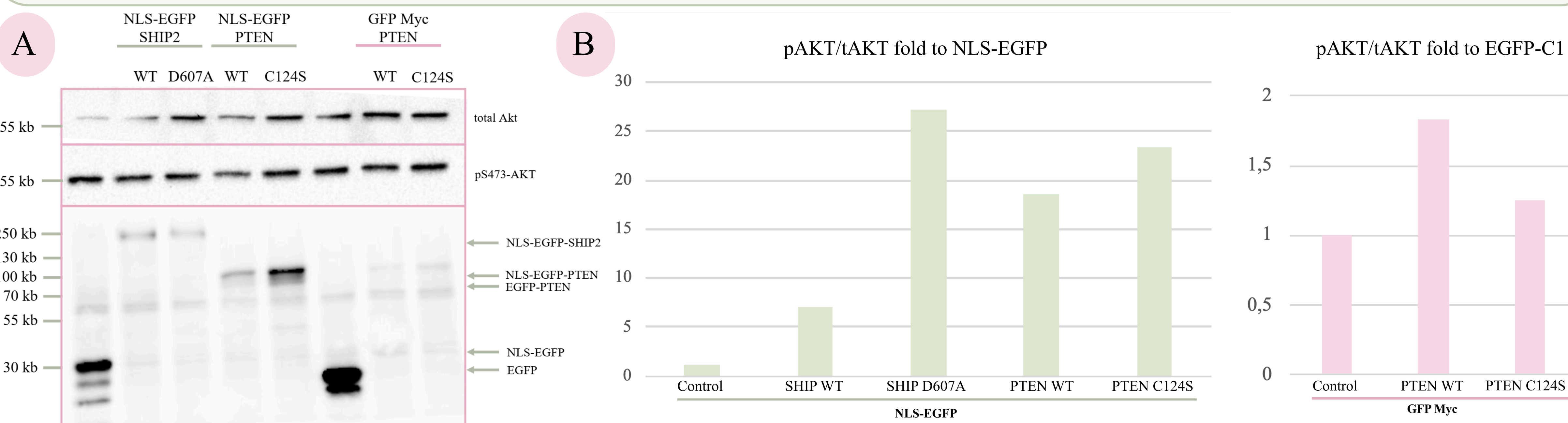


Figure 2: Overview of the experimental work flow.
SHIP2 (WT, D607A) were cloned into NLS-EGFP-C1, and PTEN C124S was generated by site-directed mutagenesis of GFP-Myc PTEN WT prior to transfection into MFE319 cells.
Bottom-left pathway (1): methods used to assess the effect of PTEN and SHIP2 on PI3K pathway activity by western immunoblotting.
Middle pathway (2): fluorescence microscopy to visualize sub-cellular localisation of the molecular tools created with or without NLS.
Bottom-right pathway (3): RT-PCR of the alternative splicing of mRNA target *NUMB*.

Results

1 Western blot: Effect of PTEN and SHIP2 expression on Akt phosphorylation, which indicates PI3K/Akt pathway activity



2 Fluorescence Microscopy: EGFP-PTEN and -SHIP2 Subcellular Localization Patterns

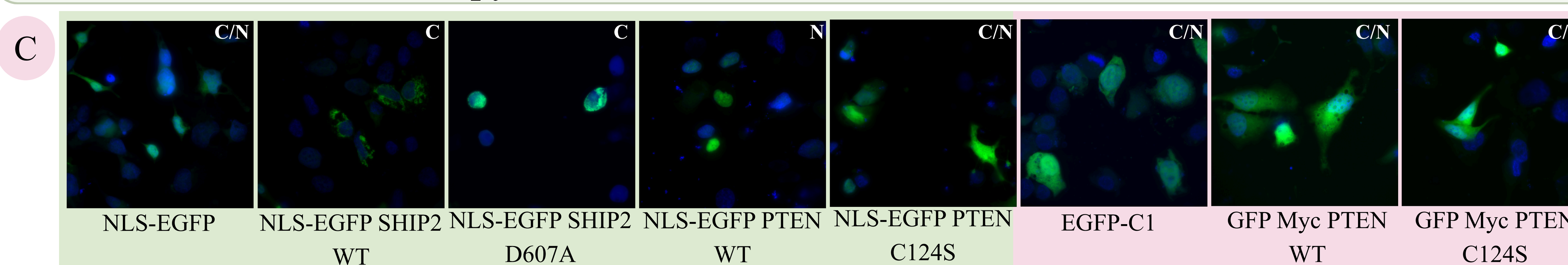


Figure 3: Endometrial cancer MFE-319 cells transfected with NLS-EGFP-SHIP2 (WT, D607A) and -PTEN (WT, C124S) and EGFP-myc-PTEN (WT, C124S). A) Cell lysates were analyzed by Western immunoblotting. **B)** pAKT levels normalized to total AKT are shown as fold change relative to the appropriate controls. **C)** Fluorescence microscopy images of EGFP tagged PTEN and SHIP2 labelled with DAPI. N: Nuclear, C: Cytoplasmic, C/N: Cytoplasmic and nuclear

3 Agarose gel electrophoresis: Effect of PTEN and SHIP2 on *NUMB* mRNA processing

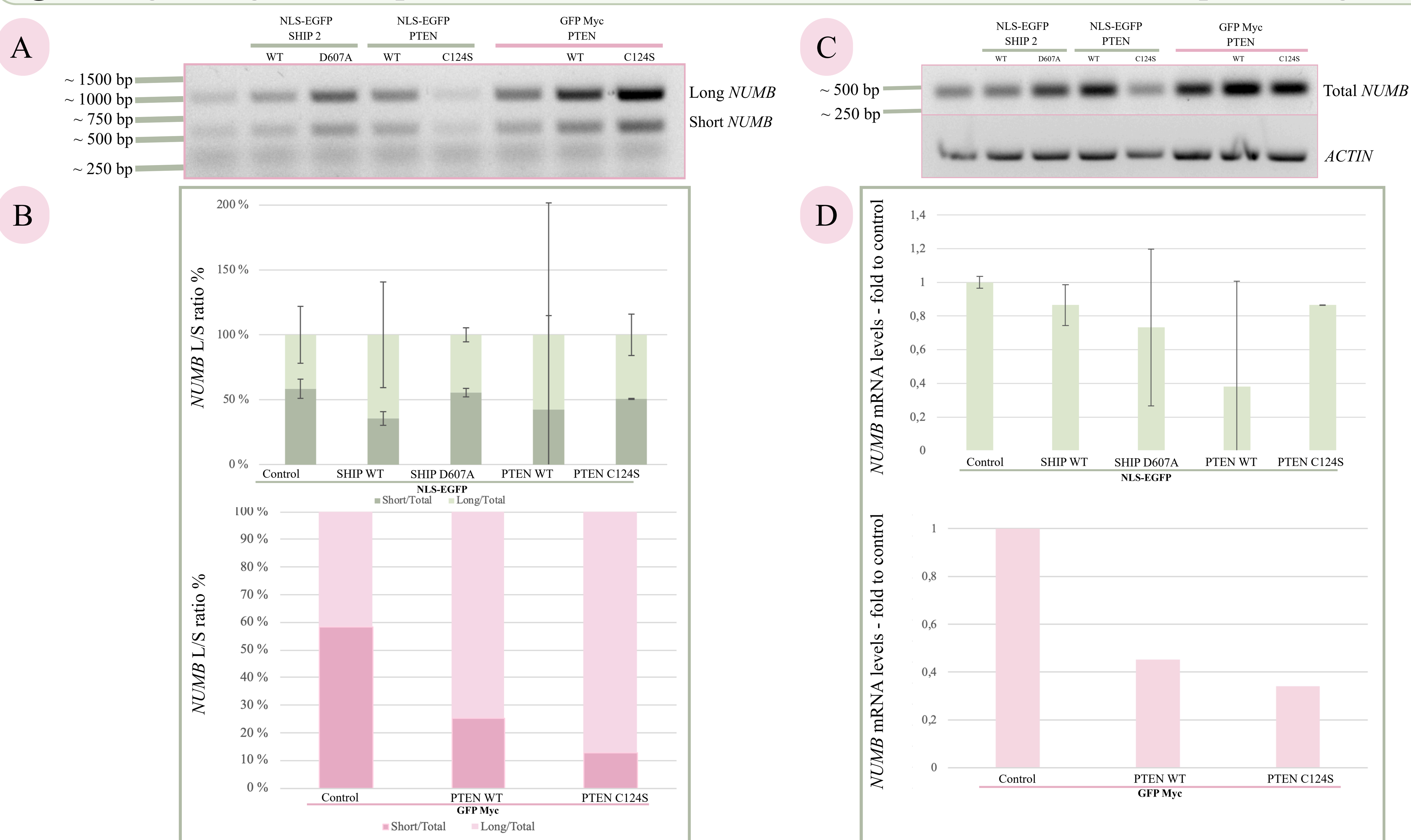
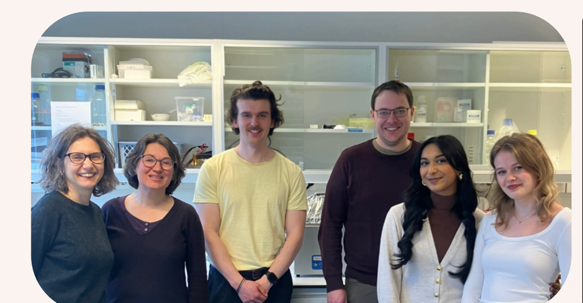


Figure 4: **Effect of PTEN and SHIP2 on *NUMB* mRNA processing in MFE-319 cells**. RT-PCR of MFE319 cells expressing EGFP-SHIP2 (WT, D607A) and -PTEN (WT, C124S) with or without NLS. **A)** *NUMB* Long (+ Exon 12, **oncogenic**) and Short (- Exon 12) PCR products separated by agarose gel electrophoresis. **B)** Quantification of *NUMB* Long and Short mRNA variants. Bars represent the percentage of each variant relative to total *NUMB*. **C)** *NUMB and* b-*ACTIN* PCR products separated by agarose gel electrophoresis. **D)** Quantification of total *NUMB* mRNA levels normalised to b-*ACTIN*.

Conclusion and Future Work

1. NLS-EGFP-PTEN and SHIP2 fusion proteins are well expressed, but the effect on the PI3K pathway is inconclusive.
2. NLS targets PTEN to the nucleus, but SHIP2 remains in the cytoplasm.
3. *NUMB* alternative splicing: no difference were observed.
   total *NUMB* mRNA level: a small decrease was observed in response to the expression of NLS-EGFP-SHIP2.

Targeting the PI3K pathway in cancer cells by re-expressing SHIP2 may affect *NUMB* mRNA levels.
Further experiments are required to confirm this, along with testing of other mRNA cancer targets.



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References

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