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Melanoma is a skin cancer with an ever-increasing incidence and a poor prognosis at the metastatic stage. Most of patients with late-stage disease develop resistance to their treatment, especially targeted therapies, leaving them in a therapeutic limbo. Bufalin is an endogenous cardiotonic steroid, derived from toad venom, which decreases the proliferation and induces the apoptosis of targeted therapy-sensitive and resistant melanoma cells.

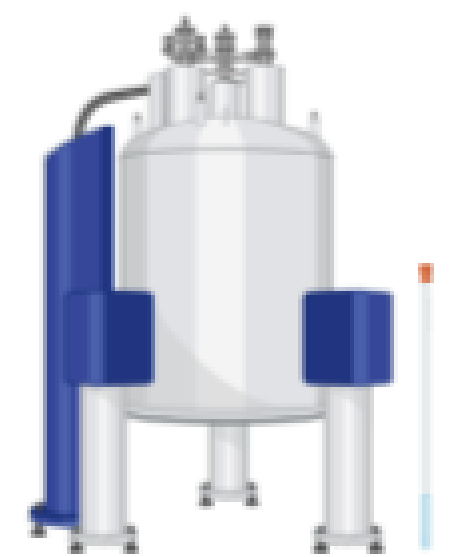
 Investigate Bufalin effects on general metabolism and translation

## MATERIAL & METHODS

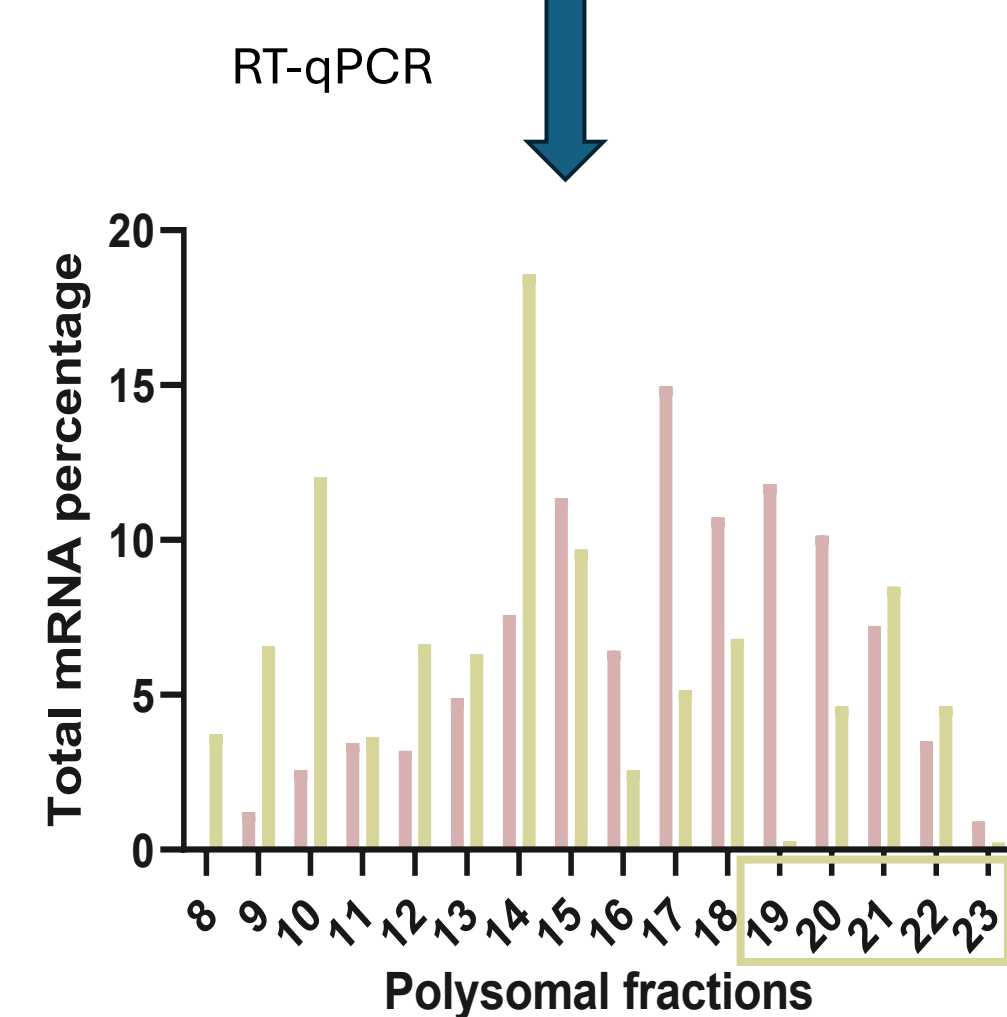
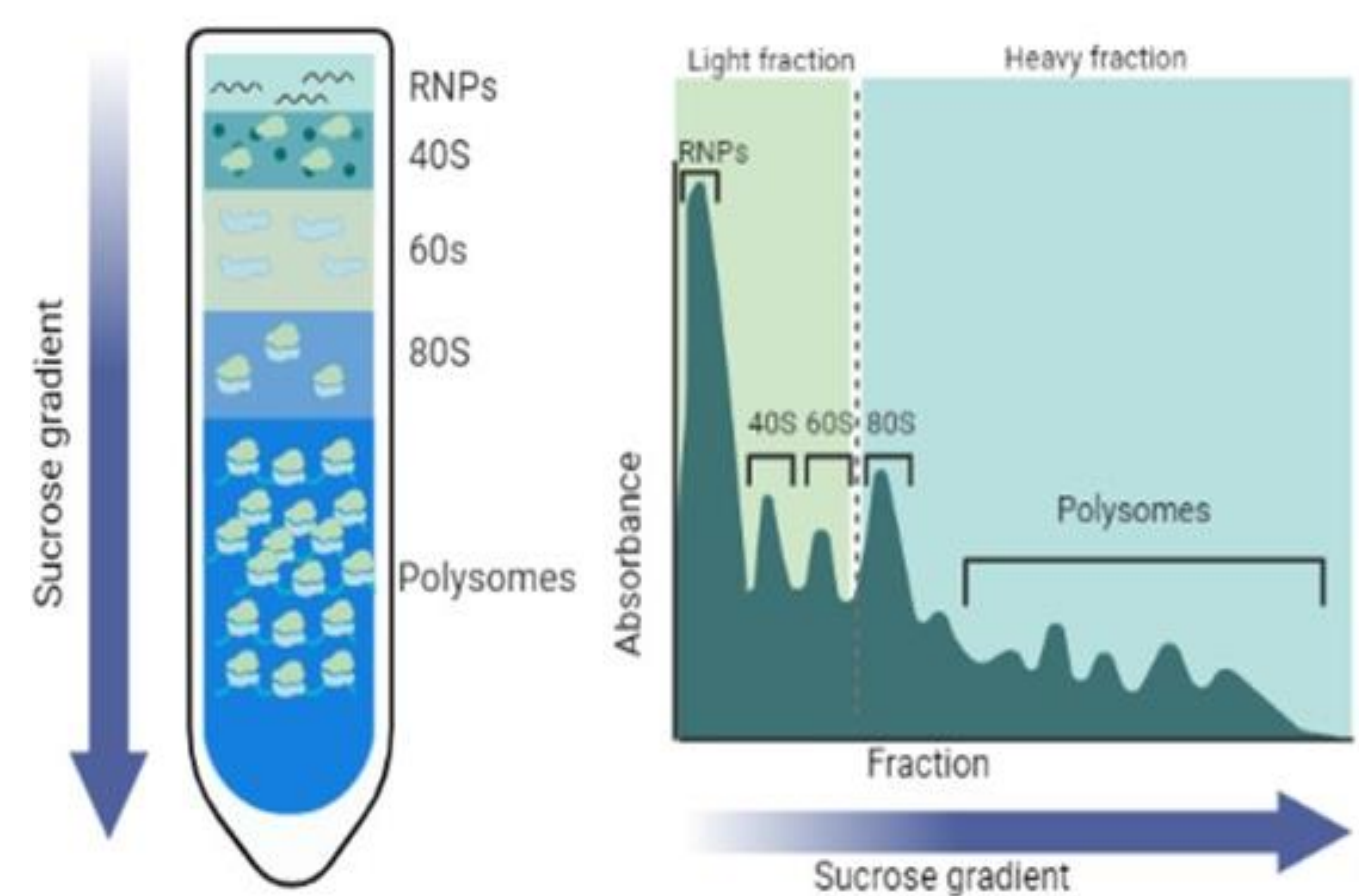
### Cell lines:

V600E BRAF mutated cells → MM074 Dabrafenib & trametinib sensitive  
MM074R Dabrafenib & trametinib resistant

### Techniques:



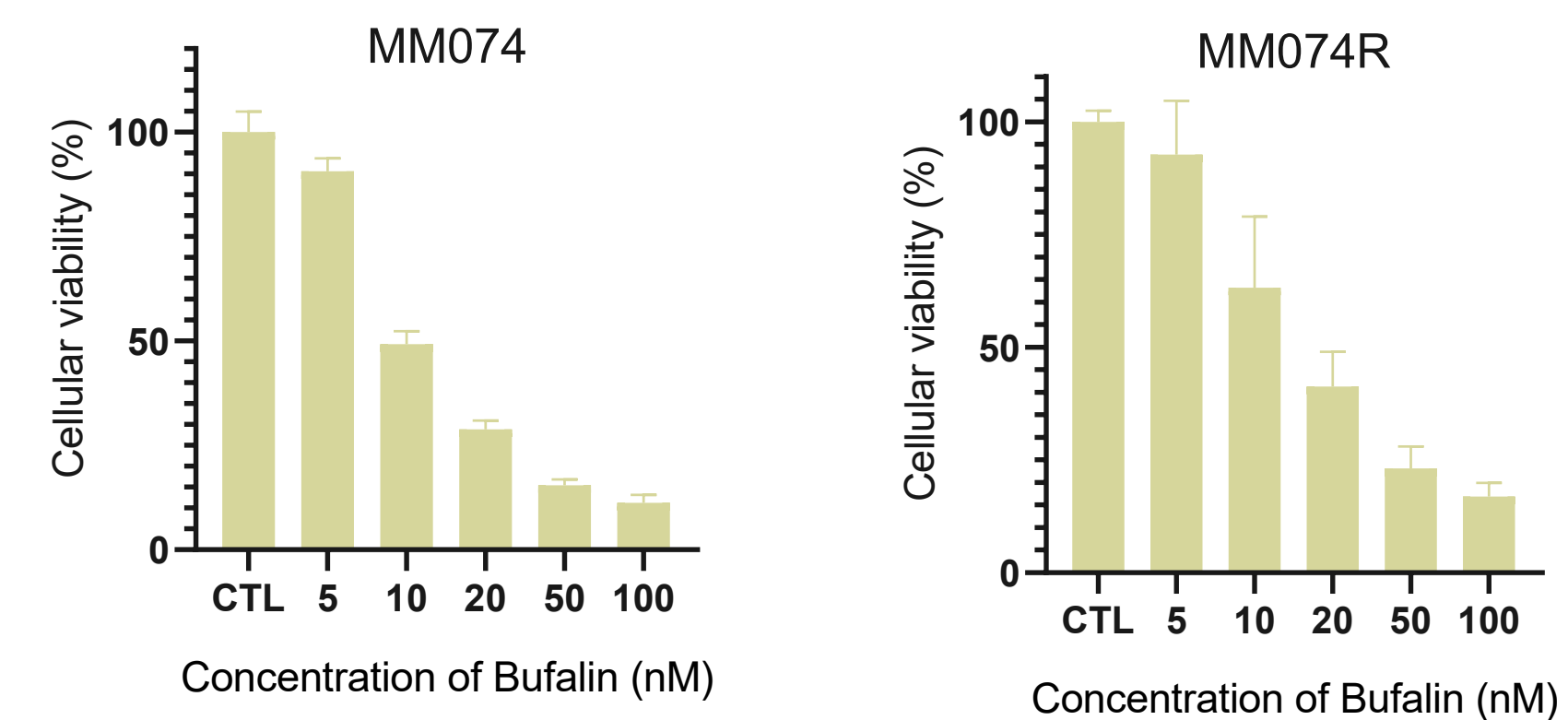
<sup>1</sup>H-NMR spectroscopy technique is used to study all the metabolites of our cells treated with bufalin



The polysome profiling technique is used to study the most highly translated mRNAs by separating the free RNA from the polysomes using a sucrose gradient. Different fractions are formed, ranging from 8 to 23, and are studied using RT-qPCR. An expression score was obtained by multiplying the last 5 fractions (19 to 23, representing fractions containing polysomes) by 1, 2, 3, 4 and 5, respectively.

## RESULTS

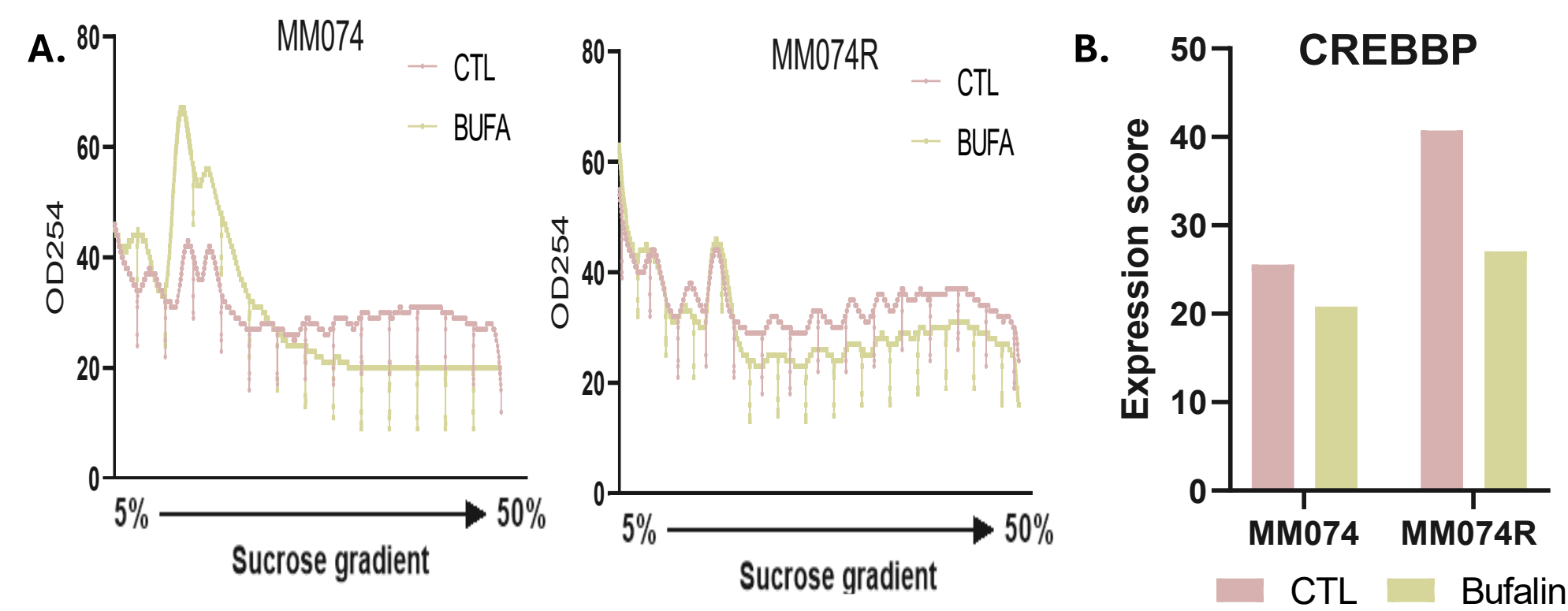
### Bufalin decreases the cellular viability of melanoma cells



**Figure 1: Bufalin effect on cellular viability in sensitive and resistant BRAF-mutated melanoma cells**

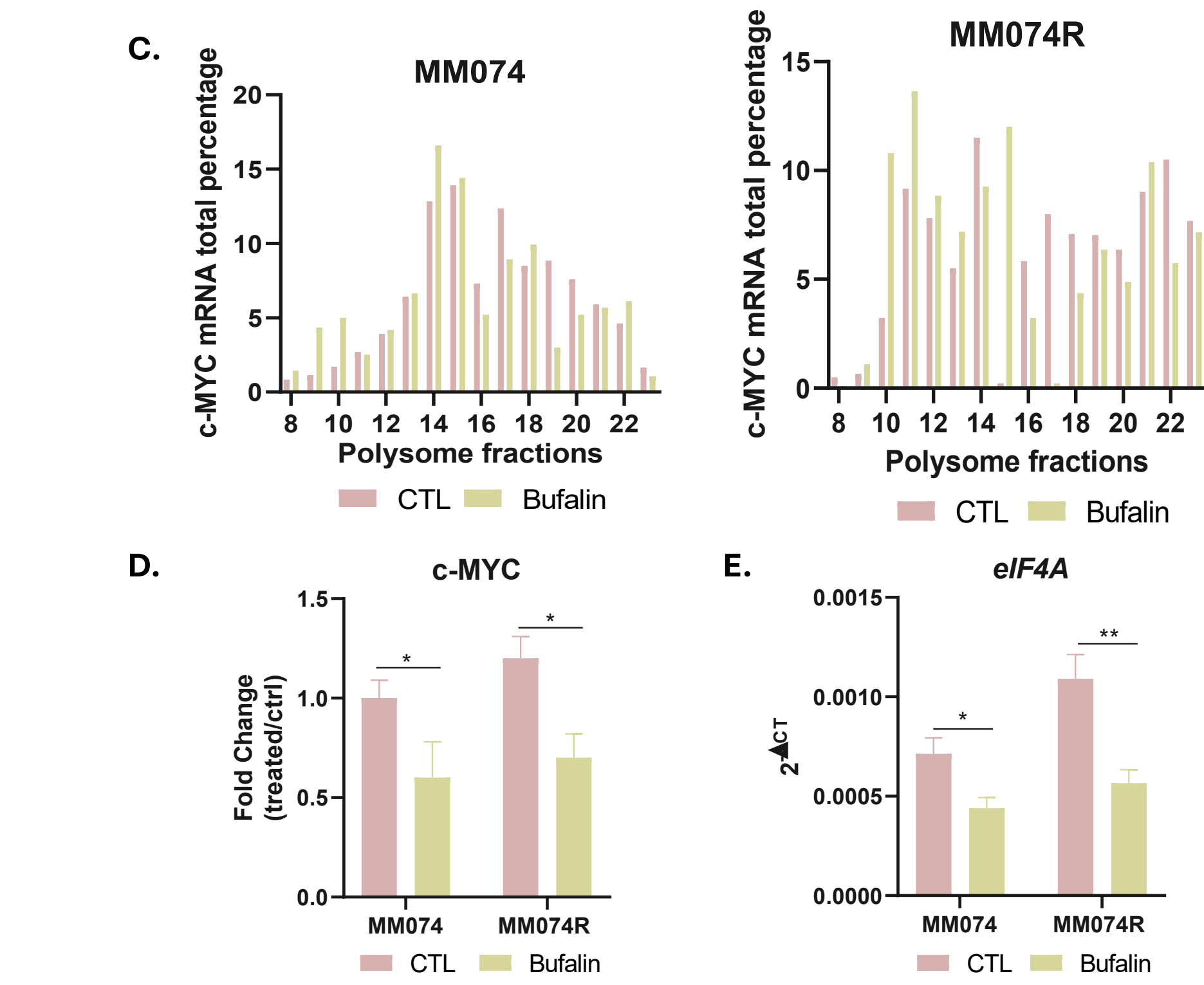
Cellular viability of melanoma cells treated with nanomolar concentrations of bufalin

### Bufalin decreases the general translation in melanoma sensitive and resistant to targeted therapies by acting on the c-MYC/eIF4A loop

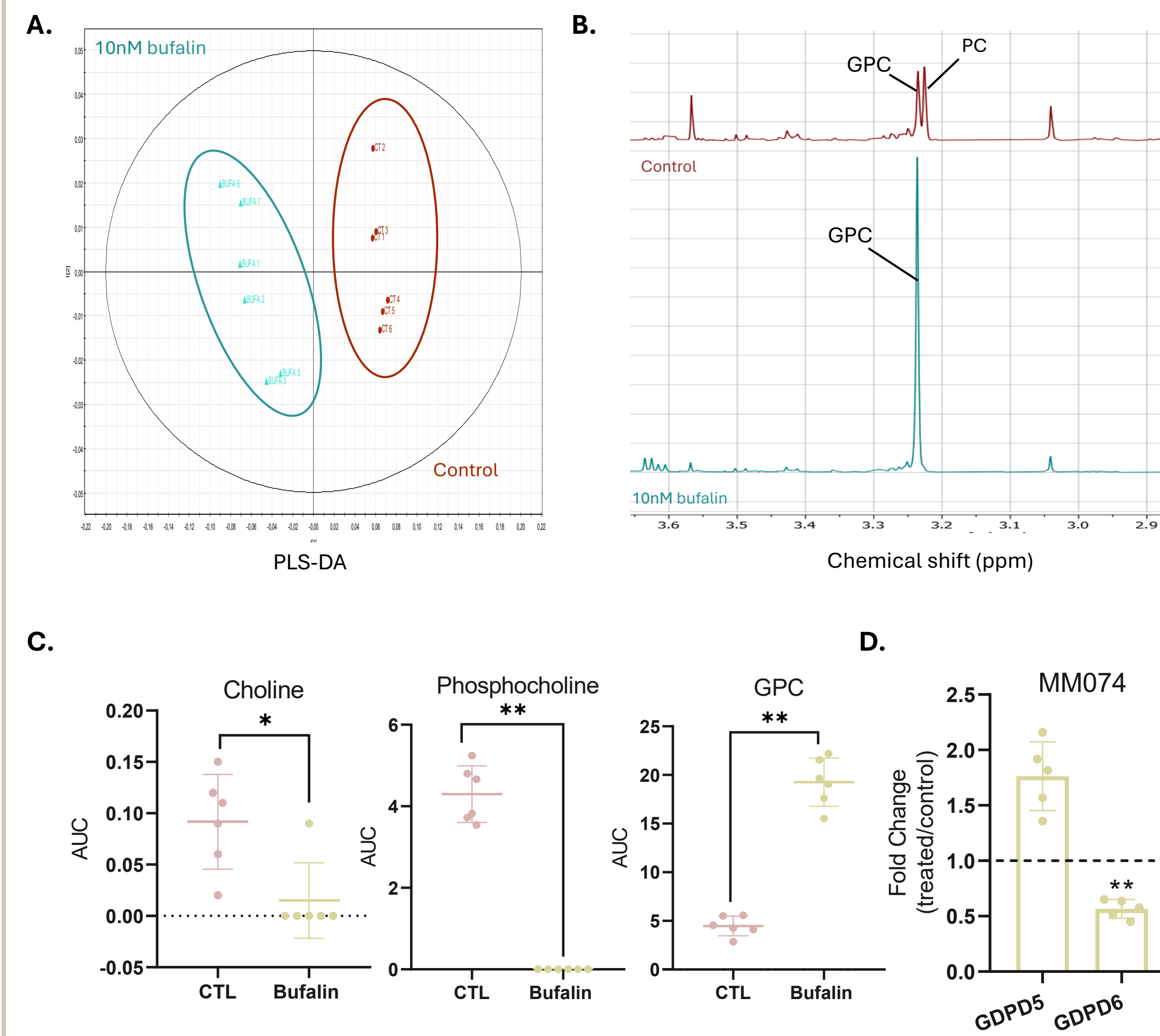


**Figure 2: Bufalin effect on general translation in sensitive and resistant BRAF-mutated melanoma cells by acting on c-MYC/eIF4A loop**

A. General translation of sensitive and resistant melanoma cells treated with 10 nM of bufalin during 12h  
B. Expression score of CREBBP (known target of eIF4F complex) after a treatment with 10 nM of bufalin during 12h



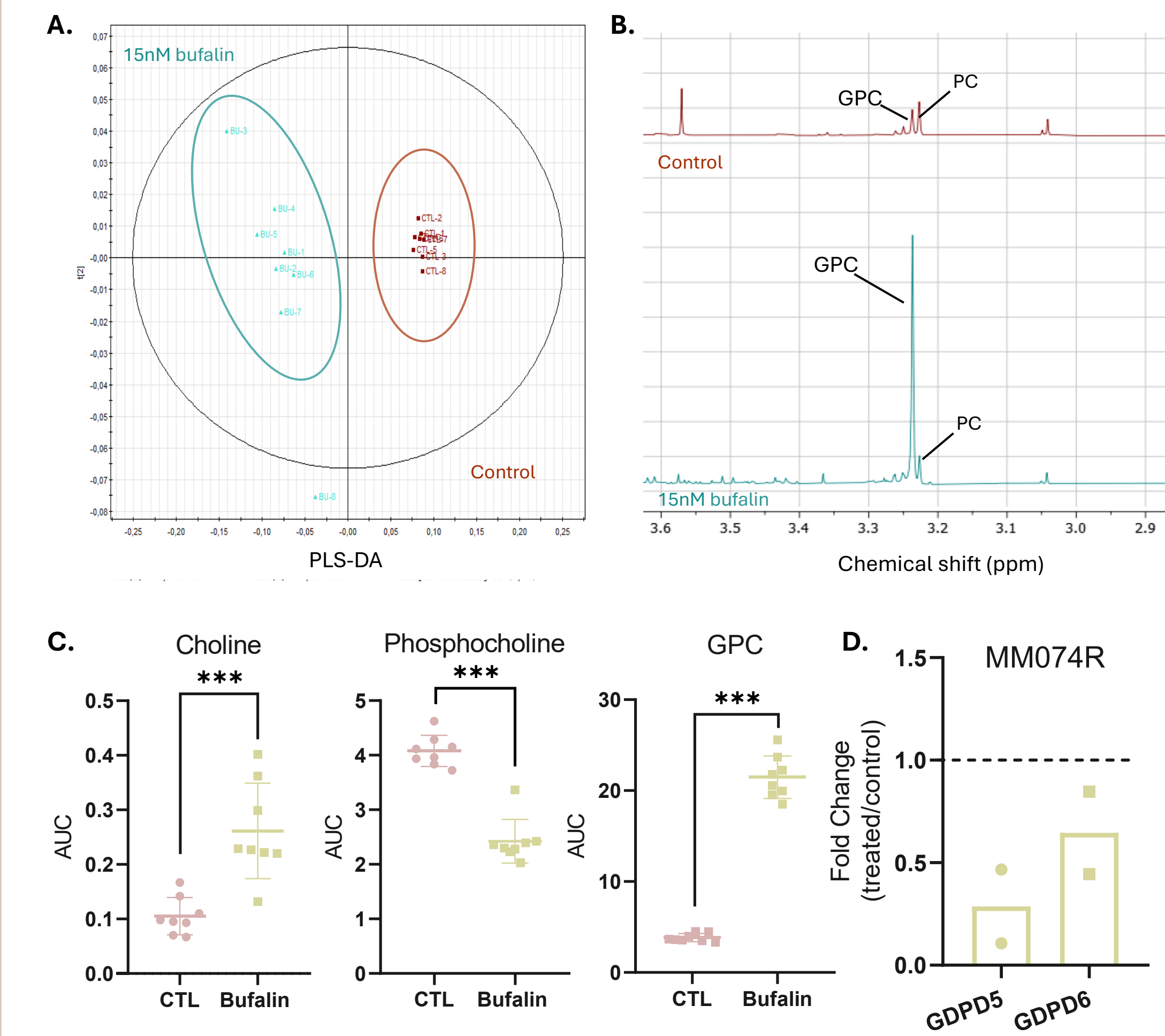
### Bufalin acts on general metabolism and particularly affects the Kennedy Pathway in targeted therapy sensitive melanoma cells



**Figure 3: Bufalin effect on general metabolism in sensitive cells assessing by <sup>1</sup>H-NMR and RT-qPCR**

A. Partial Least Squares Discriminant Analysis (PLS-DA) of <sup>1</sup>H-NMR spectra from samples treated with 10 nM bufalin for 48h vs control.  
B. Spectra showing a major increase in glycerophosphocholine (GPC) and a decrease in choline and phosphocholine (PC). MM074 cells were treated for 48h with 10 nM of bufalin.  
C. Graphs representing the AUC fold change of discriminating metabolites (n=6, mean ± SD, bivariate Wilcoxon test)  
D. Graphs showing GDPD5 and GDPD6 mRNA fold change in MM074 cells treated for 24h with 20 nM of bufalin. (n=5, t-test)

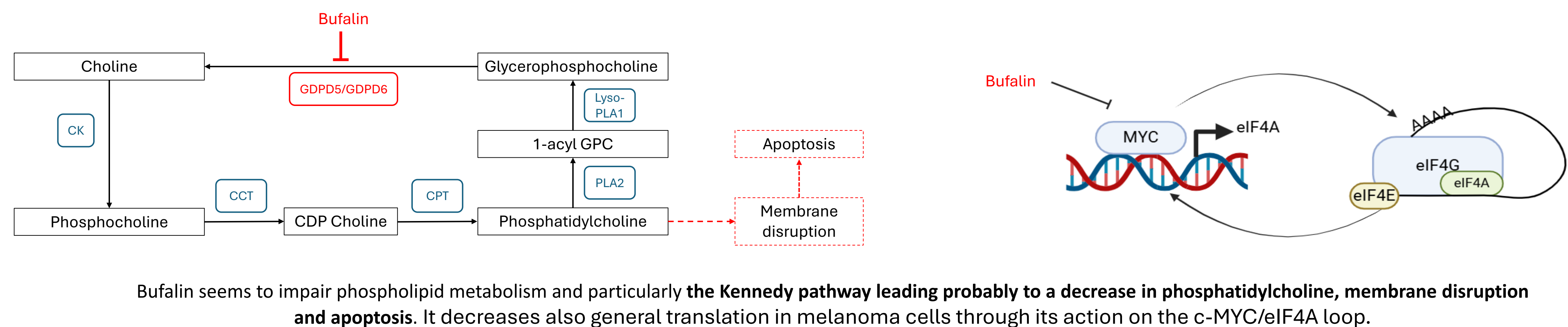
### Bufalin acts on general metabolism and particularly affects the Kennedy Pathway in targeted therapy resistant melanoma cells



**Figure 4: Bufalin effect on general metabolism in resistant cells assessing by <sup>1</sup>H-NMR and RT-qPCR**

A. Partial Least Squares Discriminant Analysis (PLS-DA) of <sup>1</sup>H-NMR spectra from samples treated with 15 nM bufalin for 48h vs control.  
B. Spectra showing a major increase in choline and glycerophosphocholine (GPC) and a decrease in phosphocholine (PC). MM074R cells were treated for 48h with 15 nM of bufalin.  
C. Graphs representing the AUC fold change of discriminating metabolites (n=8 mean ± SD, bivariate Wilcoxon test)  
D. Graphs showing GDPD5 and GDPD6 mRNA fold change in MM074R cells treated for 24h with 30 nM of bufalin.

## DISCUSSION



## ACKNOWLEDGEMENT

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## CONTACT