

Exploring oligonucleotide-based silencing strategies to prevent anthracycline-induced cardiotoxicity

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INTRODUCTION



Doxorubicin chemotherapeutic treatment leads to **cardiotoxicity** through its effect on **topoisomerase IIβ**¹

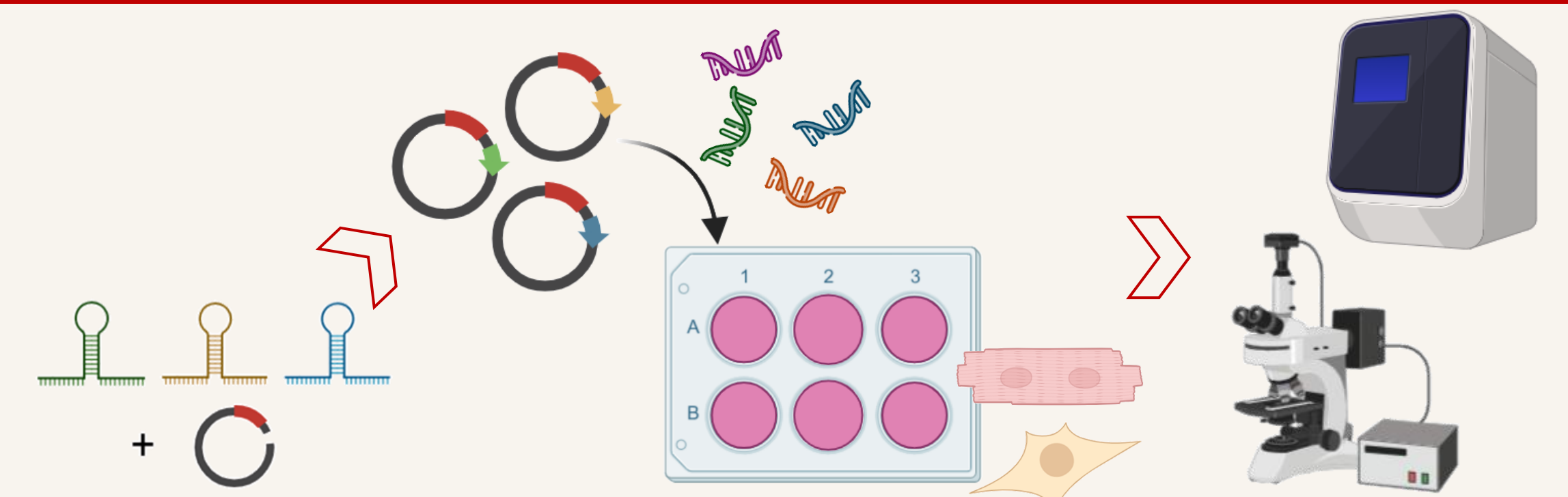
Cardiomyocyte-specific conditional **Top2b** knockout prevents doxorubicin **cardiotoxicity**²

GOALS

1. Development of a oligonucleotide-based strategy for gene **silencing** of **TOP2B**.
2. **Characterization** of the specificity and efficacy of the oligonucleotide-based candidates.

MATERIALS & METHODS

- **Design** of 3 **shRNA** candidates, 6 first-generation **ASOs** and 3 **phosphorothioate-modified second-generation ASOs** targeting **TOP2B**.
- **Cloning** of **shRNA** candidates into an inducible tdTomato expression vector.
- Transient transfection of **HEK293 cells** (shRNA and ASOs) and **iCMs** (ASOs).
- Assessment of **TOP2B** silencing specificity and efficacy at the mRNA (**qPCR**) and protein (**immunofluorescence**) levels.

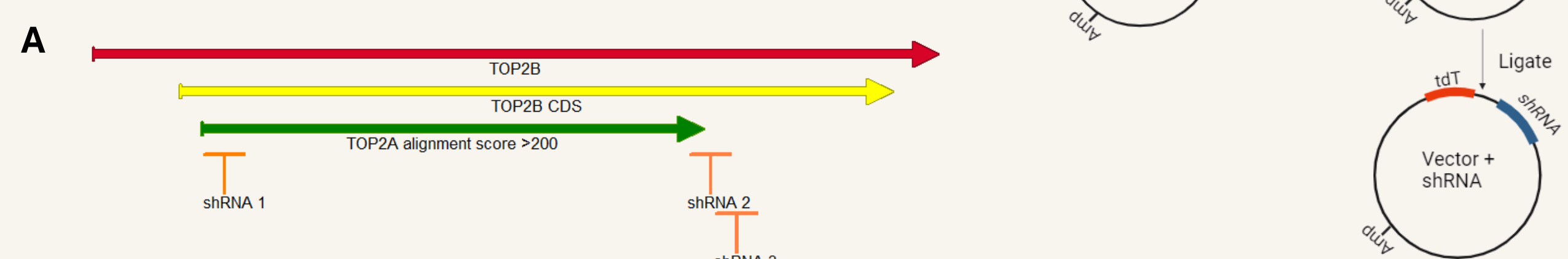


RESULTS

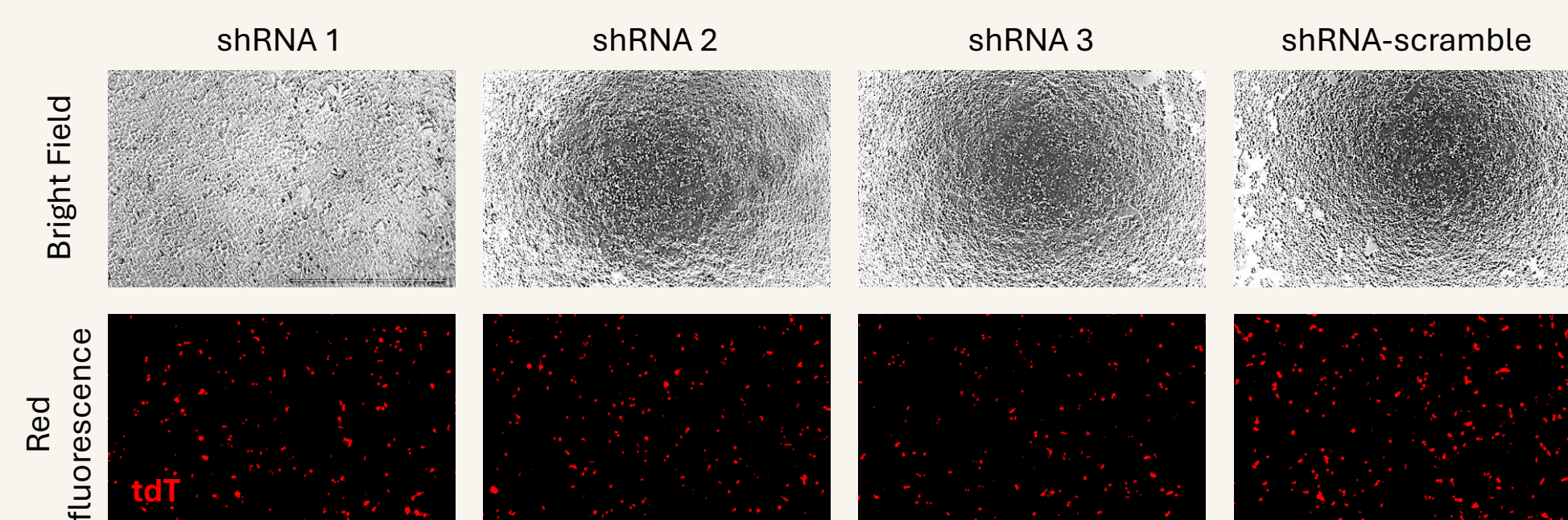
1. shRNA-based TOP2B silencing

Design and cloning of shRNA-TOP2B candidates

Three **shRNA** candidates targeting **TOP2B** were designed and selected in sequence regions with **low homology to TOP2A** (A) and **cloned** into an inducible expression vector (B).

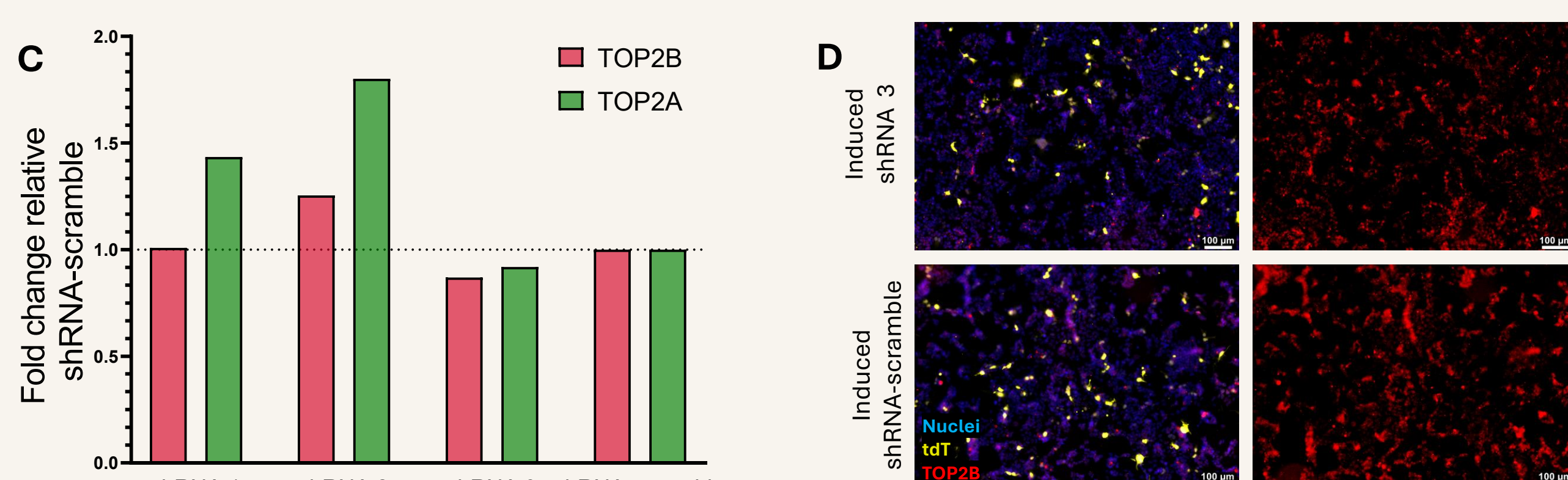


Transfection of recombinant shRNA-TOP2B vector into HEK293 cells



Doxycycline induction triggered **tdTomato** expression, confirming inducible shRNA expression following transient transfection.

Assessment of TOP2B silencing specificity and efficiency

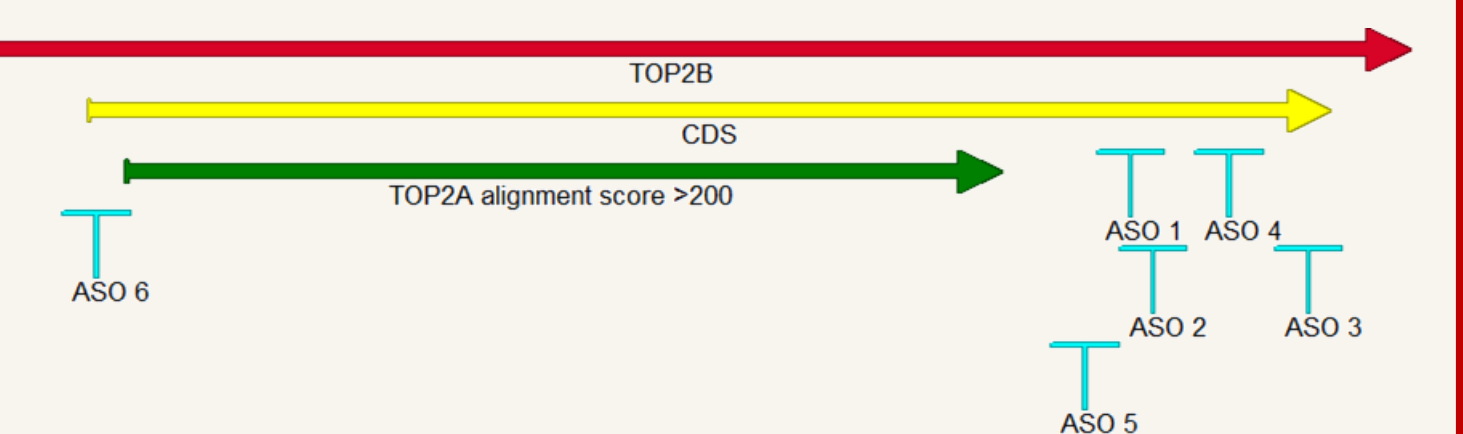


shRNA 3 showed the **strongest reduction of TOP2B** expression at the mRNA level, with a minor effect on **TOP2A** (C). Immunofluorescence analysis revealed a qualitative decrease in TOP2B signal for this candidate (D). However, **none of the shRNA candidates achieved robust silencing efficiency**.

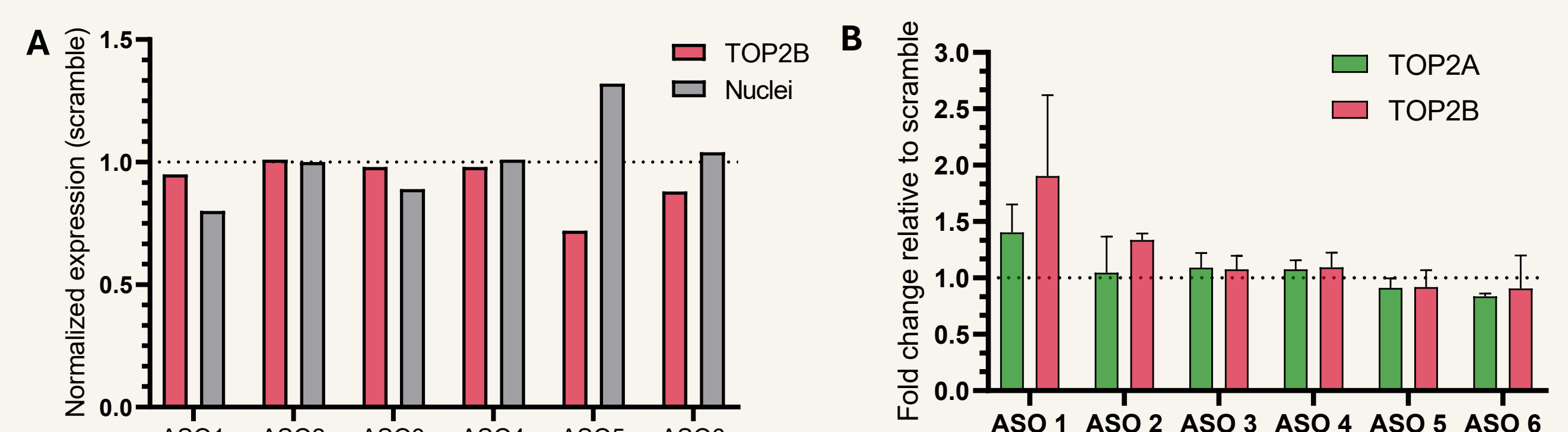
2. ASO-based TOP2B silencing

Design of first-generation ASOs

Six first-generation ASOs targeting **TOP2B** were designed in regions with **low homology to TOP2A** and distributed across the transcript to maximize silencing efficiency.



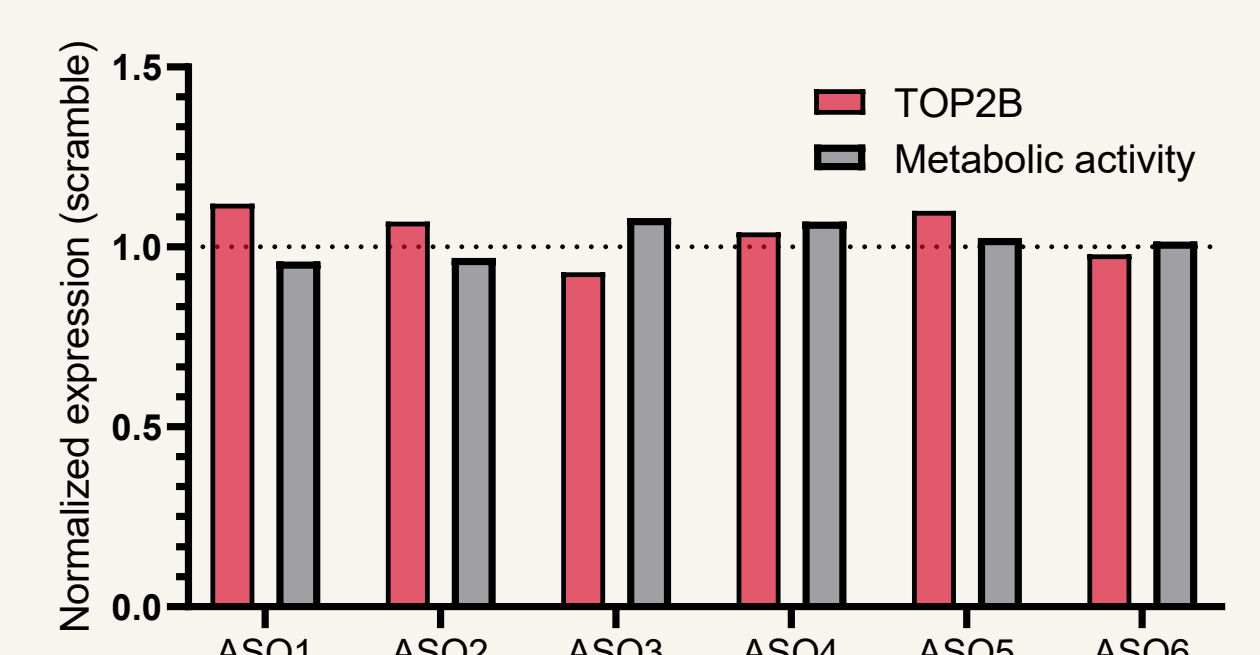
Assessment of first-generation ASOs performance in HEK293 cells



First-generation ASOs showed **variable inhibitory effects** in HEK293 cells. While reducing **TOP2B protein** expression by **up to 28%** (A), qPCR analyses revealed only **modest reductions in mRNA** levels, reaching 8% inhibition (B).

Assessment of first-generation ASOs performance in iCMs

ASO performance differed between HEK293 cells and iCMs, highlighting the importance of evaluating candidates in physiologically relevant cardiac models.



Although selected ASOs showed **partial TOP2B silencing**, none achieved the level of inhibition required for therapeutic application. These results guided the rational design of a **second generation of phosphorothioate-modified ASOs**. Functional evaluation is currently ongoing.



CONCLUSIONS

- Neither shRNA nor first-generation ASO candidates achieved **robust TOP2B silencing**.
- Assessment in iCMs highlighted the importance of **physiologically relevant cardiac models**.
- These findings supported the development of **phosphorothioate-modified second-generation ASOs**, currently under evaluation..

REFERENCES

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2. Zhang, S. *et al.* Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat. Med.* 2012 **18**, 1639–1642 (2012).

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