


Bufalin inhibits c-MYC/eIF4A loop and β -oxidation enzyme translation, fighting melanoma resistance to targeted therapies

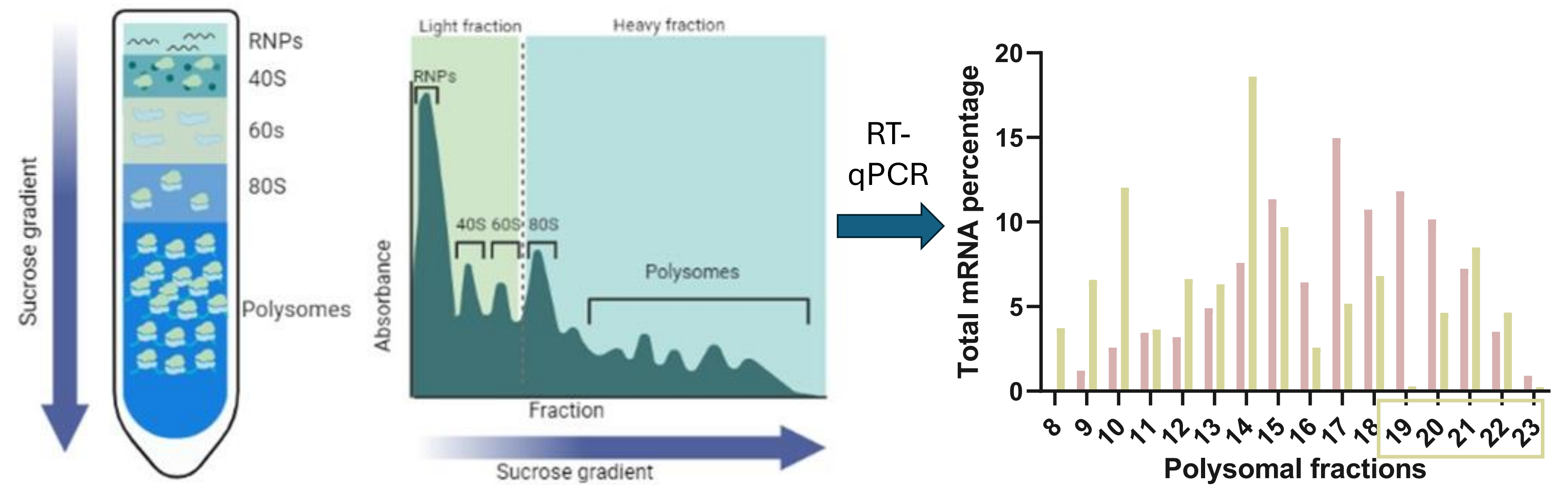
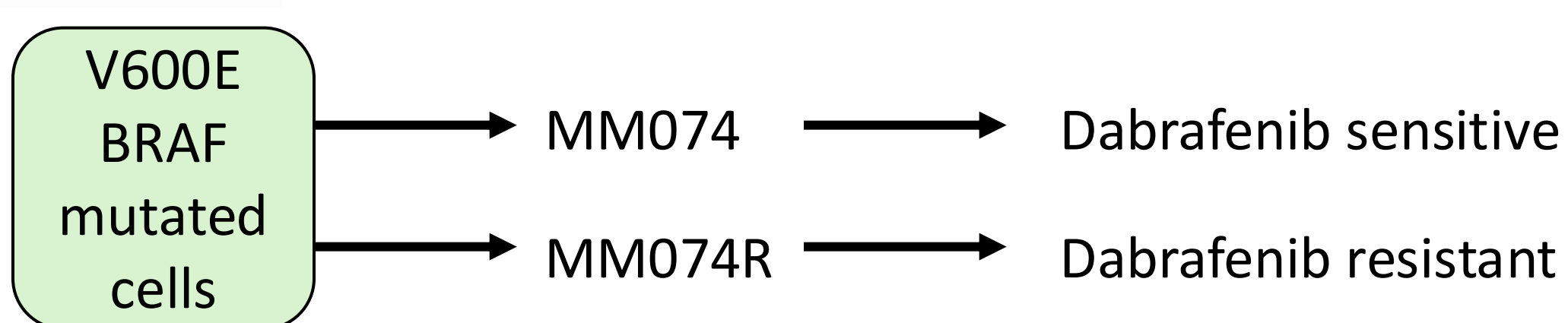
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Melanoma is a skin cancer with an ever-increasing incidence and a poor prognosis at the metastatic stage. Most of patients with late-stage disease develop resistance to their treatment, especially targeted therapies, leaving them in a therapeutic limbo. 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's effects on translation and β -oxidation to prevent resistance occurrence.

Cell lines:



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 general translation in melanoma sensitive and resistant to Dabrafenib

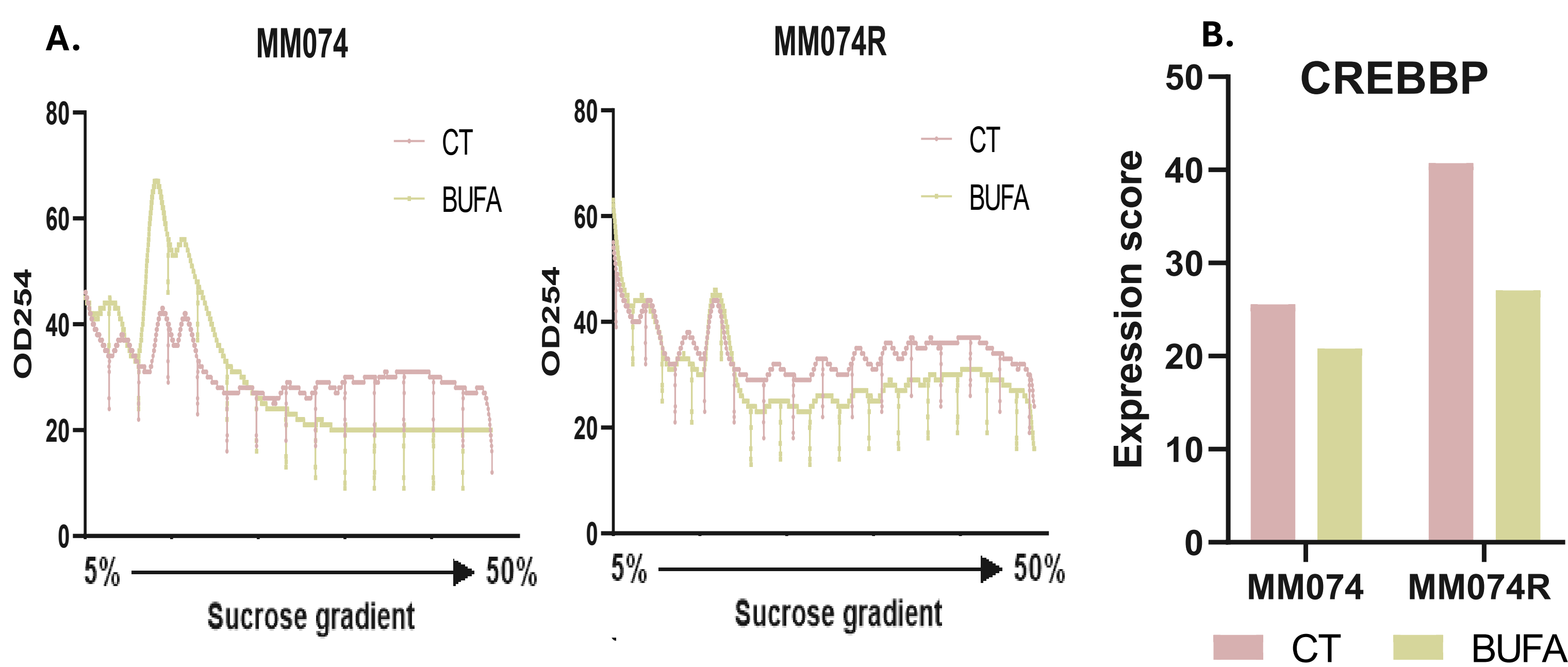


Figure 1: Bufalin effect on general translation in sensitive and resistant BRAF-mutated melanoma cells
A. General translation of sensitive and resistant melanoma cells treated with 10 nM of bufalin during 12h
B. Expression score of CREBBP (known target of eIF4F complex) after a treatment with 10 nM of bufalin during 12h

Bufalin decreases β -oxidation enzyme translation

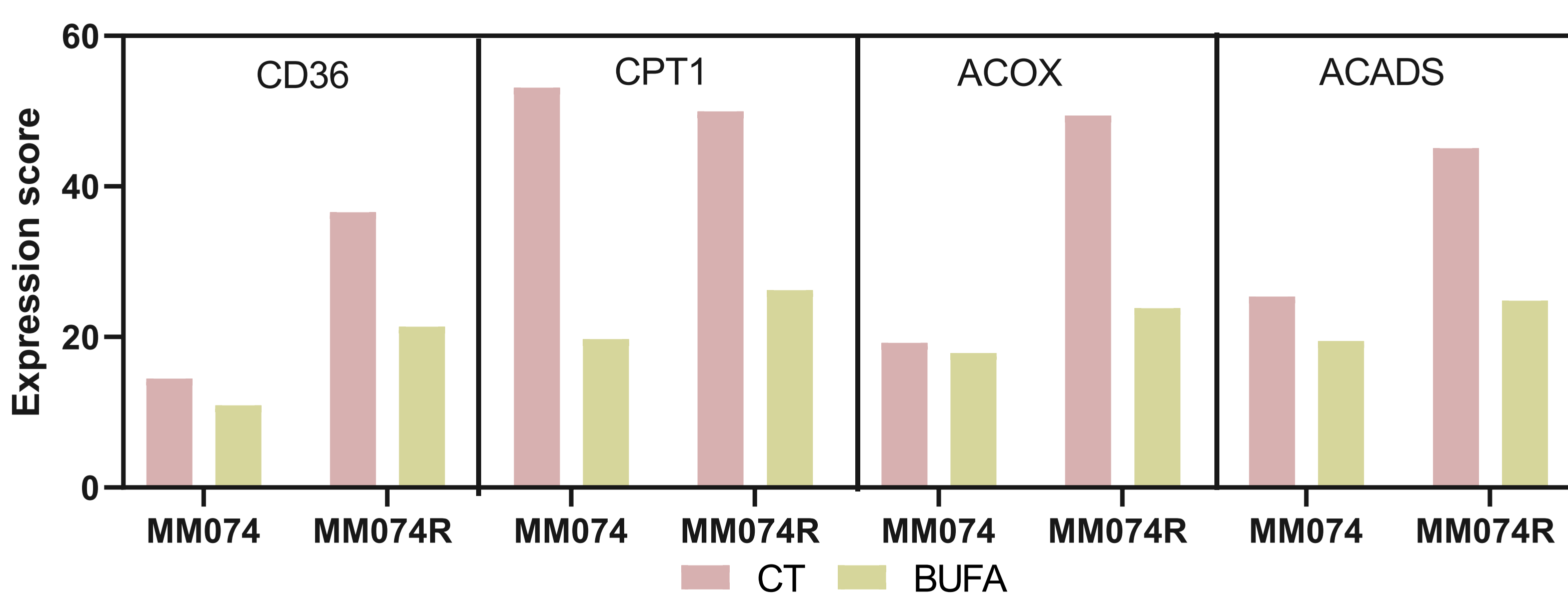


Figure 3: Bufalin effect on β -oxidation enzyme translation

Graphs representing the expression score, obtained after polysome profiling and RT-qPCR experiment, of enzymes involved in β -oxidation when treated during 12h with 10 nM of bufalin

Bufalin acts on the positive loop between c-MYC and eIF4A

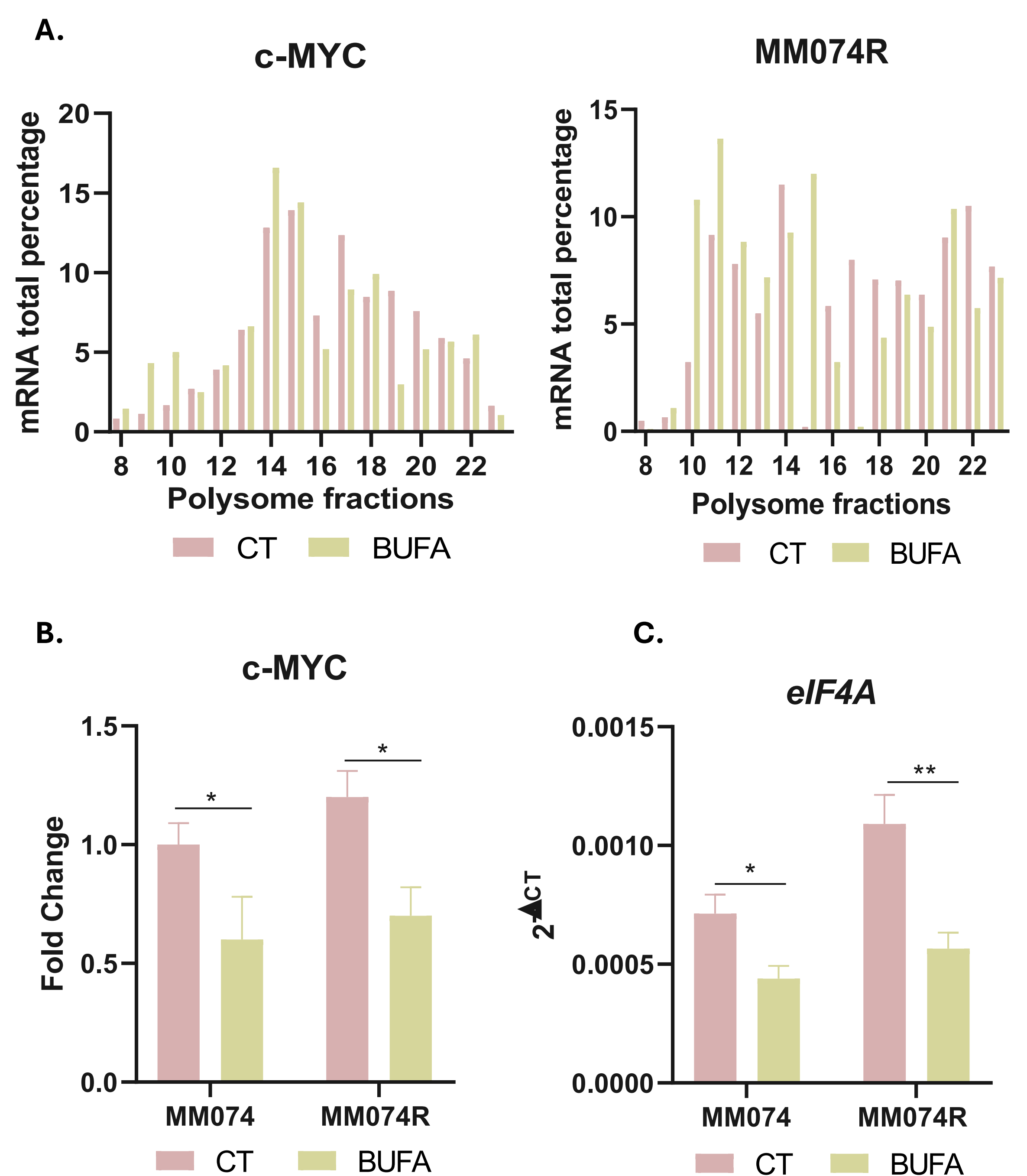
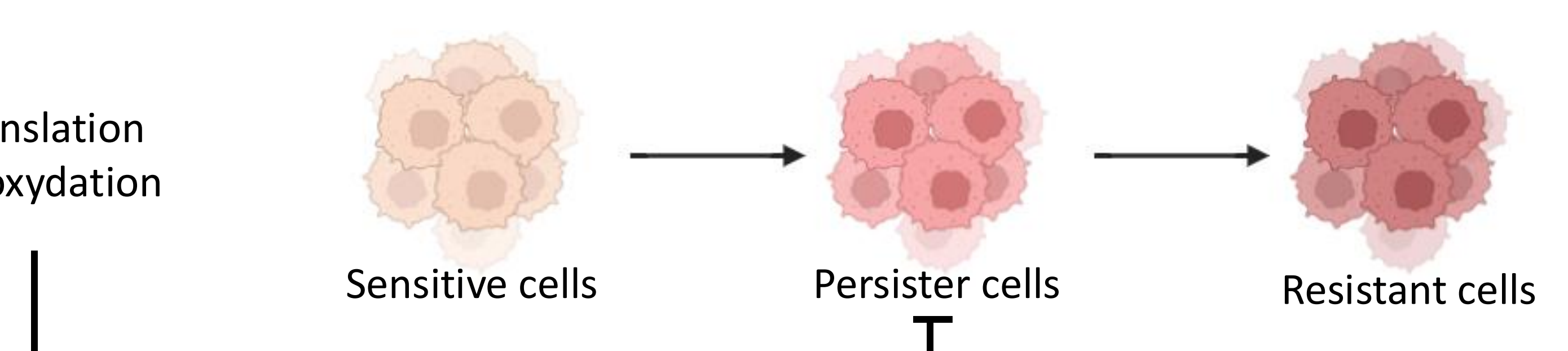
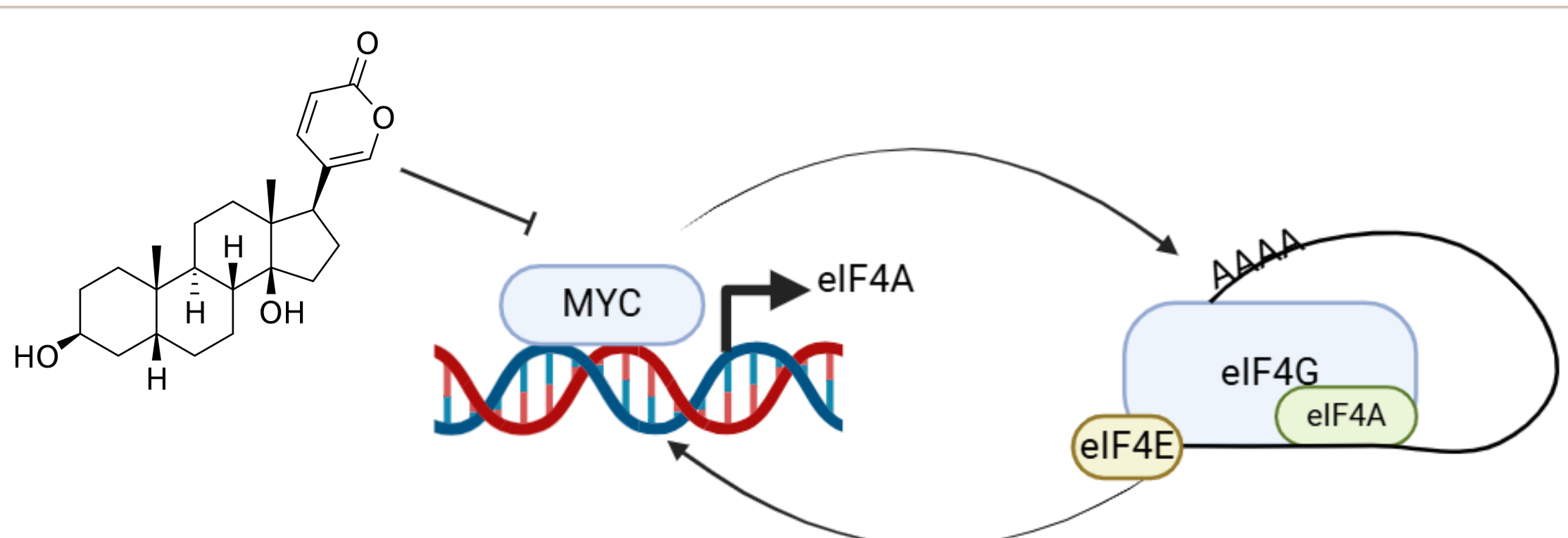


Figure 2: Bufalin effect on c-MYC/eIF4A loop

A. Graphs showing the decrease in the percentage of mRNA encoding c-MYC in each polysomal fraction in dabrafenib-sensitive (MM074) and -resistant (MM074-R) BRAF mutated melanoma cell lines. Cells were treated with 10 nM of bufalin during 12h. MM074 score: CT 13.66 vs BUFA 11.9 (p=0.047), MM074R score: CT 25.4 vs BUFA 21.17 (p=0.043)
B. Graph representing the c-MYC protein expression decrease induced by 10 nM of bufalin during 48h (n=3, mean \pm SD)
C. Graph showing the eIF4A mRNA decrease induced by 10 nM of bufalin during 24h (n=3, mean \pm SD)

Discussion



Bufalin decreases general translation in melanoma cells through its action on the c-MYC/eIF4A loop by decreasing c-MYC transcriptional activity and eIF4F complex activity. It induces a decrease in β -oxidation enzyme translation that could prevent resistance development by acting on persister cells.