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Supporting Information

Chimeric Flaviviral RNA–siRNA Molecules Resist Degradation by The Exoribonuclease Xrn1 and Trigger Gene Silencing in Mammalian Cells

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Table S1. Sequences of xrRNA constructs. Constructs with stem-loop deletions are denoted by ΔP4-L4. WT, wild type; DENV, Dengue virus; MECDV, Mercadeo virus; SLEV, Saint Louis encephalitis virus; ZIKV, Zika virus; TABV, Tamana bat virus; GBVB, GB virus B; APPV, atypical porcine pestivirus; SPgV, simian pegivirus.

Entry	Sequence (5’ to 3’)	Accession number	Range
DENV$_{\Delta P4\text{-}L4}$	AGUCAGGCCGGAUUAAGCCAUAGUACGGUAAGAGCUAUGCUGCCUGUGAGCCC	MT447147	10324-10376
MECDV$_{\Delta P4\text{-}L4}$	AGUCAGACCGAAAAGCCACUCAAUGGUUGUAAAGAGUGCUGUCUGUGACAAC	NC_027819	10594-10642
SLEV$_{\Delta P4\text{-}L4}$	AGUCAGGCCAAUCAGUUUUGCCACCGGAUGUCAGGUAAACGGUGCUGUCUGUAACCUGGC	NC_007580.2	10430-10489
ZIKV$_{\Delta P4\text{-}L4}$	AGUCAGGCCGAUAACGCCAUGGCACGGAAAAAGCCAUGCUGCCUGUGAGCC	MF510857.1	10476-10526
TABV WT	GGCAAGGUACGGAUUAGCCGUAGGGGCUUGAGAACCCCCCCUCCCCACUC	AF346759	10226-10275
GBVB WT	GGCAACAGGGGAGACCCCGGGCUUAACGACCCCGCCGAUGUGAGUU	NC_001655.1	9099-9144
APPV WT	GGGCAAGUGGCCGUAUAGGCUGGGGCGAUCGCCGUACCCCCCCUUUACCAGGCG	NC_038964.1	11108-11161
SPgV WT	GGCAGCAGCAGACGCAAGUCUGGGGGAAACGAUCGCUCCUCCCUCUGCAGAUC	U94421.1	9446-9498

Table S2. Masses calculated and found for xrRNA constructs.

Entry	Mass calculated (g/mol)	Mass found (g/mol)
DENV$_{\Delta P4\text{-}L4}$	17076.3	17076.45
MECDV$_{\Delta P4\text{-}L4}$	16747.2	16746.62
SLEV$_{\Delta P4\text{-}L4}$	19256.6	19255.83
ZIKV$_{\Delta P4\text{-}L4}$	16446.0	16445.91
TABV WT	16005.7	16004.91
GBVB WT	14863.0	14862.11
APPV WT	17379.5	17378.38
SPgV WT	17049.3	17048.22

Table S3. Sequences of xrRNA-siRNA constructs and controls. Adapters are bold. Antisense sequences are underlined. siREN, Renilla luciferase-targeting siRNA; siRND, non-targeting control siRNA; S1, strand 1; S2, strand 2; AS, antisense strand; SS, sense strand; 5' EXT, 5' extended; X, randomized nucleotide position.

Entry	Sequence (5' to 3')
DENV$_{\Delta P4\text{-}L4}$-siREN S1	AGUCAGGCC**AA**GAGCGAAGAGGGCGAGAAAUU
DENV$_{\Delta P4\text{-}L4}$-siREN S1 5' EXT	AAAGGAAAAUAAAAAAUCAAACAAGGCAAGAAGUCAGGCC**AA**GAGCGAAGAGGGCGAGAAAUU
DENV$_{\Delta P4\text{-}L4}$-siREN S2	UUUCUCGCCCUCUUCGCUCUUGCCAUAGUACGGUAAGAGCUAUGCUGCCUGUGAGCCCs
DENV$_{\Delta P4\text{-}L4}$-siRND S1	AGUCAGGCC**AA**GUGXXUAAXXAACXCAXACUU
DENV$_{\Delta P4\text{-}L4}$-siRND S2	GUXUGXGUUXXUUAXXCACUUGCCAUAGUACGGUAAGAGCUAUGCUGCCUGUGAGCCC
MECDV$_{\Delta P4\text{-}L4}$-siREN S1	AGUCAGACC**AA**GAGCGAAGAGGGCGAGAAAUU
MECDV$_{\Delta P4\text{-}L4}$-siREN S2	UUUCUCGCCCUCUUCGCUCUUGCCACUCAAUGGUUGUAAAGAGUGCUGUCUGUGACAAC
MECDV$_{\Delta P4\text{-}L4}$-siRND S1	AGUCAGACC**AA**GUGXXUAAXXAACXCAXACUU
MECDV$_{\Delta P4\text{-}L4}$-siRND S2	GUXUGXGUUXXUUAXXCACUUGCCACUCAAUGGUUGUAAAGAGUGCUGUCUGUGACAAC
SLEV$_{\Delta P4\text{-}L4}$-siREN S1	AGUCAGGCCAA**AA**GAGCGAAGAGGGCGAGAAAUU
SLEV$_{\Delta P4\text{-}L4}$-siREN S2	UUUCUCGCCCUCUUCGCUCUUUUGCCACCGGAUGUCAGGUAAACGGUGCUGUCUGUAACCUGGC
SLEV$_{\Delta P4\text{-}L4}$-siRND S1	AGUCAGGCCAA**AA**GUGXXUAAXXAACXCAXACUU
SLEV$_{\Delta P4\text{-}L4}$-siRND S2	GUXUGXGUUXXUUAXXCACUUUUGCCACCGGAUGUCAGGUAAACGGUGCUGUCUGUAACCUGGC
ZIKV$_{\Delta P4\text{-}L4}$-siREN S1	AGUCAGGCCG**AA**GAGCGAAGAGGGCGAGAAAUU
ZIKV$_{\Delta P4\text{-}L4}$-siREN S2	UUUCUCGCCCUCUUCGCUCUUCGCCAUGGCACGGAAAAAGCCAUGCUGCCUGUGAGCC
ZIKV$_{\Delta P4\text{-}L4}$-siRND S1	AGUCAGGCCG**AA**GUGXXUAAXXAACXCAXACUU
ZIKV$_{\Delta P4\text{-}L4}$-siRND S2	GUXUGXGUUXXUUAXXCACUUCGCCAUGGCACGGAAAAAGCCAUGCUGCCUGUGAGCC
siREN AS	UUUCUCGCCCUCUUCGCUCUU
siREN SS	GAGCGAAGAGGGCGAGAAAUU
siRND AS	GUXUGXGUUXXUUAXXCACUU
siRND SS	GUGXXUAAXXAACXCAXACUU

Table S4. Masses calculated and found for xrRNA-siRNA constructs and controls. For oligoribonucleotides synthesized with randomized nucleotide positions, the measured masses fall within the expected range, *i.e.*, between the mass of an oligoribonucleotide with G nucleotides at all random positions and the mass of an oligoribonucleotide with C nucleotides at all random positions.

Entry	Mass calculated (g/mol)	Mass found (g/mol)
DENV$_{\Delta P4\text{-}L4}$-siREN S1	10475.5	10474.64
DENV$_{\Delta P4\text{-}L4}$-siREN S1 5’ EXT	20642.8	20641.30
DENV$_{\Delta P4\text{-}L4}$-siREN S2	18368.9	18369.20
MECDV$_{\Delta P4\text{-}L4}$-siREN S1	10459.5	10458.57
MECDV$_{\Delta P4\text{-}L4}$-siREN S2	18684.1	18683.92
SLEV$_{\Delta P4\text{-}L4}$-siREN S1	11133.9	11132.9
SLEV$_{\Delta P4\text{-}L4}$-siREN S2	20283.0	20286.57
ZIKV$_{\Delta P4\text{-}L4}$-siREN S1	10820.7	10819.81
ZIKV$_{\Delta P4\text{-}L4}$-siREN S2	18390.0	18390.14
siREN AS	6436.8	6436.09
siREN SS	6901.3	6900.39

Table S5. Sequences of xrRNA-siRNA constructs. Adapters are bold. Antisense sequences are underlined.

Entry	Sequence (5’ to 3’)
DENV-siREN S1	AGUCAGGCC**AA**GAGCGAAGAGGGCGAGAAAUU
DENV-siREN S2	UUUCUCGCCCUCUUCGCUCUUGCCAUAGUACGGUAAGAGCUAUGCUGCCUGUGAGCCCCGUCUAAGGACGU
MECDV-siREN S1	AGUCAGACC**AA**GAGCGAAGAGGGCGAGAAAUU
MECDV-siREN S2	UUUCUCGCCCUCUUCGCUCUUGCCACUCAAUGGUUGUAAAGAGUGCUGUCUGUGACAACCCAAAUAGUCGAUGGA
ZIKV-siREN S1	AGUCAGGCCG**AA**GAGCGAAGAGGGCGAGAAAUU
ZIKV-siREN S2	UUUCUCGCCCUCUUCGCUCUUCGCCAUGGCACGGAAAAAGCCAUGCUGCCUGUGAGCCCCUCAGAGGACACUGAGU

Table S6. Sequences of mutant MECDV-siREN constructs with stem-loop P4-L4 re-installed. Mutation sites are underlined. MUT, mutant.

Entry	Sequence (5’ to 3’)
MECDV-siREN S2$_{MUT1}$	UUUCUCGCCCUCUUCGCUCUUGCCACUCAAUGGGGGGAAAGAGUGCUGUCUGUGCCCCCCCAAAUAGUCGAUGGA
MECDV-siREN S2$_{MUT2}$	UUUCUCGCCCUCUUCGCUCUUGCCACUCAAUGCCCCCAAAGAGUGCUGUCUGUGGGGGGCCAAAUAGUCGAUGGA
MECDV-siREN S2$_{MUT3}$	UUUCUCGCCCUCUUCGCUCUUGCCACUCAAUGGCGCGAAAGAGUGCUGUCUGUGCGCGCCCAAAUAGUCGAUGGA

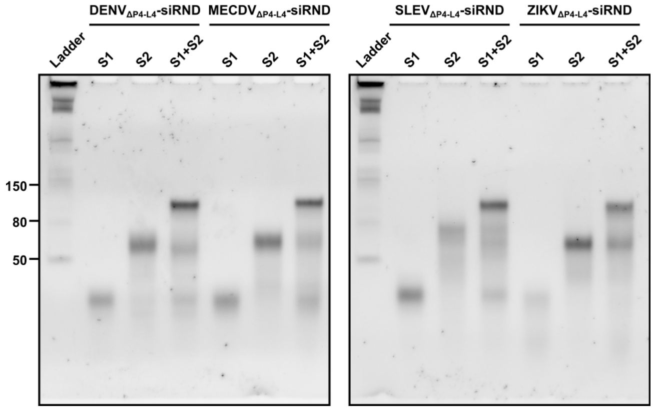

Figure S1. Non-denaturing polyacrylamide gel mobilities of control xrRNA$_{\Delta P4\text{-}L4}$-siRNA constructs. Reference bands in the ladder are single-stranded RNA.

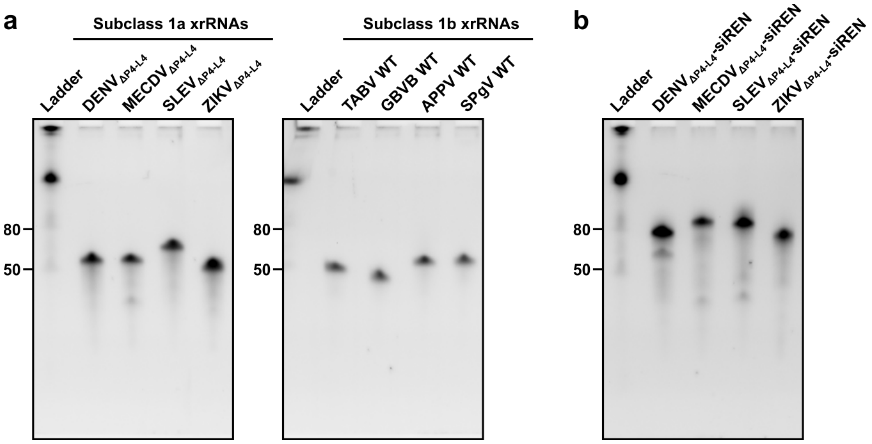

Figure S2. (a) Denaturing polyacrylamide gel mobilities of xrRNA constructs. (b) Denaturing polyacrylamide gel mobilities of xrRNA$_{\Delta P4\text{-}L4}$-siRNA constructs. Reference bands in the ladder are single-stranded RNA.

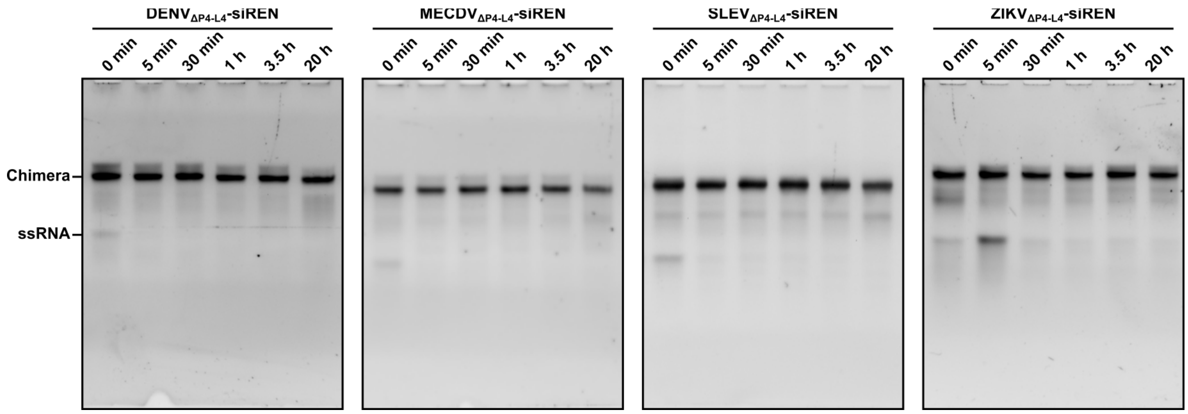

Figure S3. Non-denaturing polyacrylamide gel mobilities of xrRNA$_{\Delta P4\text{-}L4}$-siRNA constructs with a two-fold molar excess of S1 to S2 following incubation with Xrn1 at 37°C. The excess S1 is labelled as a single-stranded RNA (ssRNA). The length of incubation with Xrn1 is indicated above each lane.

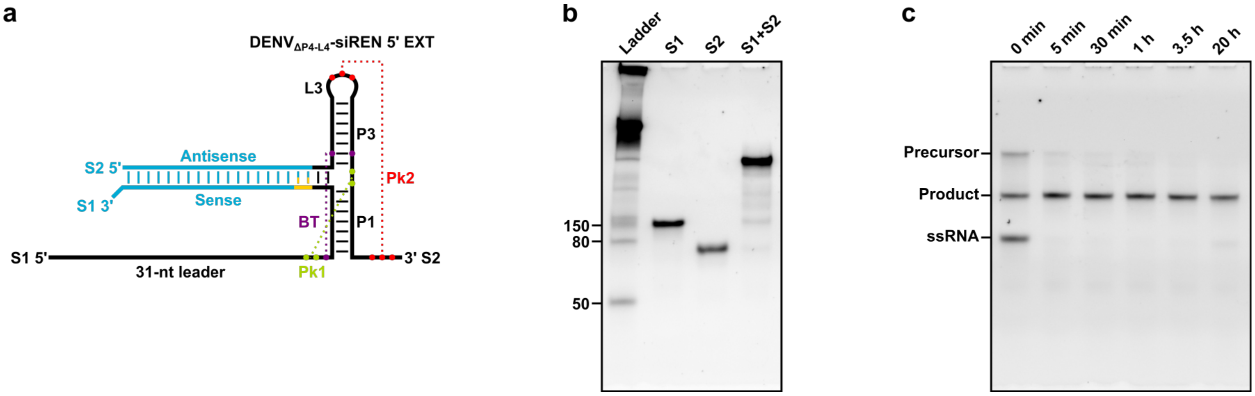

Figure S4. (a) DENV$_{\Delta P4\text{-}L4}$-siREN 5' extended (5' EXT) construct. A 31-nucleotide (nt), unstructured "leader" sequence at the 5' end of S1 is labelled. (b) Non-denaturing polyacrylamide gel mobility of the DENV$_{\Delta P4\text{-}L4}$-siREN 5' EXT construct. Reference bands in the ladder are single-stranded RNA. (c) Non-denaturing polyacrylamide gel mobility of the DENV$_{\Delta P4\text{-}L4}$-siREN 5' EXT construct with a two-fold molar excess of S2 to S1 following incubation with Xrn1 at 37°C. The excess S2 is labelled as a single-stranded RNA (ssRNA). The length of incubation with Xrn1 is indicated above each lane.

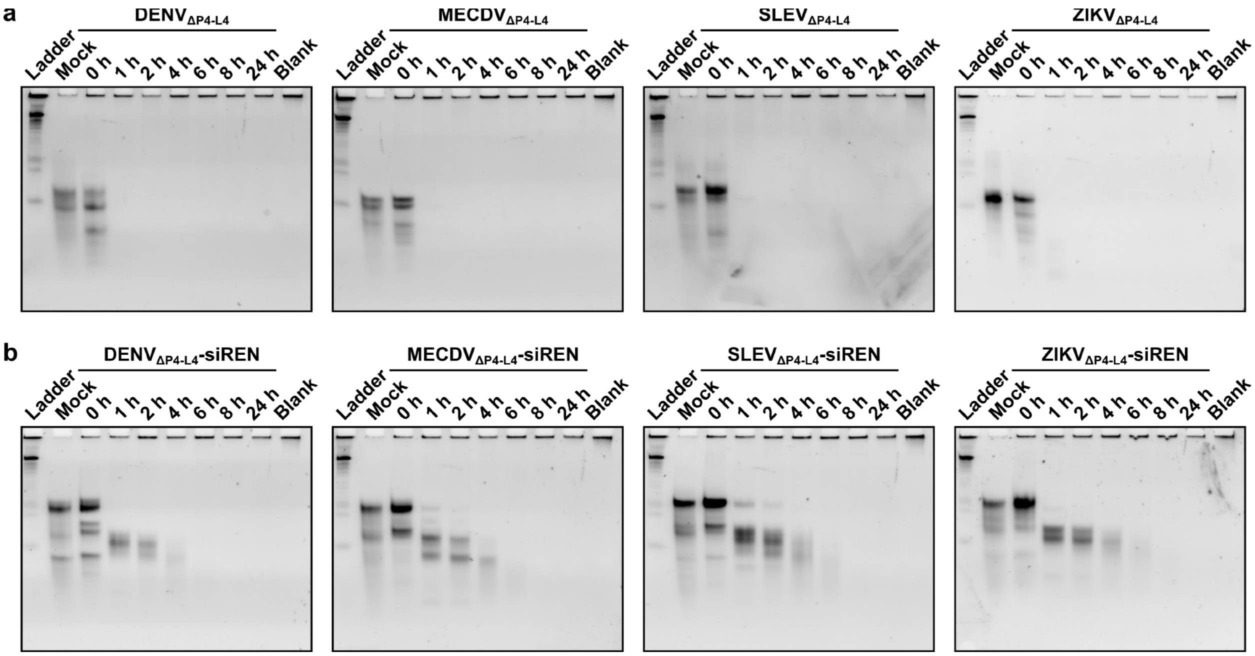

Figure S5. (a) Serum stability assays of xrRNA$_{\Delta P4\text{-}L4}$ constructs in a second batch of mouse serum. (b) Serum stability assays of xrRNA$_{\Delta P4\text{-}L4}$-siRNA constructs in a second batch of mouse serum. The length of incubation in 50% mouse serum is indicated above each lane. Mock is RNA incubated in water at 37°C for 24 h. Blank is 50% mouse serum incubated at 37°C for 24 h. Reference bands in the ladder are single-stranded RNA.

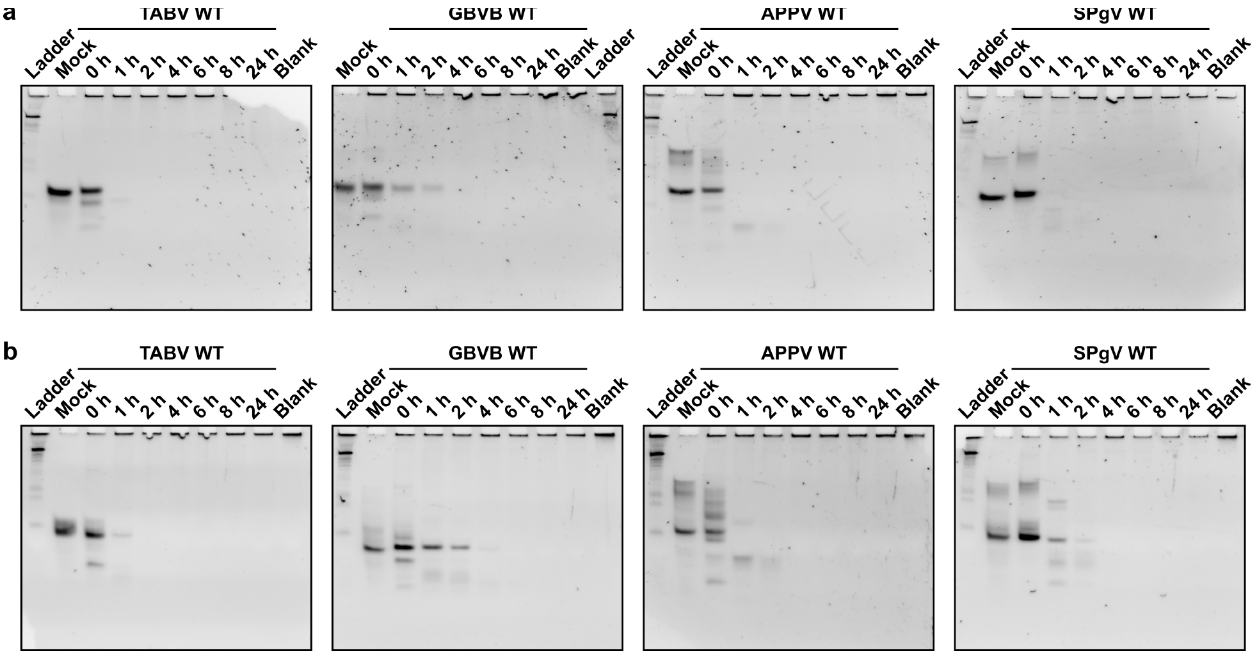

Figure S6. (a) Serum stability assays of xrRNA constructs. (b) Serum stability assays of the same constructs in a second batch of mouse serum. The length of incubation in 50% mouse serum is indicated above each lane. Mock is RNA incubated in water at 37°C for 24 h. Blank is 50% mouse serum incubated at 37°C for 24 h. Reference bands in the ladder are single-stranded RNA.

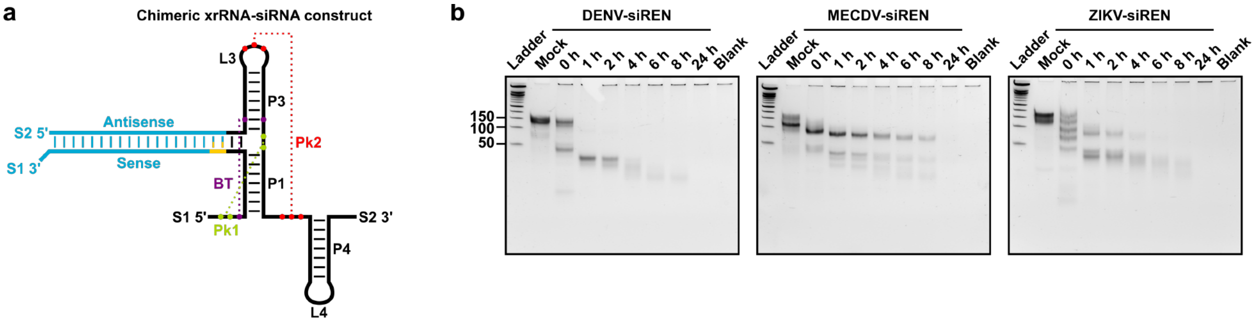

Figure S7. (a) Chimeric xrRNA-siRNA construct. (b) Serum stability assays for xrRNA-siRNA constructs annealed in 1 M NaCl, 10 mM cacodylic acid, pH 7.0. The length of incubation in 50% mouse serum at 37°C is indicated above each lane. Mock is RNA incubated in water at 37°C for 24 h. Blank is 50% mouse serum incubated at 37°C for 24 h. The reference bands in the ladder are double-stranded DNA. Each gel image is representative of three independent replicates.

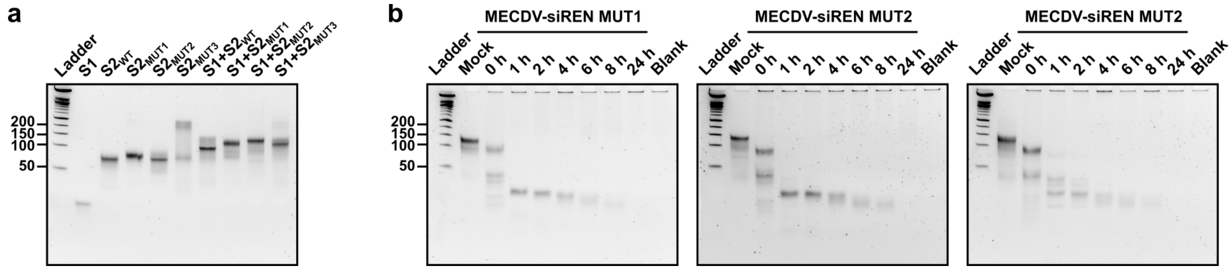

Figure S8. (a) Non-denaturing polyacrylamide gel mobilities of mutant MECDV-siREN constructs. (b) Serum stability assays for mutant MECDV-siREN constructs annealed in 1 M NaCl, 10 mM cacodylic acid, pH 7.0. The length of incubation in 50% mouse serum at 37°C is indicated above each lane. Mock is RNA incubated in water at 37°C for 24 h. Blank is 50% mouse serum incubated at 37°C for 24 h. In (a) and (b), the reference bands in the ladder are double-stranded DNA.