

# Suicide gene therapy directed by miRNA activity in lung adenocarcinoma

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## BACKGROUND AND AIMS:

**Suicide gene (SG) therapy** is a technology which consists in expressing a toxic gene into tumor cells. One of the most studied SG systems is the herpes simplex virus thymidine-kinase (**HSV-TK**) that converts the non-toxic prodrug ganciclovir (**GCV**) into a guanosine analog which induces cell apoptosis. However, there are problems regarding the expression of the toxic gene in non-tumor cells, which would cause unwanted cell death.

Micro-RNAs (**miRNAs**) are short RNA sequences that regulate gene expression by binding to messenger RNA and impeding its translation. Some miRNAs are dysregulated in tumors and they act as oncogenic miRNAs (oncomiRs) or tumor-suppressor miRNAs during tumorigenesis. **Let-7** is a tumor-suppressor miRNA family comprising 9 mature miRNAs that are **downregulated in several tumor subtypes** including lung adenocarcinoma (LUAD).

The aim for our study is to **direct the expression of a SG into LUAD cells** by taking advantage of the **let-7 downregulation in tumors**, thus increasing safety and preventing off-target cell death.

## METHODOLOGY AND RESULTS:

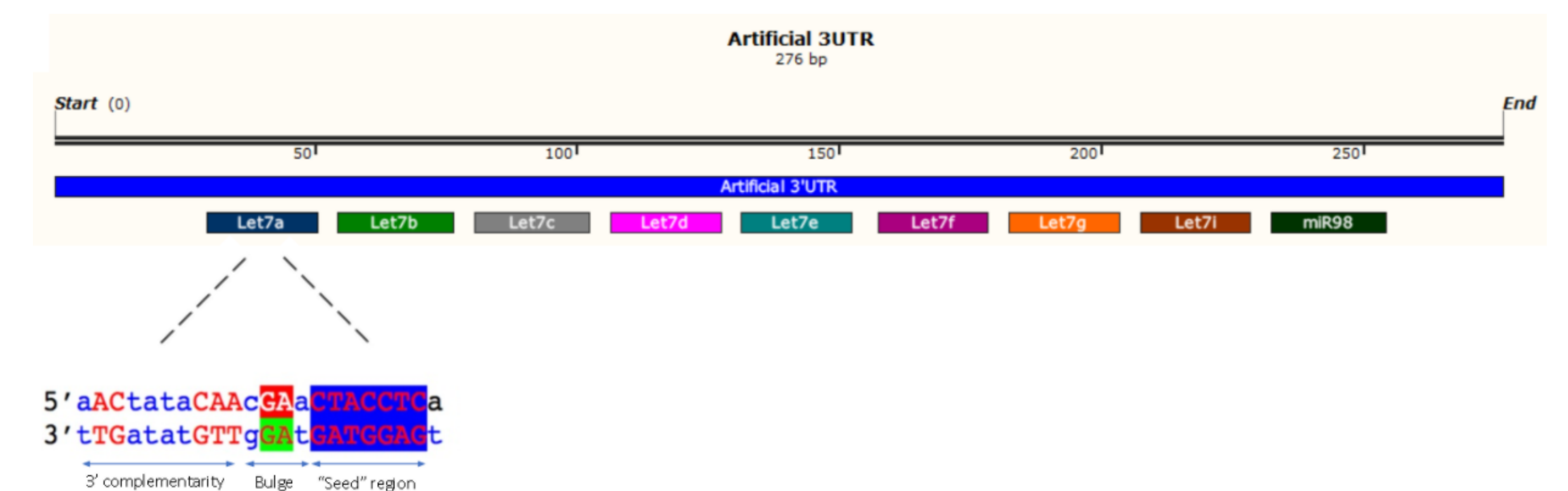


Figure 2. Artificial 3'-UTR design containing binding sites for all the members of the let-7 miRNA family.

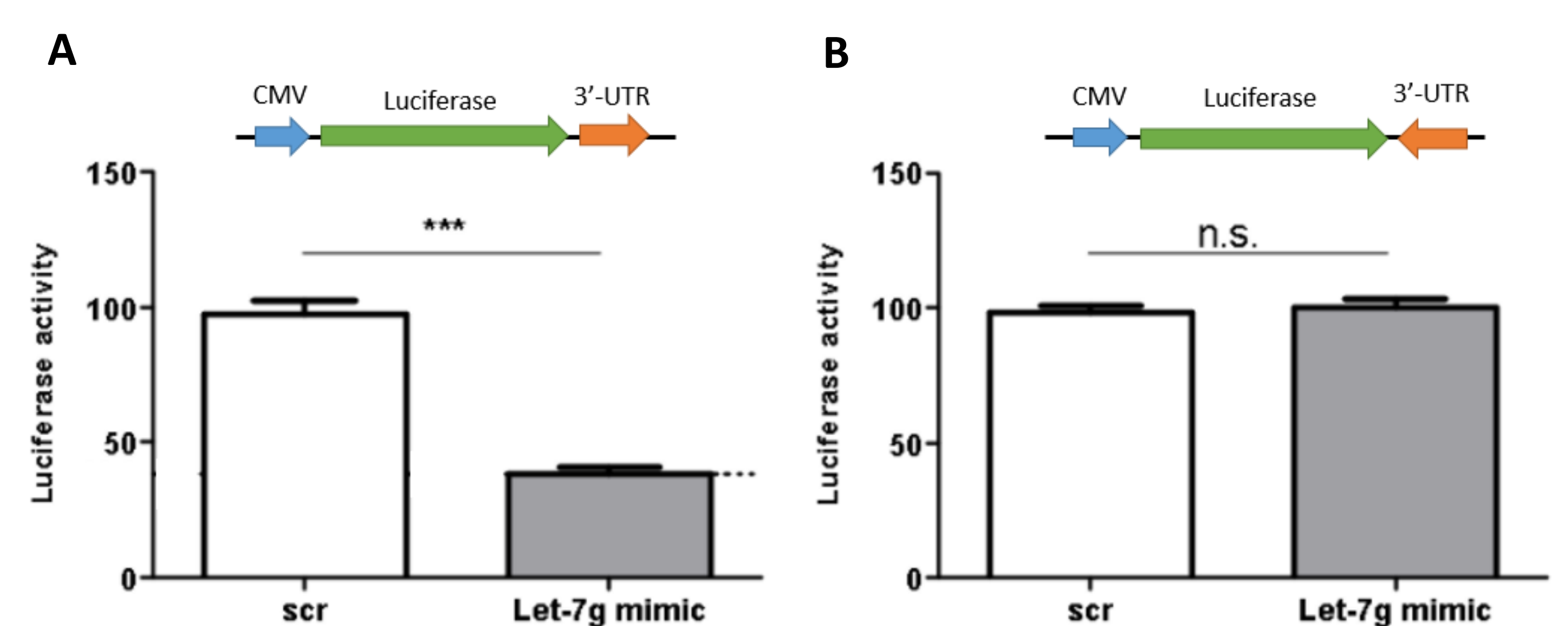


Figure 3. Luciferase assays testing the proper functioning of the artificial 3'-UTR. Introduction of a let-7 miRNA mimic impairs luciferase activity (A), but not when the 3'-UTR is inverted (B).

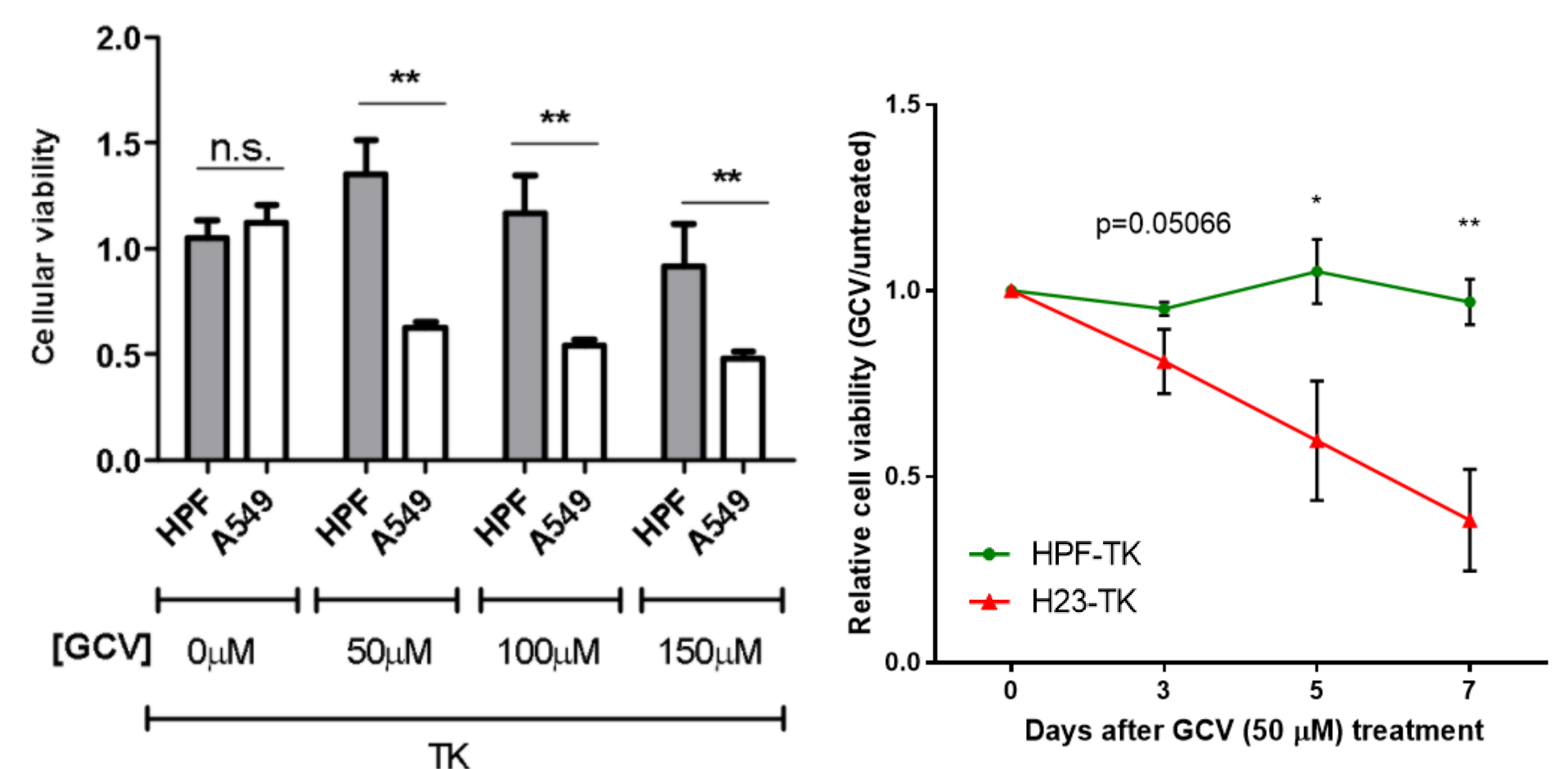


Figure 4. Cell viability assays testing GCV effect on the HSV-TK infected cell lines HPF (non-tumor, ↑ let-7 expression), A549 and H23 (LUAD, ↓ let7 expression). Cell viability was measured by resazurin fluorescence assay and normalized by the untreated condition.

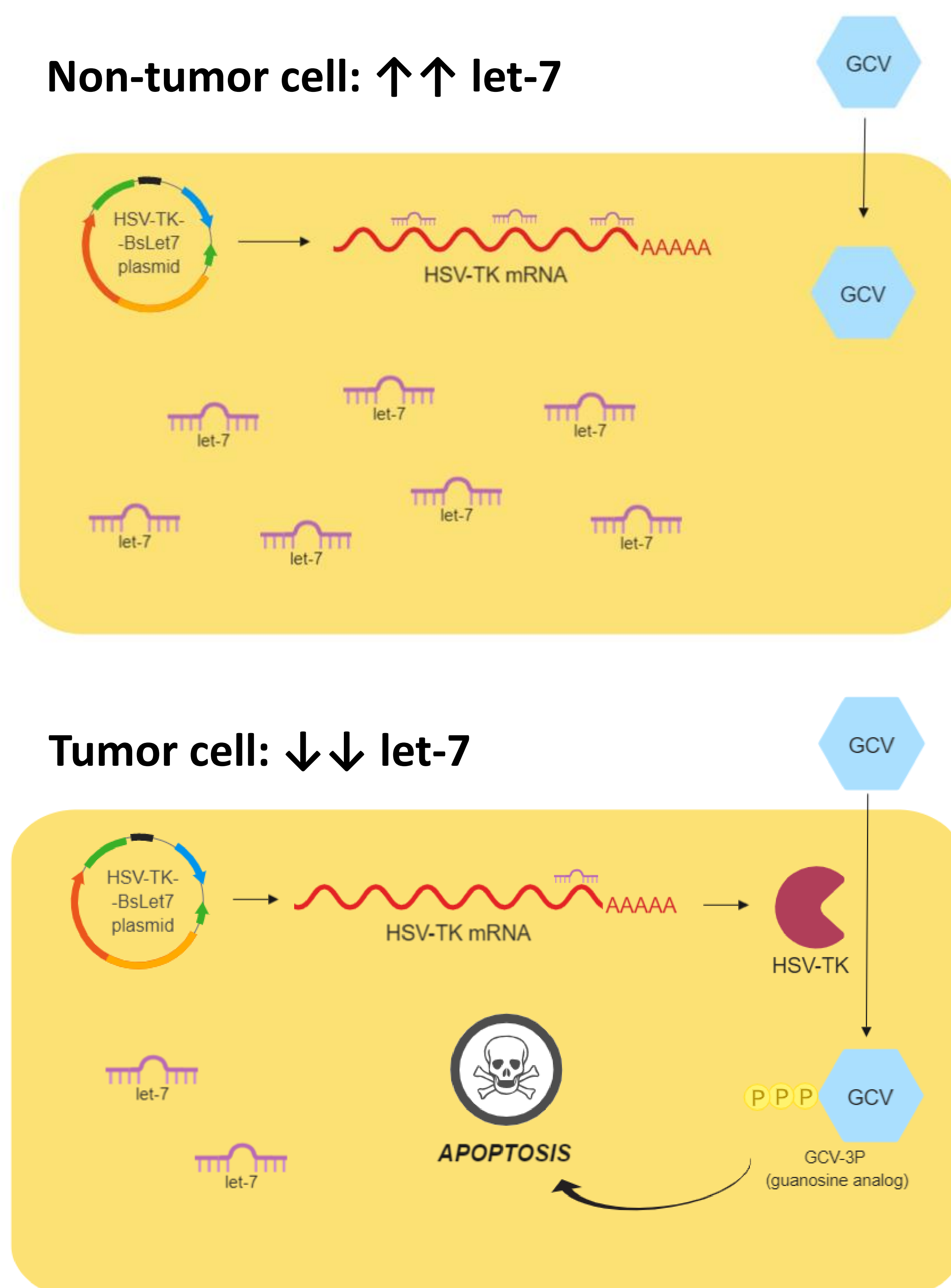


Figure 1. Scheme of our miRNA-directed suicide gene therapy strategy. Only in tumor cells, where let-7 is downregulated, there is expression of the suicide gene (HSV-TK) and cell death after the prodrug (GCV) is added.

## ACKNOWLEDGEMENTS:

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