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## **Supplemental Information**

### **GalNAc Conjugation Attenuates the Cytotoxicity of Antisense Oligonucleotide Drugs in Renal Tubular Cells**

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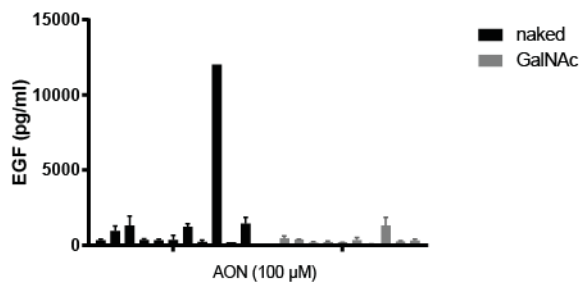
## **SUPPLEMENTARY MATERIAL**

Figure S1. Additional examples of preclinical testing of GalNAc-AON libraries in vitro

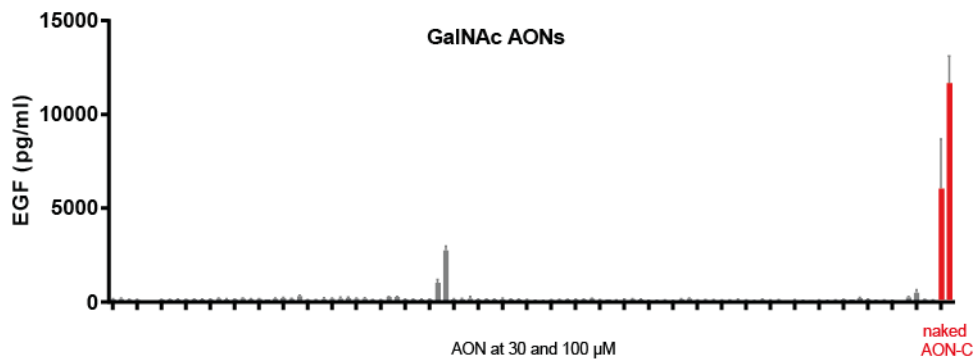
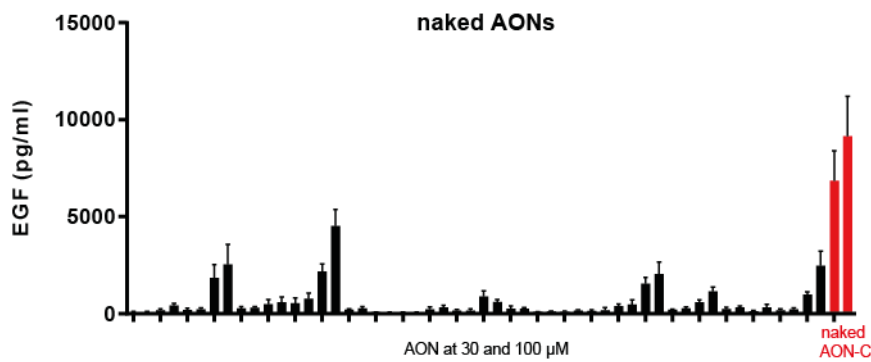
Table S1. List of AONs with corresponding sequences and backbone modifications

Table S2. Capture and detection probes for hybridization ELISA detection of AONs

**a**



**b**



**Fig. S1** Preclinical safety testing of naked and GalNAc-AON libraries in PTEC. **(a-b)** Measurement of EGF in the supernatant of PTEC cells treated for 6 days with naked or GalNAc AON libraries, revealing a generally reduced cytotoxicity effect of GalNAc-conjugated AONs compare to naked AONs. **(a)** Matched pairs of naked and GalNAc AONs sharing the same oligonucleotide sequence were tested at 100  $\mu$ M. **(b)** Libraries of naked (upper panel) and GalNAc (lower panel) AONs designed against the same target gene but consisting of different oligonucleotide sequences were tested at 30 and 100  $\mu$ M. Naked AON-C was included as a toxic AON control. Data represent means and SD (n=3).

Table S1. List of AONs with corresponding sequences and backbone modifications

AON	Target	Conjugation	Sequence (5' to 3')
A	Scramble	none (naked)	<sup>m</sup> CGTcagtatgcgAATc
		GalNAc	5'-GN2-C6ca <sup>m</sup> CGTcagtatgcgAATc
B	PCSK9	none (naked)	TG <sup>m</sup> Ctacaaaac <sup>m</sup> C <sup>m</sup> CA
		GalNAc	5'-GN2-C6caTG <sup>m</sup> Ctacaaaac <sup>m</sup> C <sup>m</sup> CA
		GalNAc, uncleavable linker	5'-GN2-C6T <sup>m</sup> Ctacaaaac <sup>m</sup> C <sup>m</sup> CA
C	PCSK9	none (naked)	G <sup>m</sup> CtgtgtgagcttGG
		GalNAc	5'-GN2-C6caG <sup>m</sup> CtgtgtgagcttGG

Single strand oligonucleotide sequence is indicated in blue font

Uppercase: Wing segments of nucleotides with locked nucleic acid (LNA) sugar modifications. <sup>m</sup>C: 5-methylcytidine.  
Lower case: Gap segment of 2'-deoxynucleotides.  
Internucleotide linkages are phosphorothioated.

5'-GN2: N-acetylgalactosamine (GalNAc).  
Molecular weight: 1476.5 g/mol  
Empirical formula: C60H106N7O33.Na.

C6ca: triantennary linker  
C6T: uncleavable triantennary linker

Table S2. Capture and detection probes for hybridization ELISA detection of AONs

AON	Detection probe	Capture probe
A	5'-DIG-GATTCGCA-3'	5'-TACTGACG-BIO-3'
B	5'-DIG-TGGGTTT-3'	5'-TGTAGCA-BIO-3'
C	5'-DIG-CCAAGCTC-3'	5'-ACACAGC-BIO-3'