


# Engineering Cell-Specific delivery of therapeutic programs via cell-classifier circuits encoded on self-amplifying RNA.

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Background*:
The clinical successes of cancer immunotherapy have transformed the landscape of cancer treatment, demonstrating the potential of harnessing the patient's immune system to achieve durable tumor control. Despite these advances, challenges remain in precisely targeting and modulating specific cell populations within the tumor microenvironment to enhance the effectiveness and safety of these treatments.

Methods*:

To improve the precision and efficacy of cancer immunotherapy, we are developing multi-input cell classifier circuits encoded on self-amplifying RNA (replicon RNA). These genetic circuits are designed to sense endogenous microRNA profiles and, in response, express therapeutic programs exclusively to desired cell types within the tumor microenvironment, enabling more refined and potent therapeutic interventions.

## Results*:

We demonstrated that miRNA sensing modules inserted at the 3' UTR of a replicon RNA can detect the activity of endogenous microRNAs and control the expression of a genetically encoded payload accordingly. Additionally, we demonstrated that we can achieve either coordinated or independent regulation of two genetic payloads encoded on a single replicon RNA molecule by strategically positioning miRNA-sensing modules between two distinct subgenomic RNA units. Building on these findings, we developed microRNA-regulated circuits that activate the expression of a genetically encoded payload only when a specific microRNA is active in the recipient cell.

## Conclusion*:

Together, our results demonstrate the feasibility of encoding microRNA-regulated genetic circuits on single-replicon RNA molecules for cell-specific expression of genetic programs in different cell types within the tumor microenvironment.