

Machine Learning-Accelerated Guide RNA Design for ADAR-Based Therapeutics



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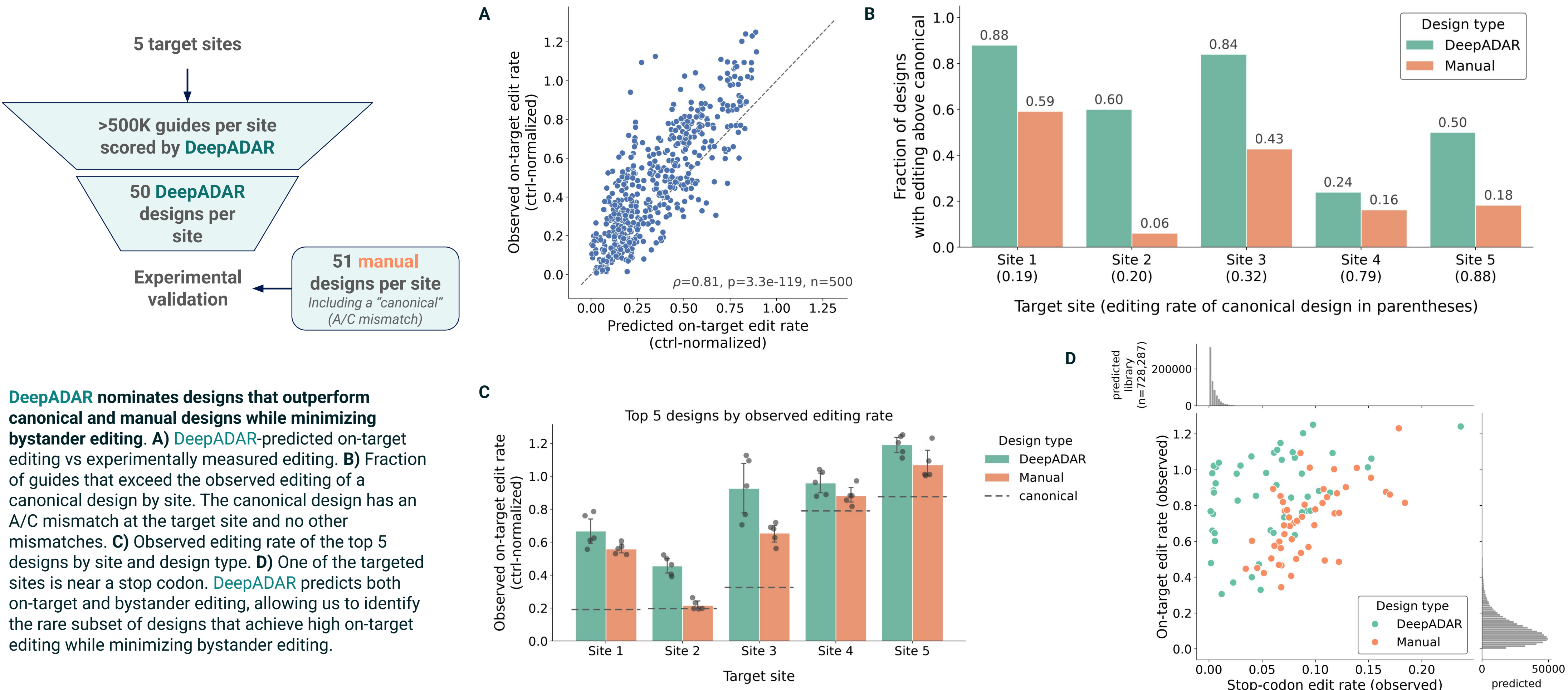
1. Introduction

ADAR enzymes catalyze the deamination of adenosine to inosine in double-stranded RNA. ADAR-guided RNA editing has emerged as a promising oligonucleotide modality with the potential to restore protein function, modulate protein abundance, and address mutations that have proven difficult for other approaches.

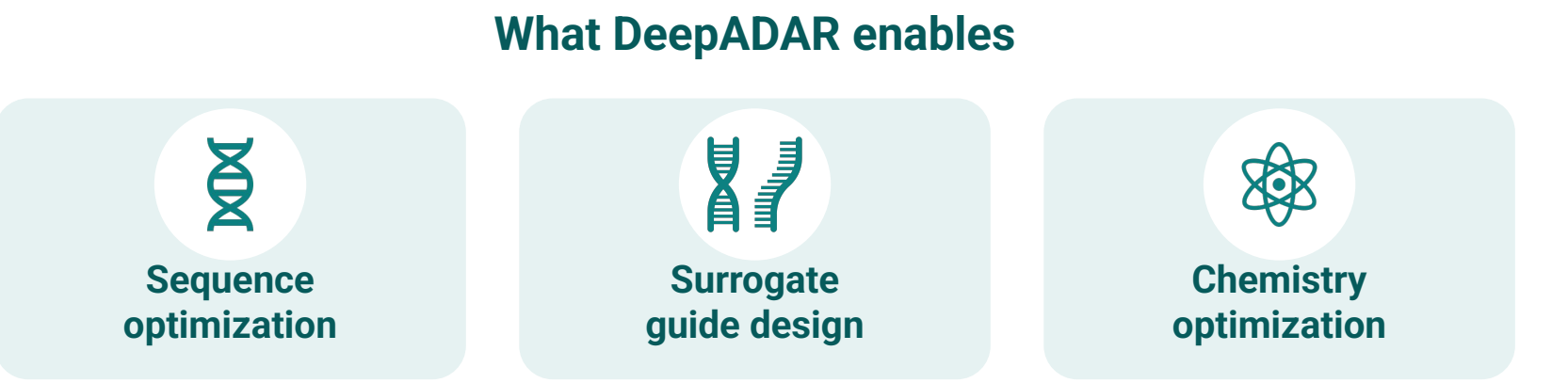
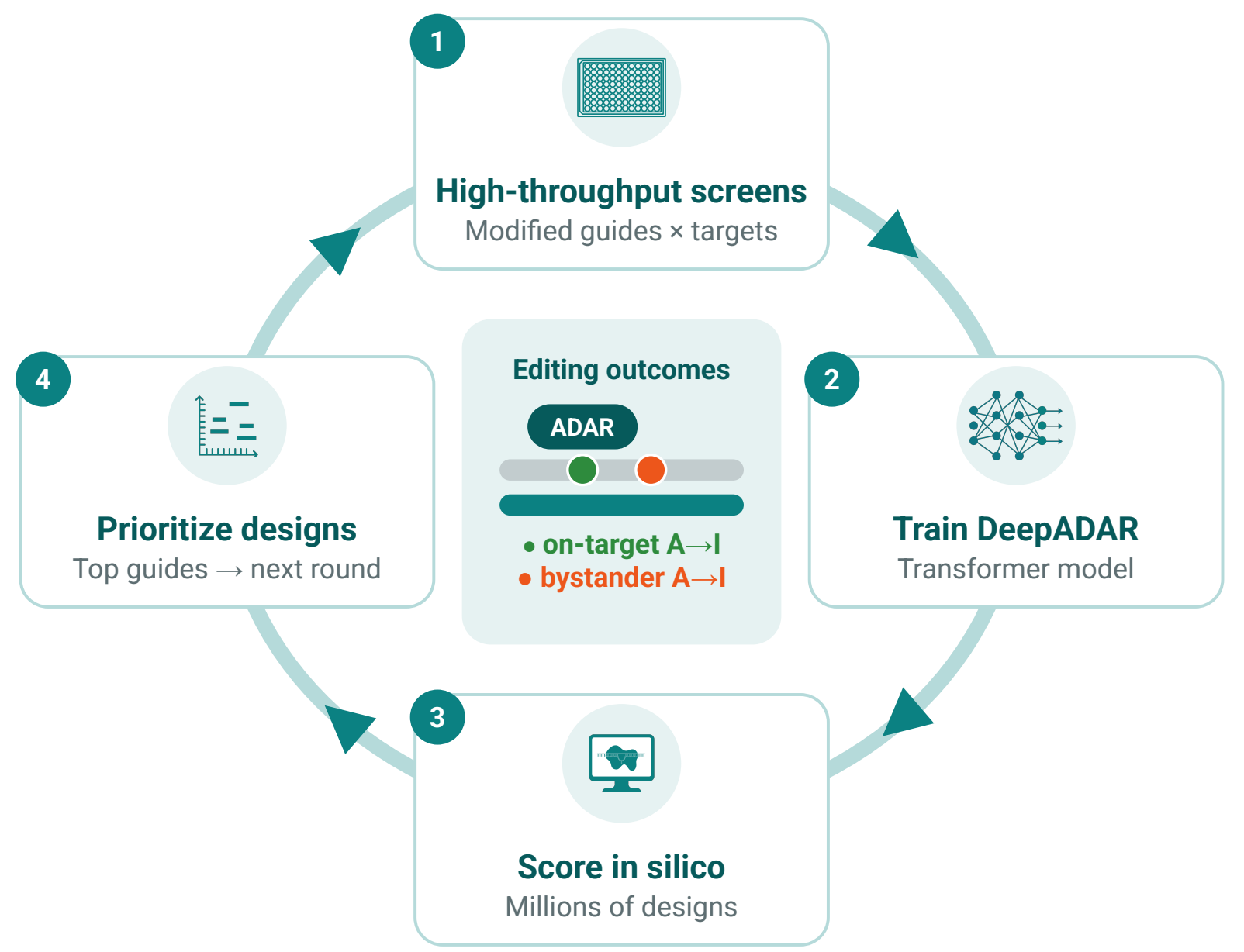
Realizing ADAR's therapeutic potential requires jointly optimizing guide RNA sequence for on-target editing efficiency, bystander editing minimization, chemical stability, and cross-species translatability across a vast design space. Site selection, guide sequence optimization, and identification of non-human primate surrogate guides for translational studies are typically driven by iterative experimental screening, limiting how many sites and targets can be advanced in parallel.

We previously developed DeepADAR-Endogenous [1], a deep learning model for predicting endogenous levels of ADAR editing. Here, we present a major extension, **DeepADAR**, which is designed for advancing novel ADAR therapeutics with exogenous gRNAs. **DeepADAR** is actively deployed across multiple stages of therapeutic program development, helping to shorten development times and de-risk early design decisions.

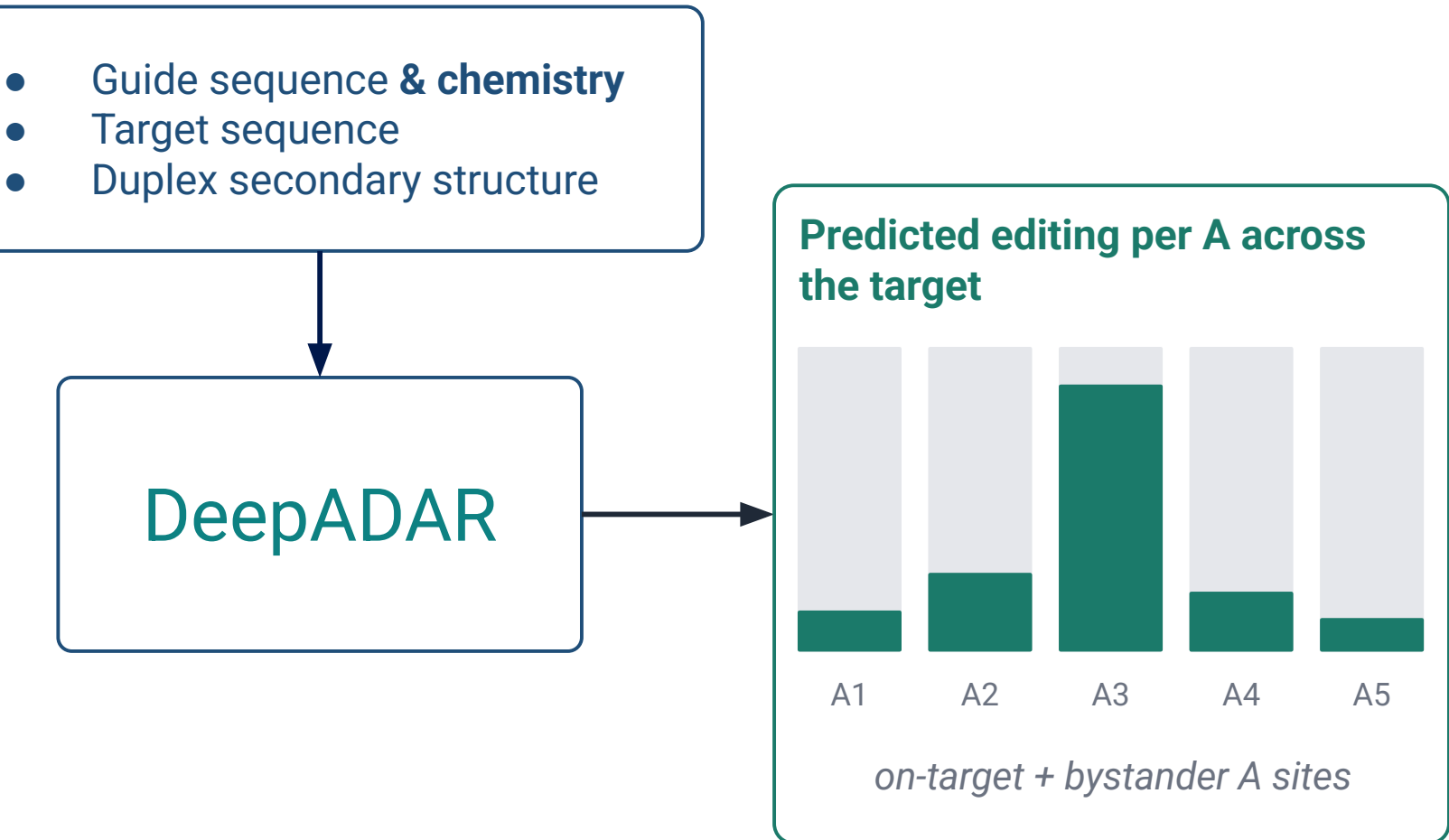
3. DeepADAR helps identify guides that jointly achieve good on-target and low bystander editing



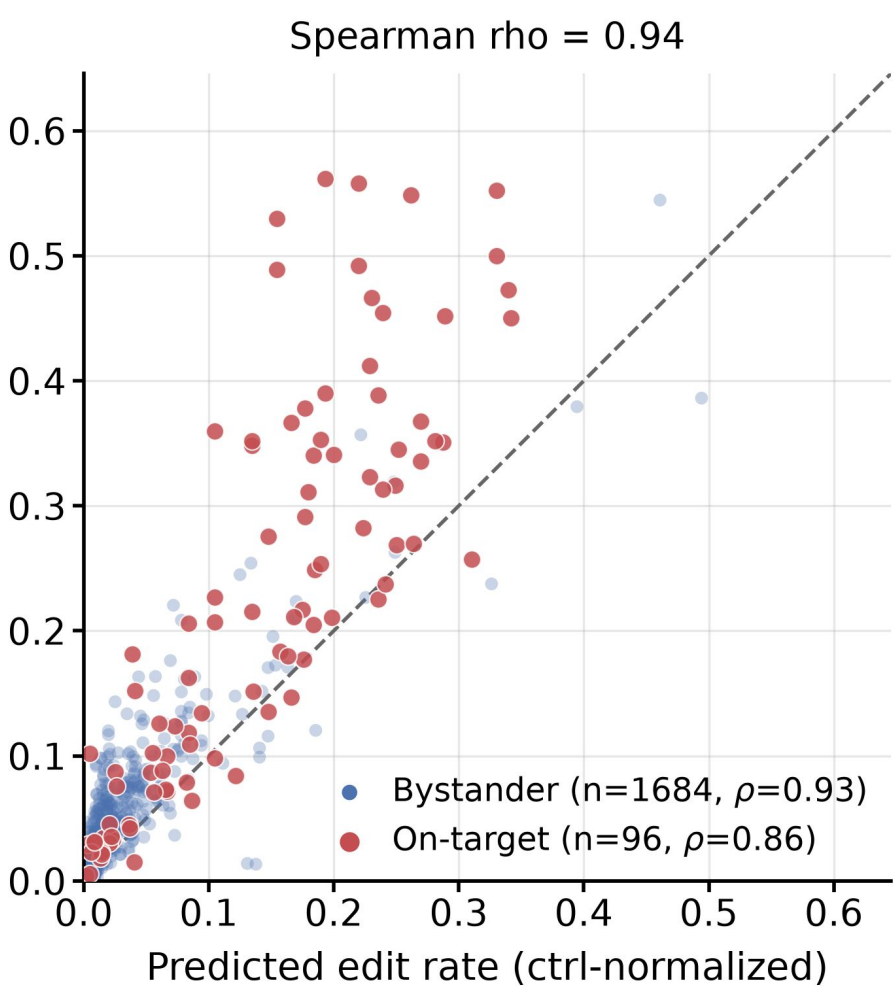
2. Iterative design and DeepADAR training



- DeepADAR is trained using data from high-throughput screens, measuring on-target and bystander off-target editing of chemically modified guides against diverse targets in therapeutically relevant cell contexts.
- Once trained, DeepADAR is employed to perform in silico screening enabling efficient sequence and chemistry optimization.
- DeepADAR allows exploration of large design spaces and helps us solve for multiple design constraints, such as avoidance of bystander edits or cross-species reactivity.

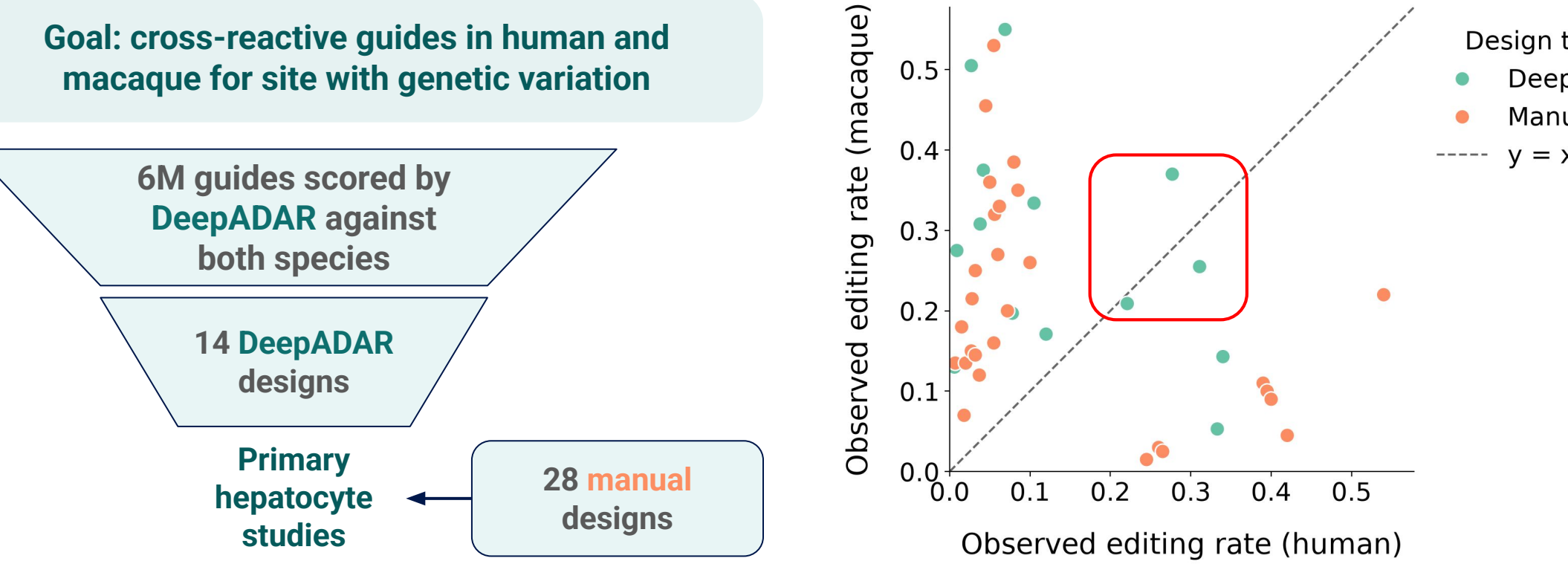
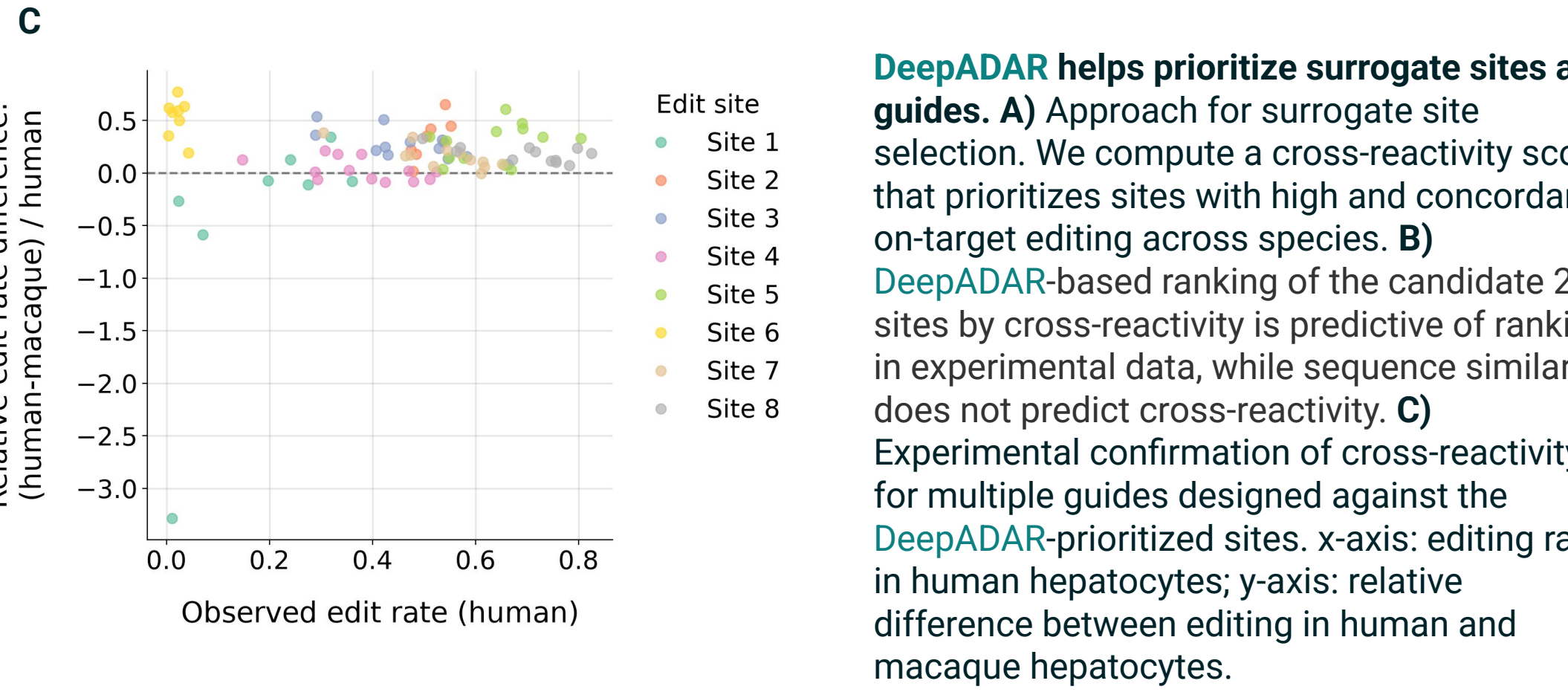
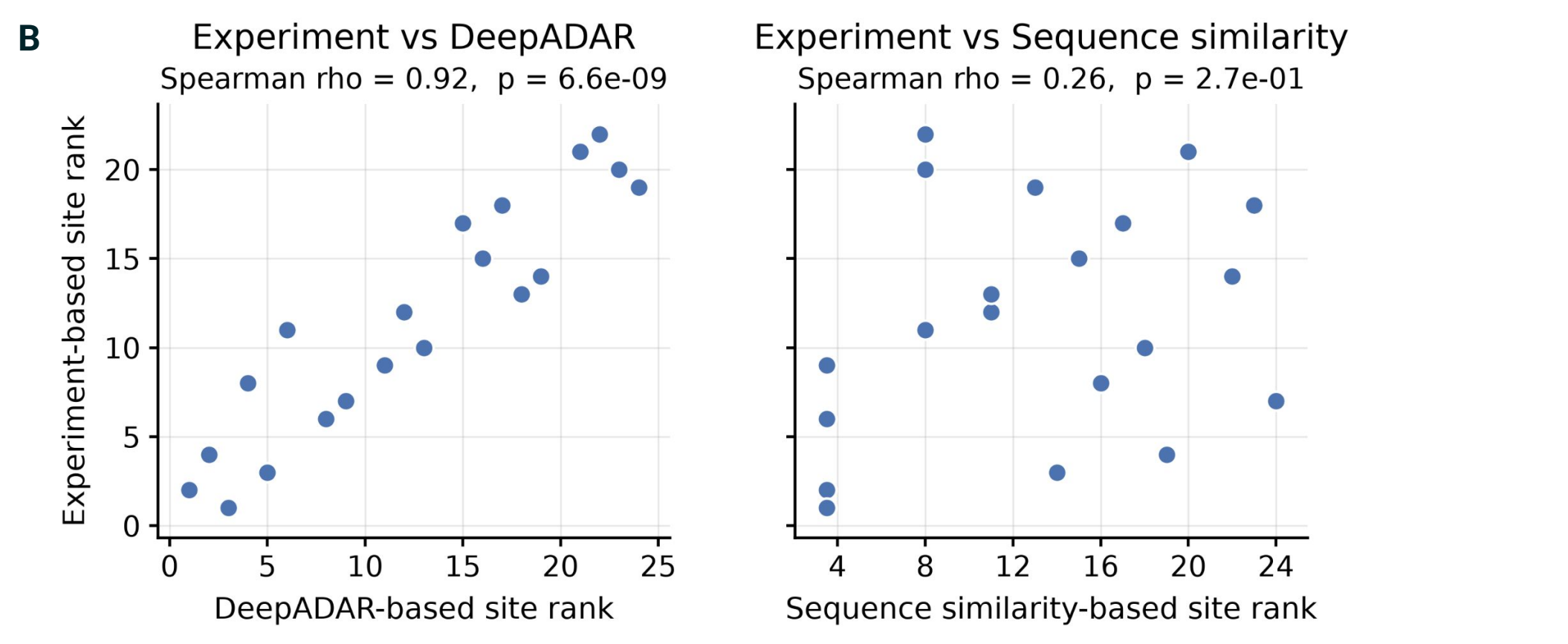
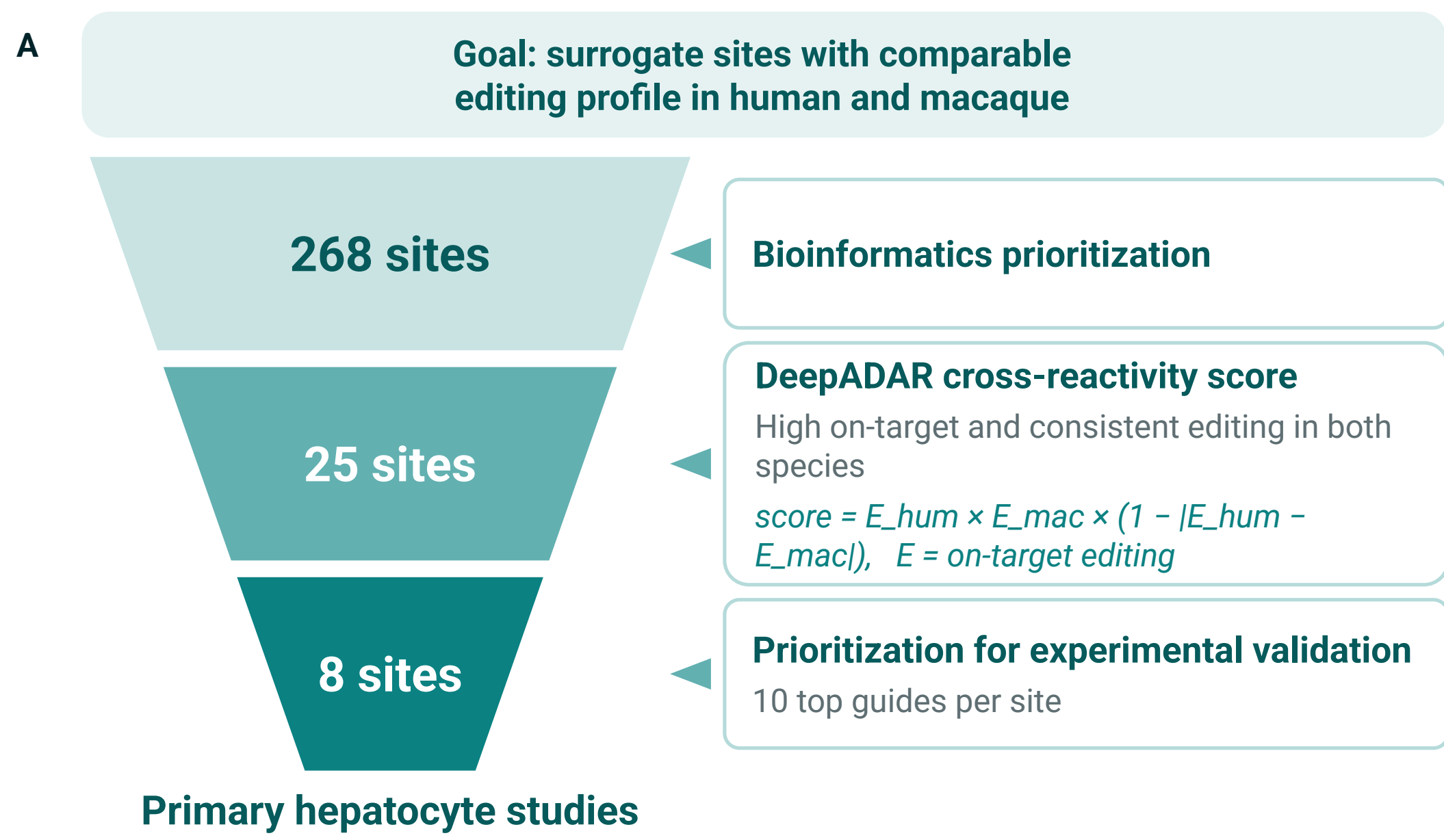


DeepADAR is a 100M-parameter transformer-based model that predicts on-target and bystander off-target editing rates for chemically modified ADAR guides.



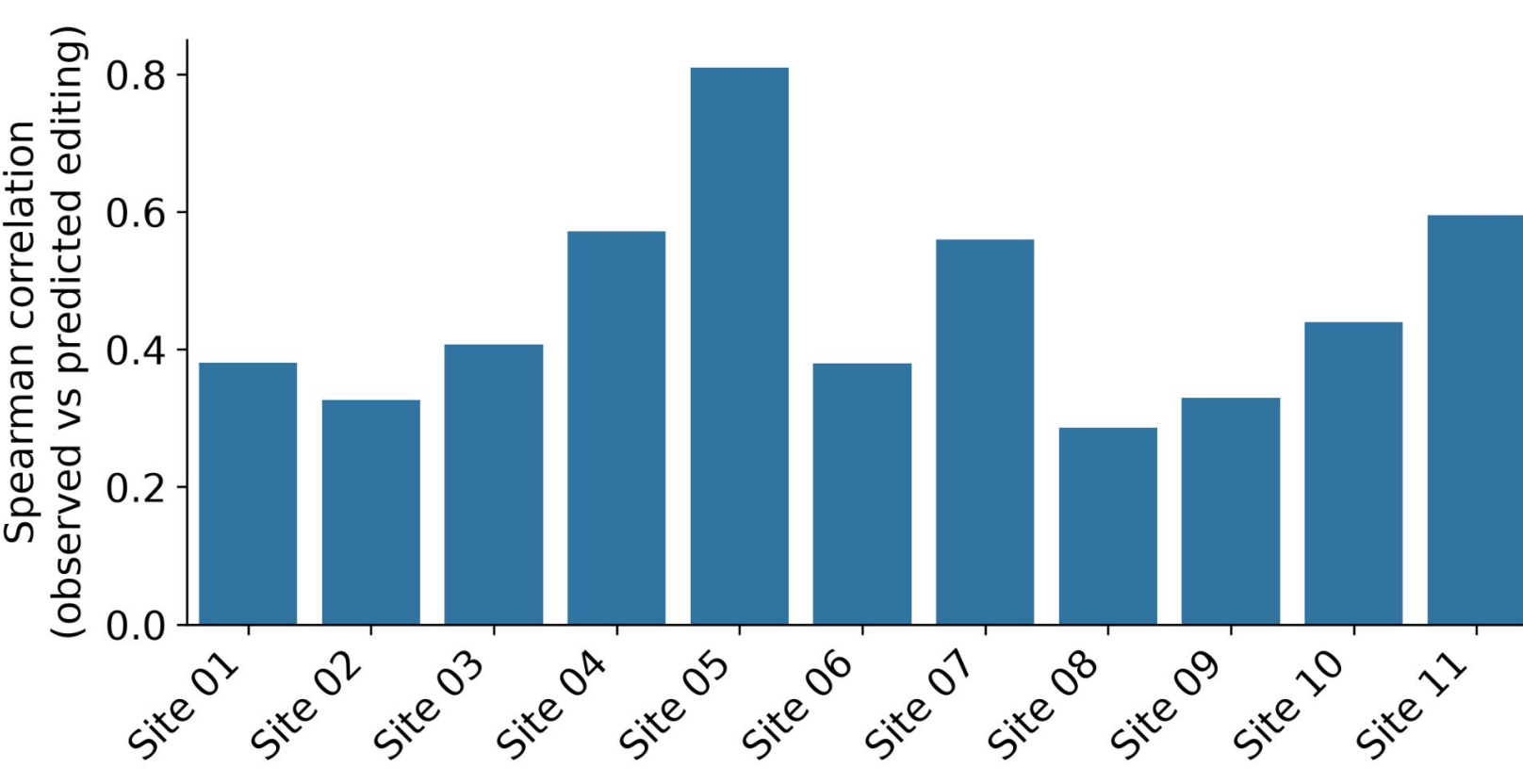
DeepADAR predicts on target and bystander editing for unseen target sites. We evaluated DeepADAR on guides designed against a target site on a gene entirely absent from the training data. We show predicted vs experimentally measured editing rates, for on-target A sites as well as off-target bystander sites.

4. DeepADAR identifies surrogate sites and guides to enable NHP studies



- Genetic variation between human and macaque in close proximity to the targeted A can make identification of cross-reactive guides challenging.
- For a site with such genetic variation, we used DeepADAR to score 6M guides against the sequences of both species. The vast majority of scored guides were not predicted to be cross-reactive. A very small subset was selected for primary hepatocyte studies. Unlike rationally designed guides, some of the DeepADAR nominated guides show comparable editing in both species (red box).

5. DeepADAR ranks chemically modified guides by editing



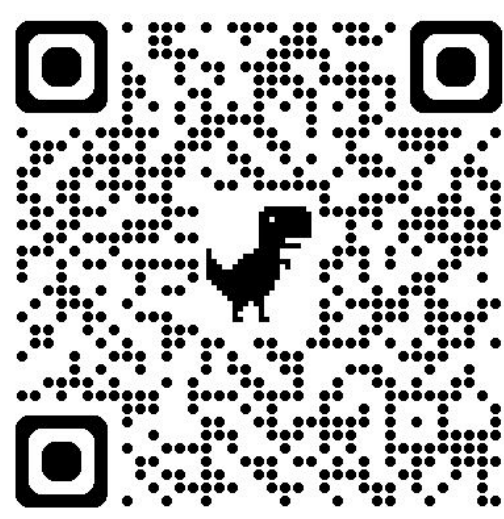
DeepADAR ranks guides against the same target site with different chemistry patterns. Chemically modified guides with different patterns of sugar modifications were manually designed against 11 target sites on a gene entirely unseen during training.

We used DeepADAR to rank the guides for each site by predicted editing and compared to experimental results.

6. Conclusions

- In silico scoring with DeepADAR allows us to explore very large design spaces and to solve for multiple design constraints, such as avoidance of bystander edits and cross-species reactivity.
- In many cases, it would have been extremely inefficient to resolve such constraints with manual iterative design. For example, for our cross-reactivity search, only a handful out of nearly 6M in silico screened designs were cross-reactive.
- DeepADAR enables both initial hit identification and chemistry optimization, even for sites unseen in the training data.
- DeepADAR exemplifies a broader platform strategy for in silico design of genetic medicines, applied here to a modality in which guide selection and optimization has been a key bottleneck for parallel advancement of multiple targets.

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See also our poster on DeepRNAi, our model for siRNA design (P-021)

References

- Jung, A.-J., Gao, A.-J., Lee, L.-J., & Frey, B. (2024). DeepADAR: A deep learning approach to model regulatory elements of ADAR-based RNA editing and its application to gRNA design. *NeurIPS 2024 Workshop on AI for New Drug Modalities*.