The STRIKE Platform Enables Kidney-Selective Gene Silencing via Megalin-Mediated Uptake

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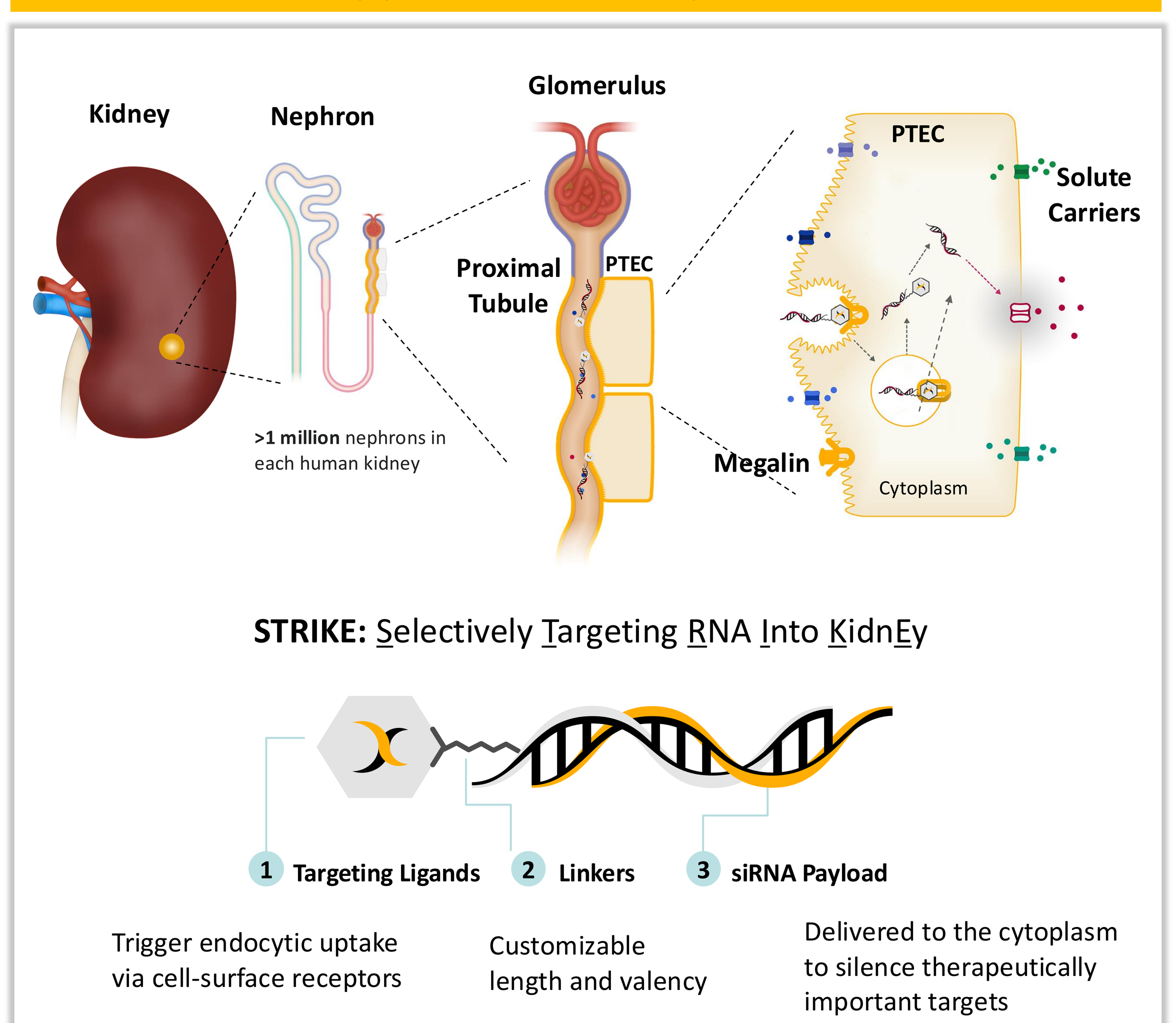
Proximal tubular epithelial cells (PTECs) in the kidney are critical to solute reabsorption and systemic homeostasis, making them an attractive target for therapeutic intervention in both renal and systemic diseases. However, oligonucleotide-based therapeutics such as siRNAs have shown limited effectiveness in this compartment due to inefficient delivery.

Judo Bio developed the STRIKE (Selectively Targeting RNA Into KidnEy) platform to achieve kidney-specific and PTEC-selective delivery of ligand-conjugated siRNAs. The platform leverages megalin, a highly expressed, rapidly internalized, and recycled receptor on PTECs, to enable receptor-mediated uptake and durable gene silencing within the kidney.

Megalin-binding ligand-siRNA conjugates (STRIKERs) were designed and evaluated in vitro and in vivo to assess delivery and activity. Fluorescence imaging, mass spectrometry, and stem-loop qPCR confirmed selective siRNA distribution to the kidney, particularly in PTECs, while qPCR and in situ hybridization demonstrated robust and durable target knockdown following a single subcutaneous dose, with minimal effects in non-renal tissues. By targeting a solute carrier gene, megalin-STRIKER induced specific solute excretion in urine, confirming functional engagement.

The STRIKE platform enables potent, megalin-mediated delivery of siRNAs to PTECs, providing robust and selective gene silencing. These results demonstrate cross-species translatability and establish a foundation for targeted RNA-based therapeutics for renal and systemic diseases. STRIKE represents a promising platform for specifically targeting solute carrier proteins for therapeutic benefit.

The STRIKE platform enables targeted delivery of siRNA to kidney proximal tubule epithelial cells



- The kidney is a complex organ, containing dozens of unique and specialized cell types. Proximal tubule epithelial cells (PTECs) on the nephrons play a central role in solute reabsorption.
- Megalin is a highly expressed endocytic receptor on PTECs that recognizes diverse substrates. Its rapid internalization, slow degradation, and efficient recycling make it an ideal gateway for intracellular delivery of ligand–siRNA conjugates.
- The STRIKE platform selectively delivers siRNA to PTECs via megalin-mediated endocytosis, silencing mRNA expression of specific solute carrier proteins and thereby reducing the uptake of circulating solutes implicated in renal and systemic diseases.
- The Judo ligand was modified to achieve an optimal combination of cellular uptake and tissue specificity and was conjugated to siRNA via a proprietary linker optimized for stability and functional performance.

Megalin-STRIKERs distribute specifically to PTECs in kidneys

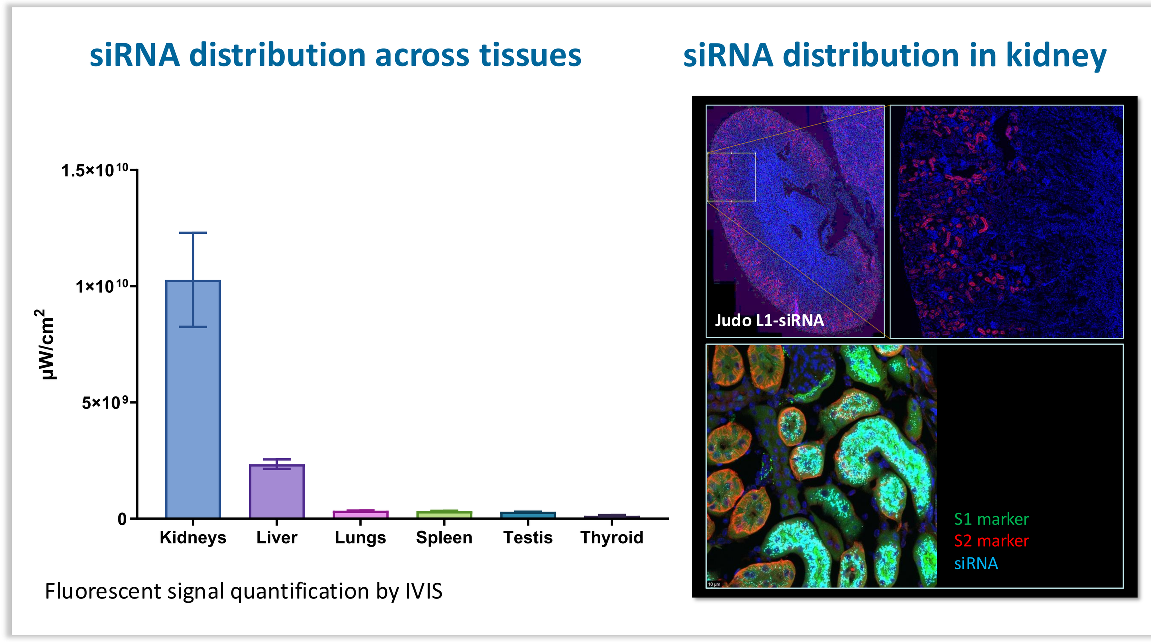


Figure 1. Mice were administered megalin-STRIKERs conjugated to a fluorescent dye (5 mg/kg) and sacrificed 4 hours post-dose. Fluorescence intensity across different organs was quantified by IVIS imaging (graph). Tissues sections were analyzed by fluorescence microscopy and immunofluorescence staining, showing robust, PTEC-specific uptake of Judo ligand-conjugated siRNA in the kidney.

Megalin-STRIKER platform is modular

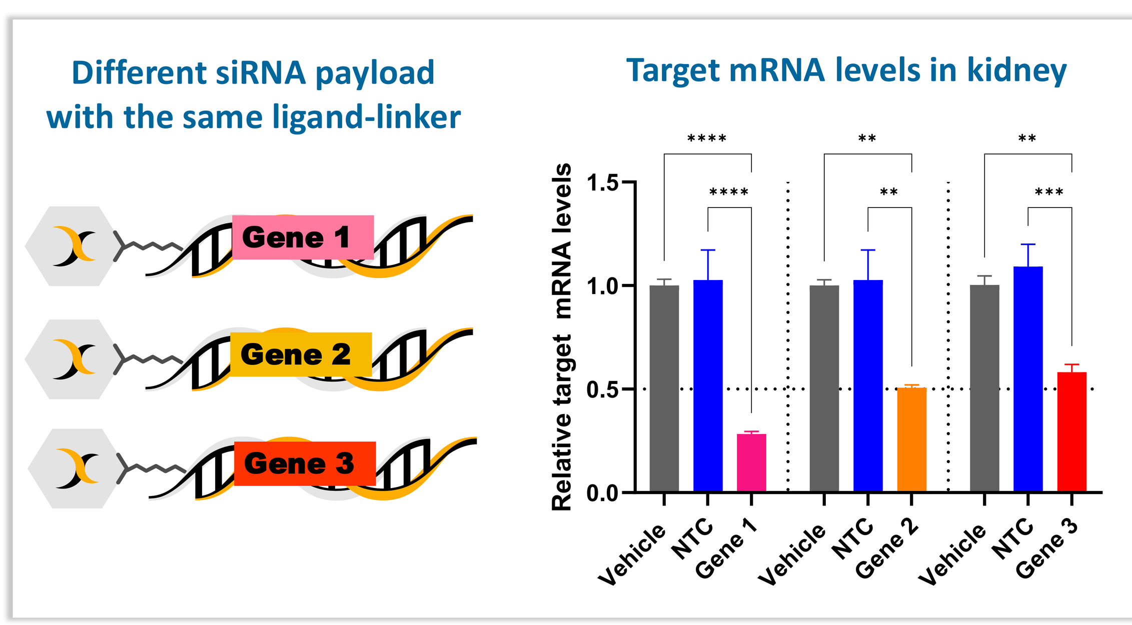


Figure 2. Mice were dosed with megalin STRIKERs targeting distinct genes (10 ml/kg) and necropsied on Day 7. Across all targets, 40 to 70% mRNA knockdown was achieved in the kidney. The relative PTEC expression levels of these tool genes followed the order: Gene 1 > Gene 2 > Gene 3. These results demonstrate the modularity of the STRIKE platform, enabling application of a single ligand-linker design across diverse siRNA targets. Statistical analysis by one-way ANOVA, **: P<0.01, ***: P<0.001, ****: P<0.0001.

~70% durable target gene knockdown was attained in mouse kidneys with Megalin-STRIKERs

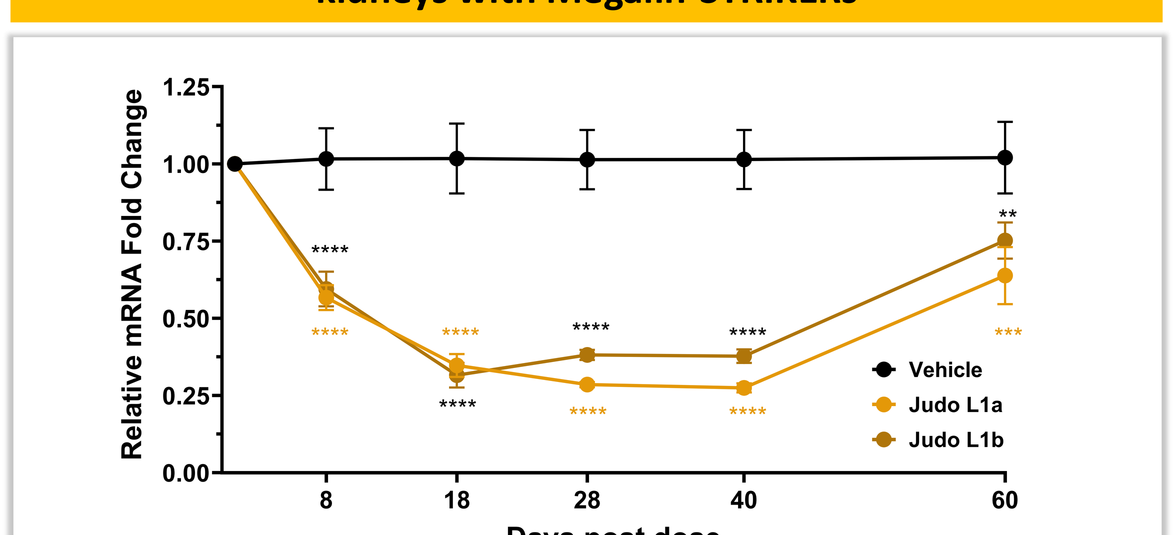


Figure 3. Mice were administered a single subcutaneous dose (10 ml/kg) of two variants of megalin STRIKERs (Judo L1a and L1b), and gene silencing duration was monitored over 60 days. Both L1a- and L1b-conjugated siRNAs achieved sustained kidney gene knockdown, reaching up to 70% reduction. Statistical analysis by two-way ANOVA, **: P<0.01, ***: P<0.001, ****: P<0.0001.

Dose-dependent mRNA knockdown and siRNA exposure with Megalin-STRIKERs

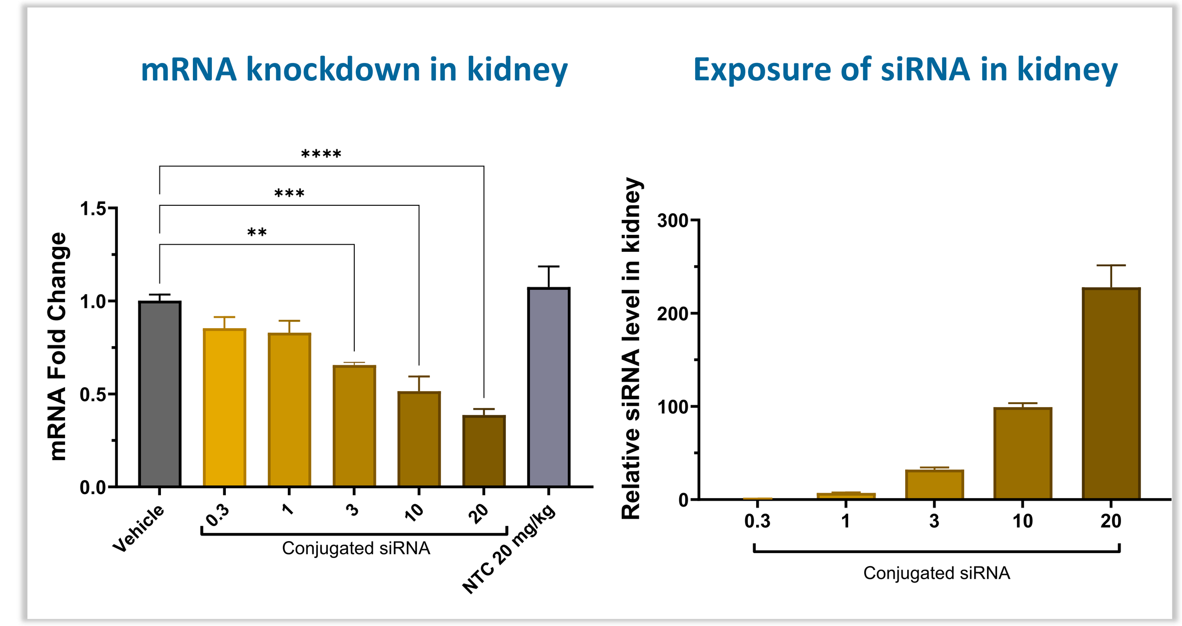


Figure 4. Mice were administered a single subcutaneous dose of megalin-STRIKERs at varying dose levels and necropsied on Day 14. Dose-dependent target gene knockdown was observed in the kidney (Left), which corresponded with a dose proportional increase in siRNA exposure, as quantified by Stem-loop qPCR (Right). Statistical analysis by one-way ANOVA, **:P<0.01,***: P<0.001,****: P<0.0001.

Characterizing Megalin-STRIKERs plasma and tissue PK

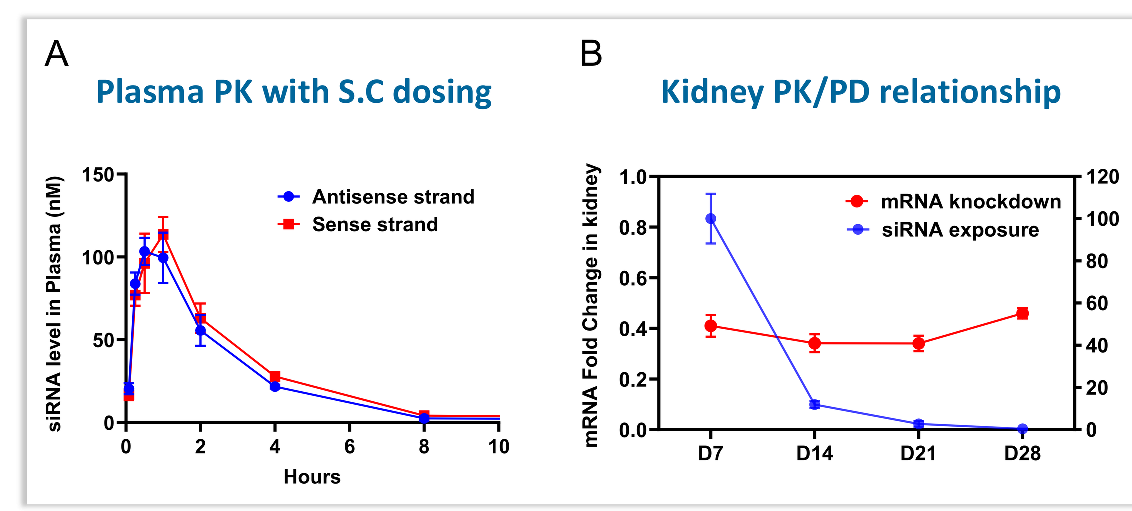


Figure 5. A) Mice were administered a single subcutaneous dose of megalin STRIKERs and necropsied at various time points. Plasma siRNA levels were quantified by LC/MS. The sense and antisense strands exhibited similar PK profiles. B) Mice received a single dose of conjugated siRNA and sacrificed on day 7, 14, 21 and 28. mRNA knockdown and siRNA exposure in kidneys were determined. Sustained kidney mRNA silencing was observed for 4 weeks post dose.

Megalin-STRIKERs primarily distributed to PTECs in kidneys in non-human primates (NHPs)

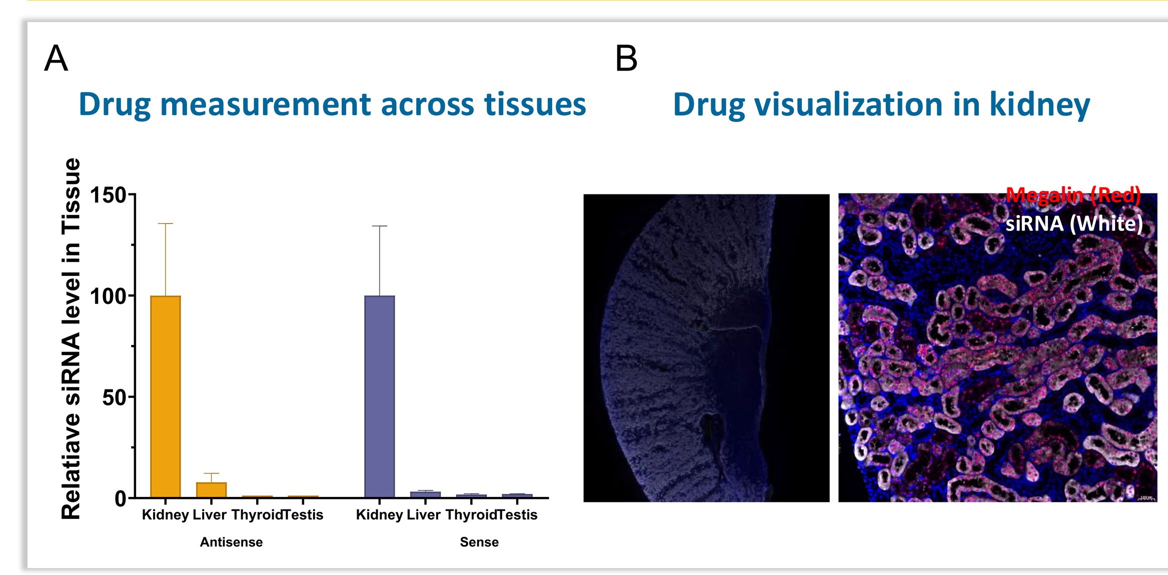


Figure 6. A) Non-human primates (NHPs) were subcutaneously dosed with the megalin STRIKERs and necropsied 4 weeks post dose. Multiple tissues were collected, and siRNA exposure was quantified by LC/MS. B) Megalin ligand conjugated siRNA was injected subcutaneously, and kidney sections were imaged 4 hours post dose by RNAscope *in situ* hybridization, showing siRNA (white) and megalin mRNA (red) localization. The megalin STRIKER signal was specifically enriched in PTECs, co-localizing with megalin expression.

Megalin-STRIKER increased urinary solute excretion via silencing target gene in proximal tubule of NHP kidneys

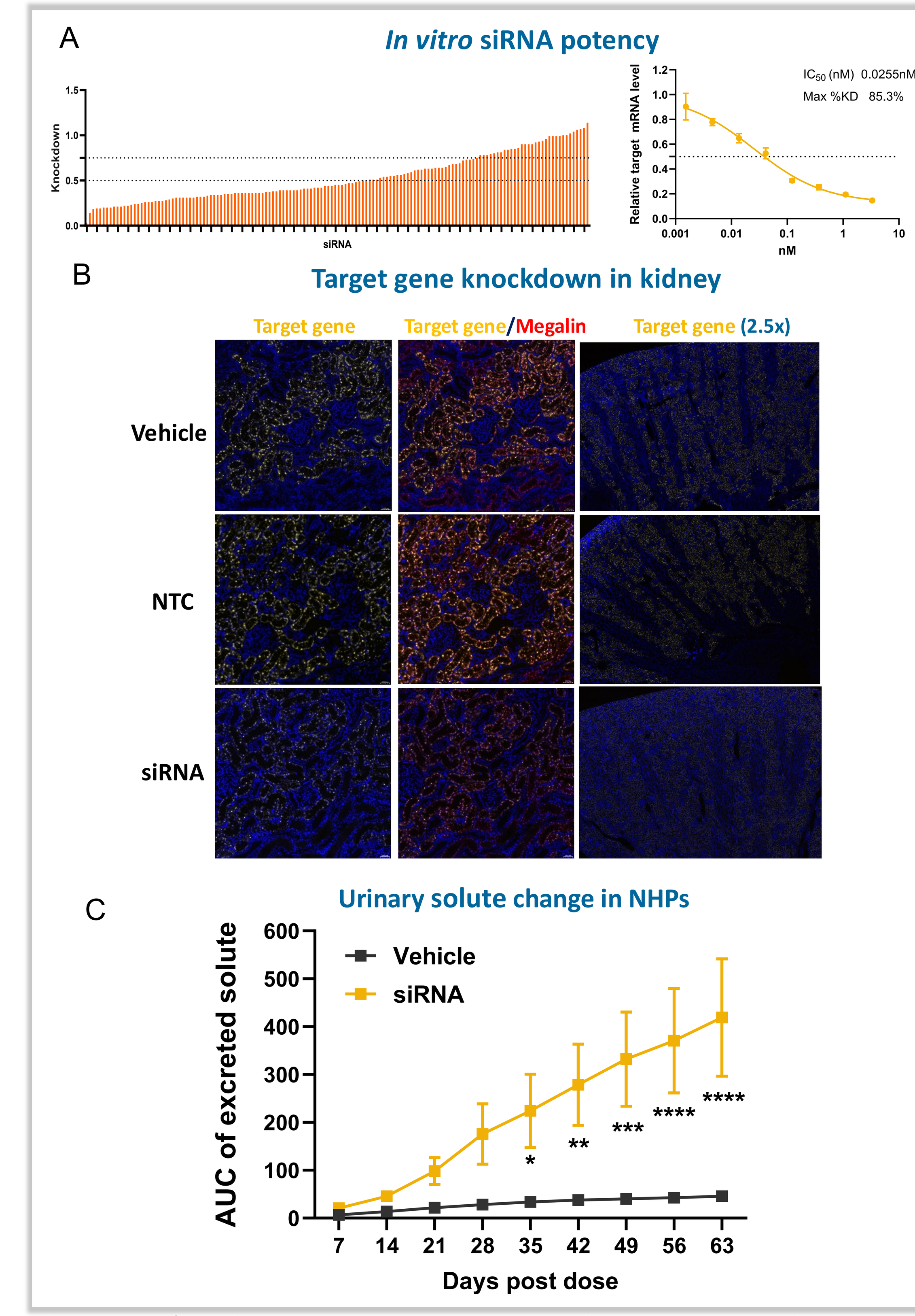


Figure 7. A) Cells were treated with siRNAs for 48 hours, and target gene knockdown efficiency was measured at single dose or multiple dose levels to calculate the IC_{50} values. The lead siRNA was scaled up for NHP studies.

B) NHPs received a single subcutaneous dose of siRNA conjugate (megalin STRIKER) targeting the solute carrier on Day 0. Kidney tissues were processed at necropsy (Day 63) to visualize target mRNA by *in situ* hybridization. Sections were also probed for megalin to mark the PTECs.

C) NHP urine samples were collected before dosing and periodically over 63 days post-dose. Concentrations of urinary solutes were quantified by LC/MS and normalized using an internal reference factor. Area under the curve (AUC) of fold changes relative to pre-dose levels were calculated. Statistical analysis by two-way ANOVA, *P<0.05, **: P<0.01, ***: P<0.001, ****: P<0.0001

SUMMARY

The STRIKE (Selectively Targeting RNA Into KidnEy) platform represents a significant advancement in targeted siRNA delivery for renal applications. Megalin-STRIKERs specifically target PTECs by engaging the megalin receptor and achieved ~70% knockdown with the tool gene following subcutaneous dosing. The platform demonstrated dose-dependence, sustained efficacy and modularity, allowing multiple siRNA payloads to be efficiently delivered using a common ligand-linker design. In NHPs, the megalin-STRIKER selectively accumulated in PTECs, driving functional effects such as increased urinary solute excretion. Additionally, no adverse effects were observed in both mice and NHPs. There was no change in hematology and serum chemistry or kidney injury markers. These findings highlight the utility of our approach in optimizing gene silencing for therapeutic intervention in systemic and renal diseases. Future research will focus on linking molecular knockdown to functional outcomes and disease modification in both rodent and NHP models.