SCIENTIFIC POSTER

A new siRNA design strategy for the modern transcriptome.

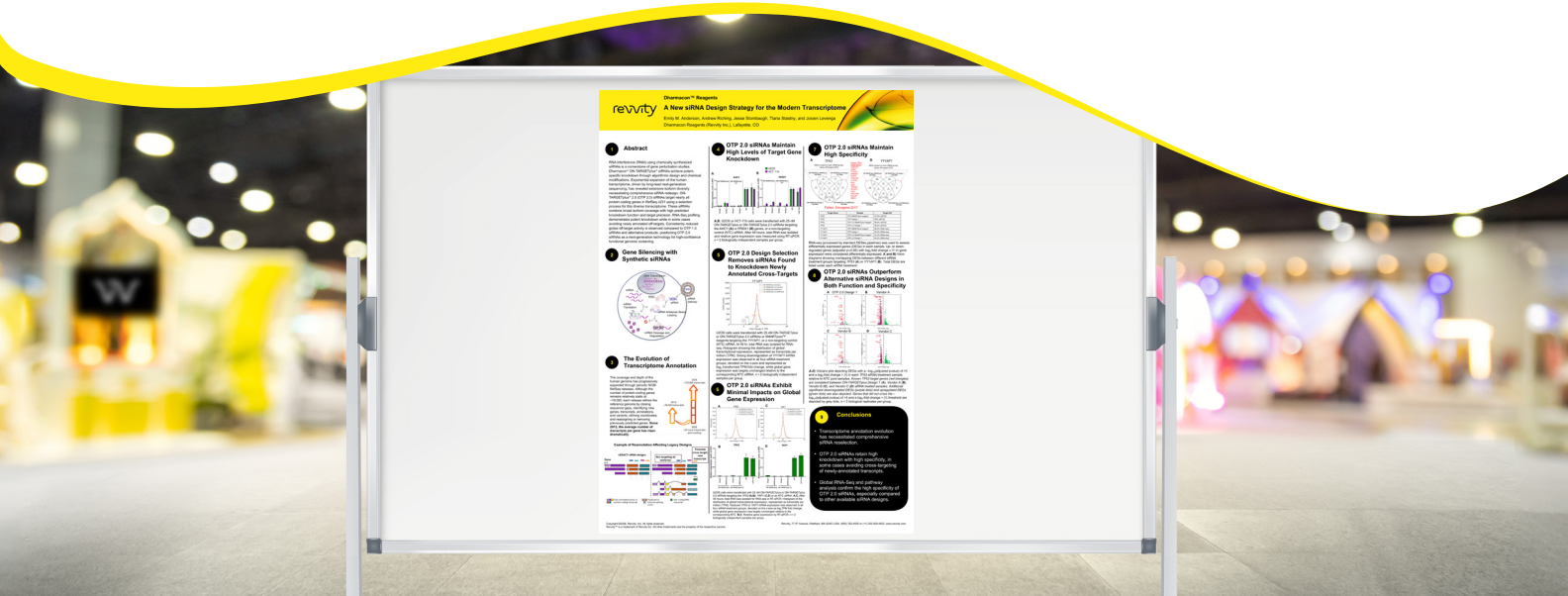
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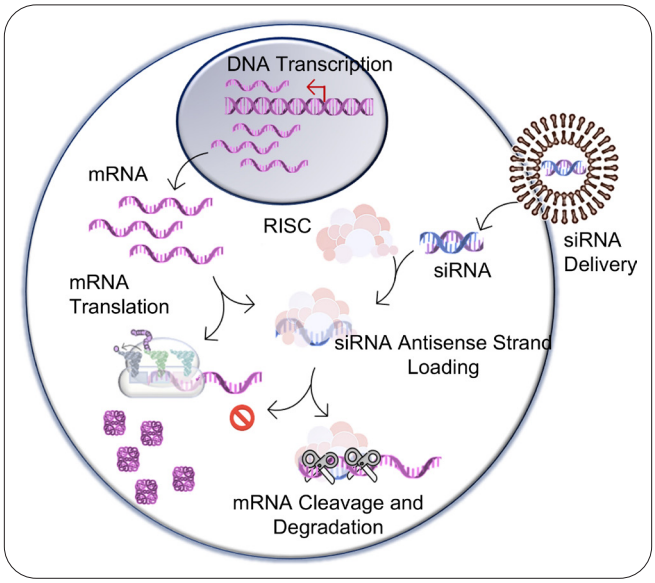
Revvity

Abstract

RNA interference (RNAi) using chemically synthesized siRNAs is a cornerstone of gene perturbation studies. Dharmacon™ ON-TARGETplus™ siRNAs achieve potent, specific knockdown through algorithmic design and chemical modifications. Exponential expansion of the human transcriptome, driven by long-read next-generation sequencing, has revealed extensive isoform diversity necessitating comprehensive siRNA redesign. ONTARGETplus 2.0 (OTP 2.0) siRNAs target nearly all protein-coding genes in RefSeq v231 using a selection process for this diverse transcriptome. These siRNAs combine broad isoform coverage with high predicted knockdown function and target precision. RNA-Seq profiling demonstrates potent knockdown while in some cases avoiding newly annotated off-targets. Consistently reduced global off-target activity is observed compared to OTP 1.0 siRNAs and alternative products, positioning OTP 2.0 siRNAs as a next-generation technology for high-confidence functional genomic screening.

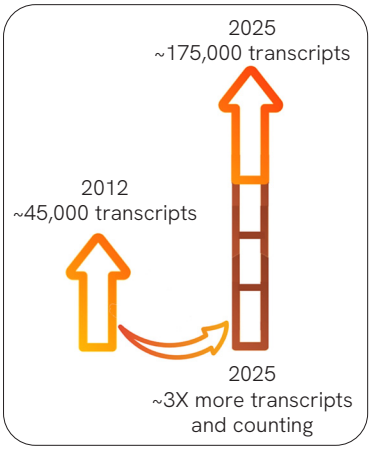


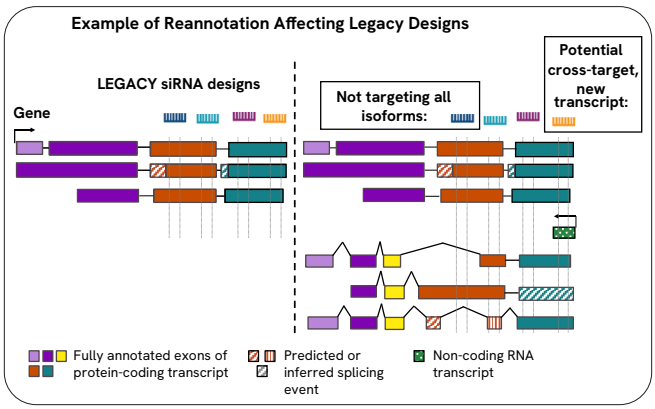
Gene silencing with synthetic siRNAs



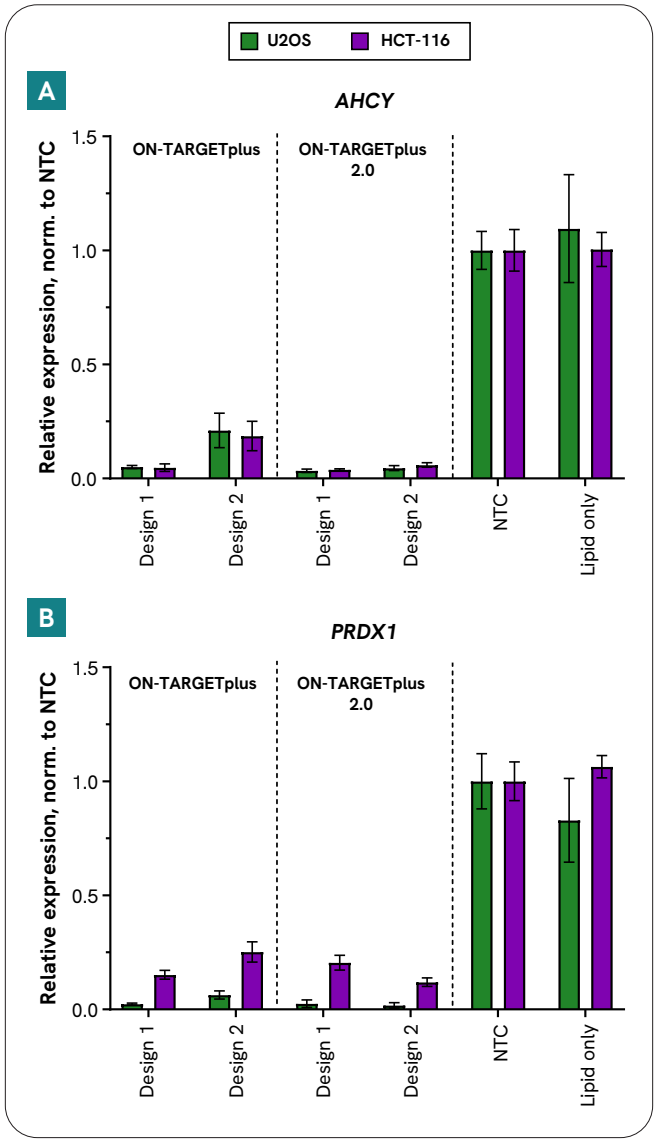
The evolution of transcriptome annotation

The coverage and depth of the human genome has progressively expanded through periodic NCBI RefSeq releases. Although the number of protein-coding genes remains relatively static at ~19,000, each release refines the reference genome by closing sequence gaps, identifying new genes, transcripts, annotations, and variants, refining coordinates, and reassigning or removing previously predicted genes. Since 2012, the average number of transcripts per gene has risen dramatically.



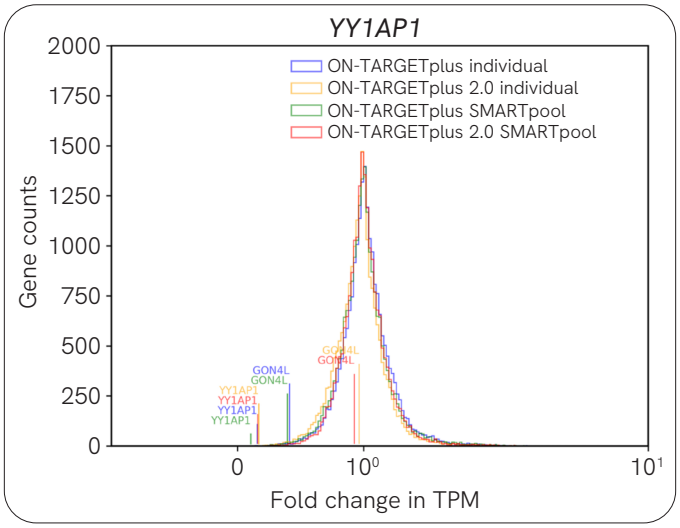


OTP 2.0 siRNAs maintain high levels of target gene knockdown



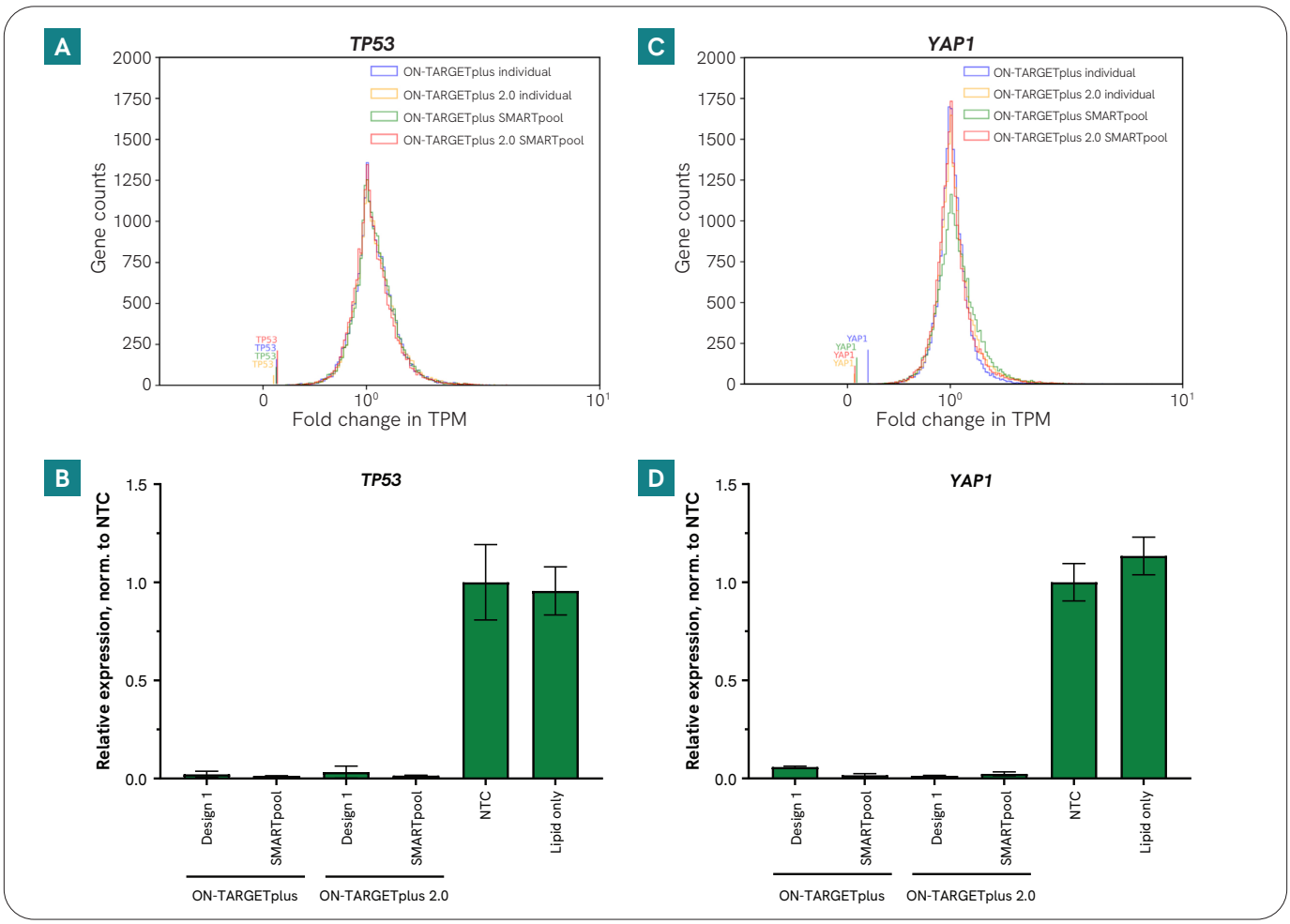
A,B. U2OS or HCT-116 cells were transfected with 25 nM ON-TARGETplus or ON-TARGETplus 2.0 siRNAs targeting the AHCY (A) or PRDX1 (B) genes, or a non-targeting control (NTC) siRNA. After 48 hours, total RNA was isolated and relative gene expression was measured using RT-qPCR. n=2 biologically independent samples per group.

OTP 2.0 design selection removes siRNAs found to knockdown newly annotated cross-targets



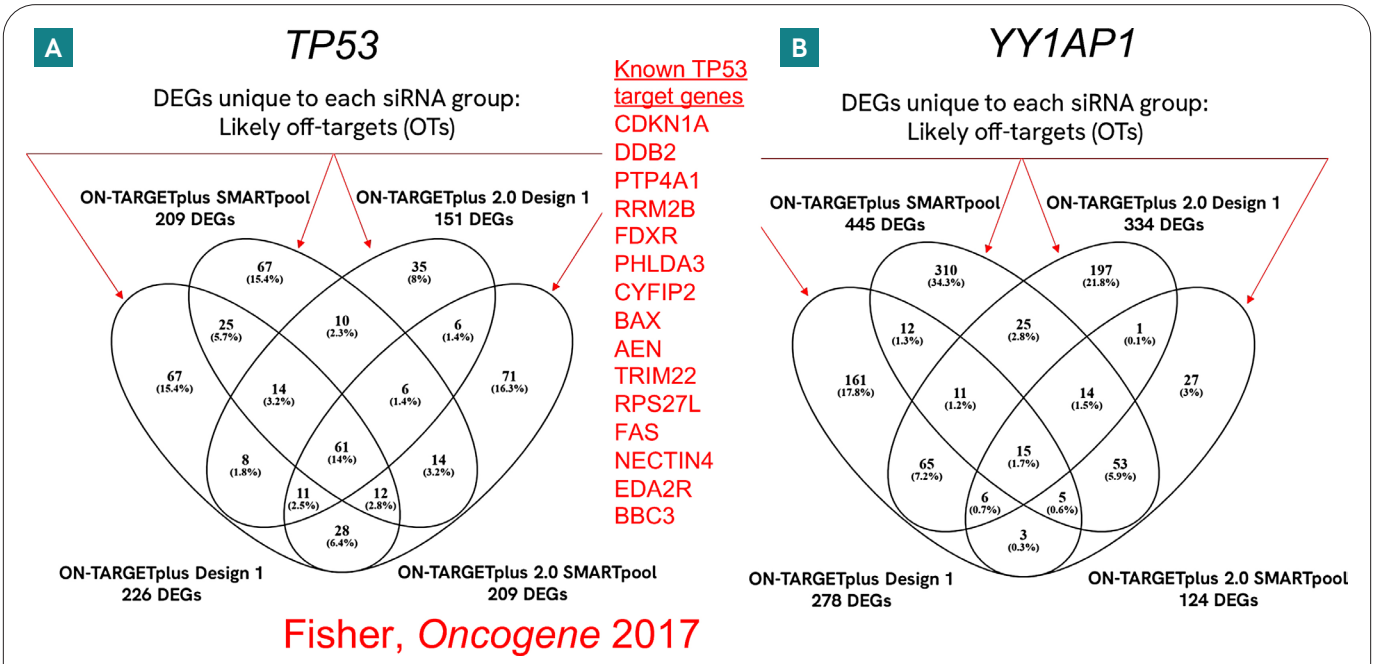
U2OS cells were transfected with 25 nM ON-TARGETplus or ON-TARGETplus 2.0 siRNAs or SMARTpool™ reagents targeting the YY1AP1, or a non-targeting control (NTC) siRNA. At 48 hr, total RNA was isolated for RNAseq. Histogram showing the distribution of global transcriptional expression, represented as transcripts per million (TPM). Strong downregulation of YY1AP1 mRNA expression was observed in all four siRNA treatment groups, denoted on the x-axis and represented as log2 transformed TPM fold change, while global gene expression was largely unchanged relative to the corresponding NTC siRNA. n=2 biologically independent samples per group.

OTP 2.0 siRNAs exhibit minimal impacts on global gene expression



U2OS cells were transfected with 25 nM ON-TARGETplus or ON-TARGETplus 2.0 siRNAs targeting the TP53 (A,B), YAP1 (C,D) or an NTC siRNA. A,C. After 48 hours, total RNA was isolated for RNA-seq or RT-qPCR. Histogram of the distribution of global transcriptional expression, represented as transcripts per million (TPM). Reduced TP53 or YAP1 mRNA expression was observed in all four siRNA treatment groups, denoted on the x-axis as log2TPM fold change, while global gene expression was largely unchanged relative to the corresponding NTC. B,D. Relative gene expression by RT-qPCR. n=2 biologically independent samples per group.

OTP 2.0 siRNAs maintain high specificity

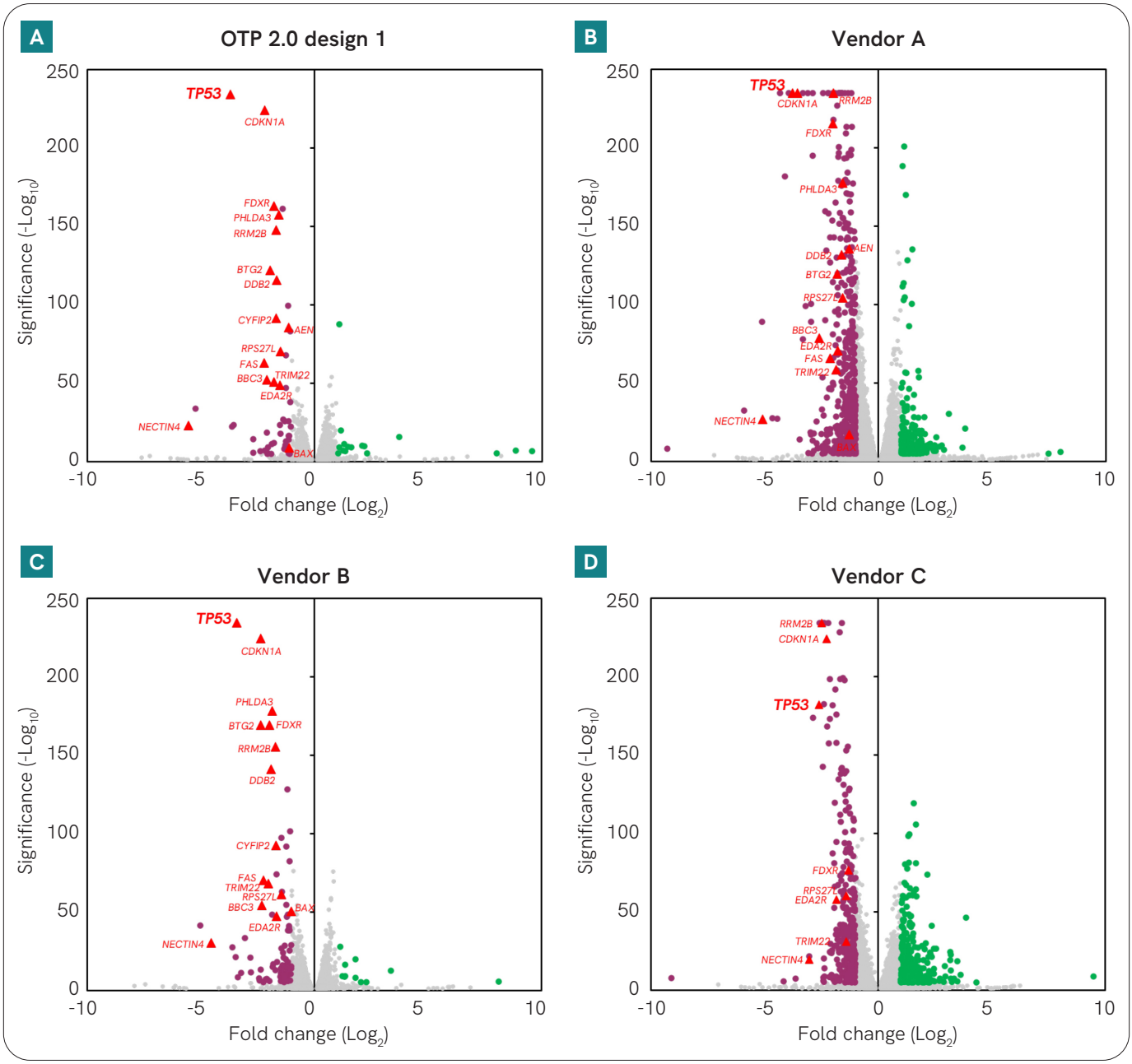


Fisher, *Oncogene* 2017

Target gene	Sample	Target KD
TP53	OTP SMARTpool reagent	97.9% (qPCR)
TP53	OTP Design 1	99% (qPCR)
TP53	OTP 2.0 SMARTpool reagent	96.8% (qPCR)
TP53	OTP 2.0 Design 1	98.5% (qPCR)
YY1AP1	OTP SMARTpool reagent	86.5% (RNA-seq)
YY1AP1	OTP Design 1	83.4% (RNA-seq)
YY1AP1	OTP 2.0 SMARTpool reagent	91.2% (RNA-seq)
YY1AP1	OTP 2.0 Design 1	85.2% (RNA-seq)

RNA-seq (processed by standard DESeq pipelines) was used to assess differentially expressed genes (DEGs) in each sample. Up- or downregulated genes (adjusted $p < 0.05$) with log2-fold change > |1| in gene expression were considered differentially expressed. A and B) Venn diagrams showing overlapping DEGs between different siRNA treatment groups targeting TP53 (A) or YY1AP1 (B). Total DEGs are listed under each siRNA treatment.

OTP 2.0 siRNAs outperform alternative siRNA designs in both function and specificity



A-D) Volcano plot depicting DEGs with a -log10(adjusted pvalue) of >5 and a log2-fold change > |1| in each TP53 siRNA treatment sample, relative to NTC pool samples. Known TP53 target genes (red triangles) are consistent between ON-TARGETplus Design 1 (A), Vendor A (B), Vendor B (C), and Vendor C (D) siRNA treated samples. Additional significant downregulated DEGs (purple dots) and upregulated DEGs (green dots) are also depicted. Genes that did not cross the - log10(adjusted pvalue) of >5 and a log2-fold change > |1| threshold are depicted by grey dots. n=2 biological replicates per group.

Conclusions

- Transcriptome annotation evolution has necessitated comprehensive siRNA reselection.
- OTP 2.0 siRNAs retain high knockdown with high specificity, in some cases avoiding cross-targeting of newly-annotated transcripts.
- Global RNA-Seq and pathway analysis confirm the high specificity of OTP 2.0 siRNAs, especially compared to other available siRNA designs.

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