Ein Unternehmen der
Johann Wolfgang Goethe-Universität
Frankfurt am Main

*Technology Offer*

# DARPins for reactivation of temperature-sensitive p53 cancer mutants

## Benefits

**Broad therapeutic approach for temperature-sensitive p53 mutant cancers. Potential to address tumors not tractable with mutation-specific small molecules.**

## Project Status

- **DARPin C10 and affinity-optimized variant C10-H82R generated and characterized.**
- **Thermal stabilization shown by DSF across representative p53 mutants.**
- **Nanomolar binding to multiple TS p53 mutants demonstrated by ITC.**
- **Delivery via mRNA/LNP as potential translational route; further delivery optimization and in vivo therapeutic development remain future steps.**

## Patents and Publications

- **WO2026013280A1 DESIGNED ANKYRIN REPEAT PROTEINS BINDING P53, AND USES THEREOF**
- **Münick et al., 2026, DARPins as pan-reactivators of temperature-sensitive p53 cancer mutants, PNAS 123(18)**

## Offer

- **Technology can be licensed or assigned**
- **Collaborations for further development welcome**

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Researchers at Goethe University Frankfurt have developed designed ankyrin repeat proteins (DARPins) that bind to the p53 DNA-binding domain and stabilize a broad set of temperature-sensitive (TS) p53 cancer mutants. This creates a differentiated therapeutic approach for reactivating mutant p53 in tumors that currently lack mutation-specific small-molecule options. The concept addresses a major challenge in p53-targeted oncology: while p53 is mutated in about 50% of cancers, only a small subset of mutants can be targeted with pocket-binding small molecules such as those developed for Y220C mutants. In contrast, the reported DARPin binds a nonfunctional surface of p53 and compensates for mutation-induced loss of conformational stability, enabling broad mutant coverage beyond cavity-forming variants.

**Key findings include:**

- DARPin C10-H82R bound tested TS p53 DNA-binding domain mutants with $K_d$ values in the 37–103 nM range, comparable to wild-type p53 binding.
- Binding increased melting temperatures by up to 10 °C and in most cases compensated for mutation-induced stability loss.
- High-resolution crystal structures were solved for complexes with multiple clinically relevant p53 mutants, including V143A, V157F, Y220C, Y234C, V272M, R282W and E285K, providing a mechanistic basis for stabilization.
- In reporter assays, DARPin C10-H82R reactivated almost all tested TS mutants and restored up to 85% of wild-type p53 activity in some variants.
- In CRISPR-engineered and patient-derived cancer cell lines, DARPin delivery restored p53 target-gene expression and induced antiproliferative effects including cell-cycle arrest and reduced clonogenic growth.

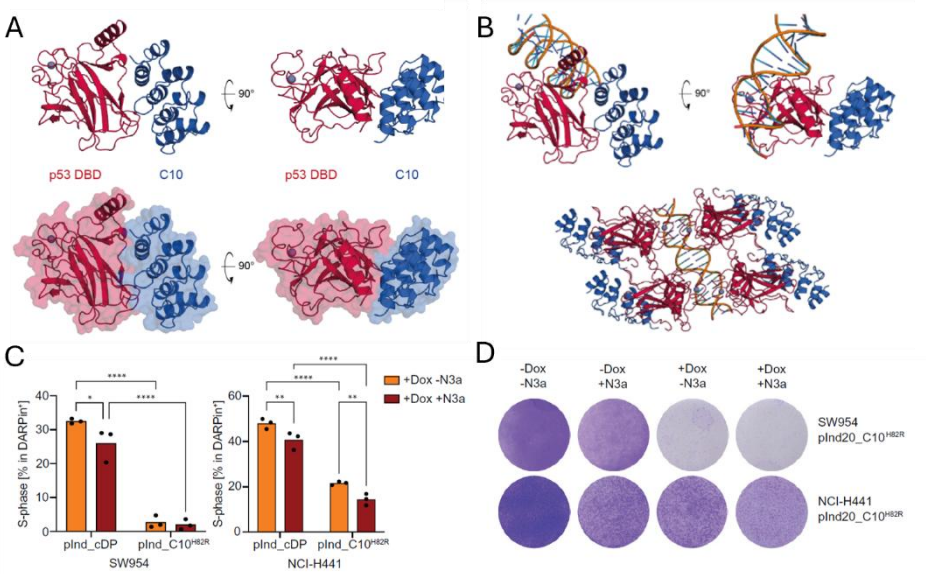



**Fig. 1 (A+B):** Crystal structures of DARPin C10 (blue) in complex with the p53 DNA-binding domain (red) indicating that DARPin C10 does not block the DNA-binding surface of the p53 DBD. (**C**) Fraction of DARPin-expressing SW954 and NCI-H441 cells in S-phase showing C10-induced cell-cycle arrest. (**D**) Long-term colony formation assays demonstrating that DARPin C10 reduces clonogenic growth in both cell lines.