Appendix

The helicase domain of human Dicer prevents RNAi-independent activation of antiviral and inflammatory pathways

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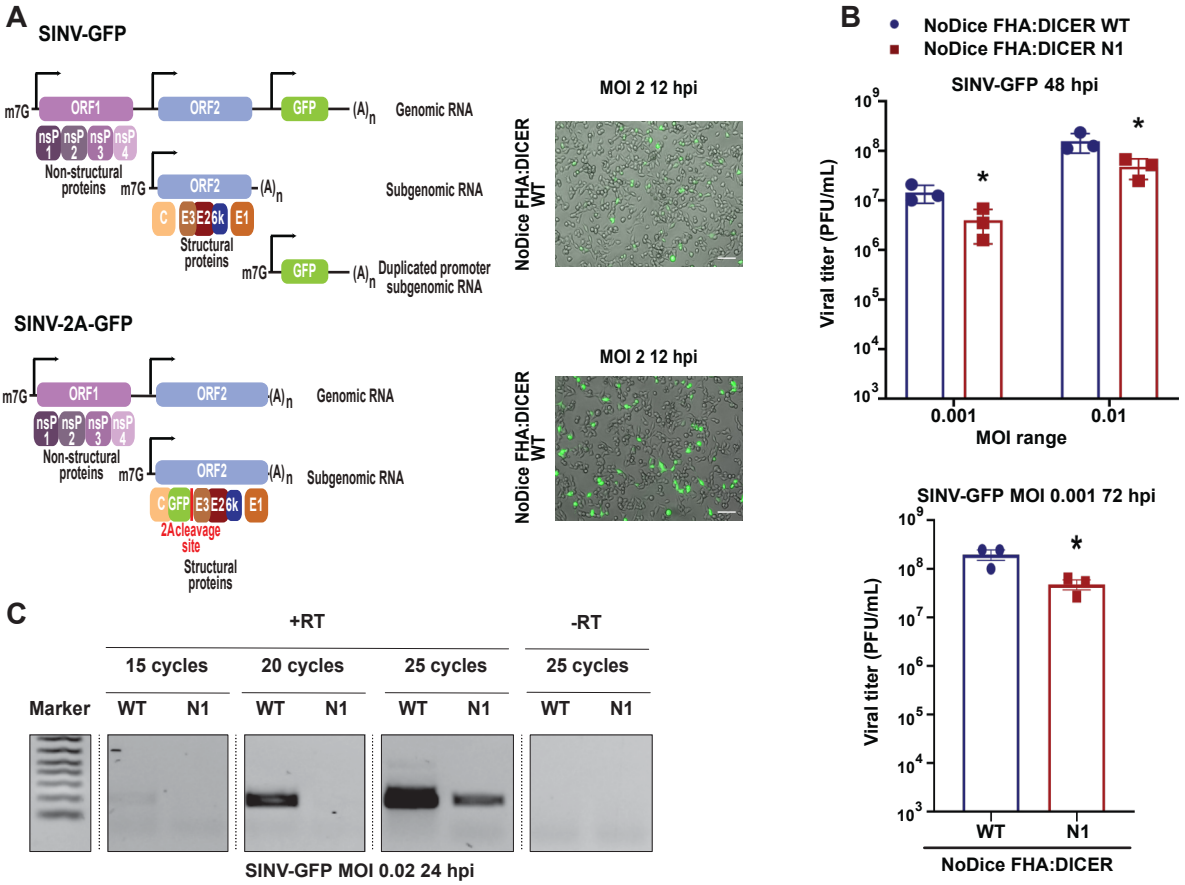
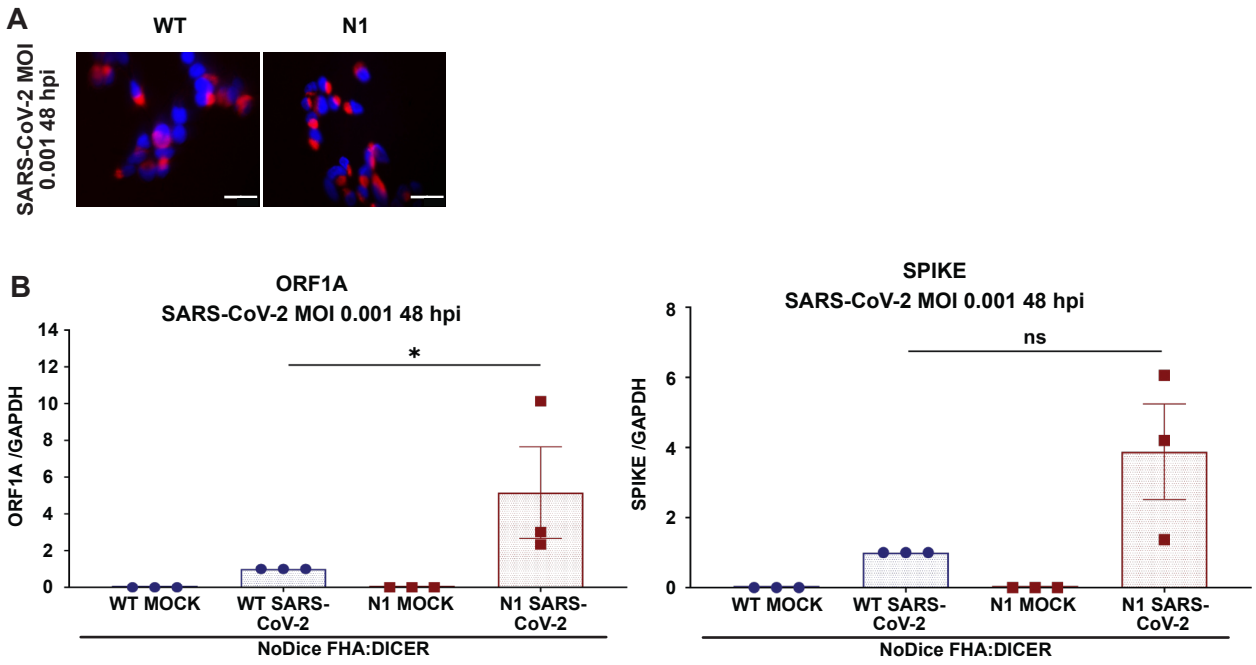


Appendix Figure S1. Dicer N1 reduces SINV replication at longer infection times and decreases antigenome production.

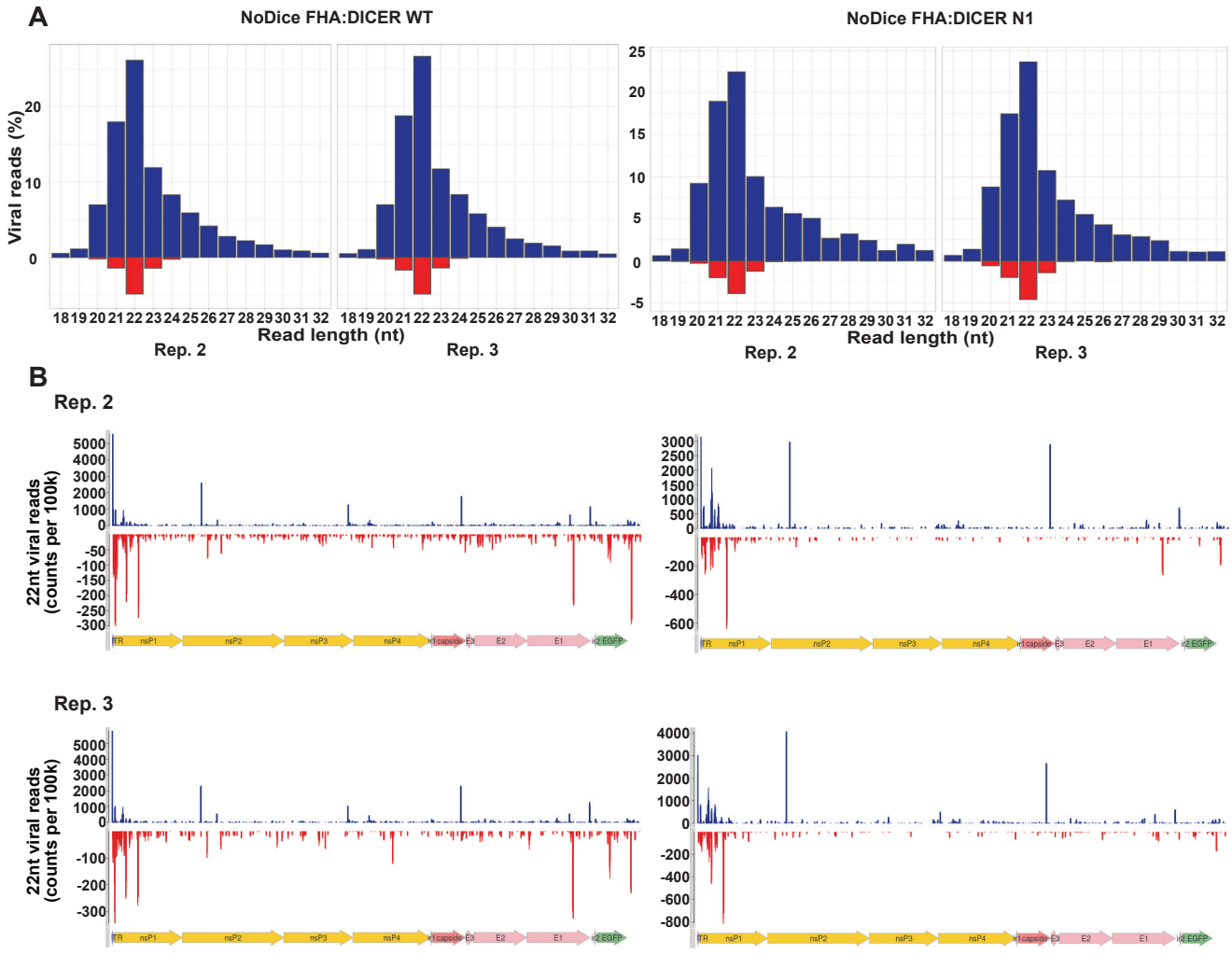
A. Schematic representation of SINV-GFP and SINV-2A-GFP genome organization and representative fluorescent pictures of GFP expressed by the two viruses upon infection of NoDice FHA:DICER WT cells at an MOI of 2 for 12h. Scale bar = 50µm, (n = 3 biological replicates). ORF: Open reading frame, nsP: non-structural proteins, C: capsid, E1 to E3: envelope proteins.

B. Mean (+/- SEM) SINV-GFP viral titer in NoDice FHA:DICER WT and N1 infected at an MOI 0.001 and 0.01 for 48h (up) and 0.001 for 72h (bottom) from plaque assay quantification (n = 3 biological replicates). Upper panel: ordinary two-way ANOVA test with Sidak's correction. *: $p < 0.05$. Bottom panel: Unpaired t-test. *: $p<0.05$.

C. Agarose gel of RT-PCR on SINV antigenome in SINV-GFP infected NoDice FHA:DICER WT and N1 cells at an MOI of 0.02 for 24h (n = 3 biological replicates).



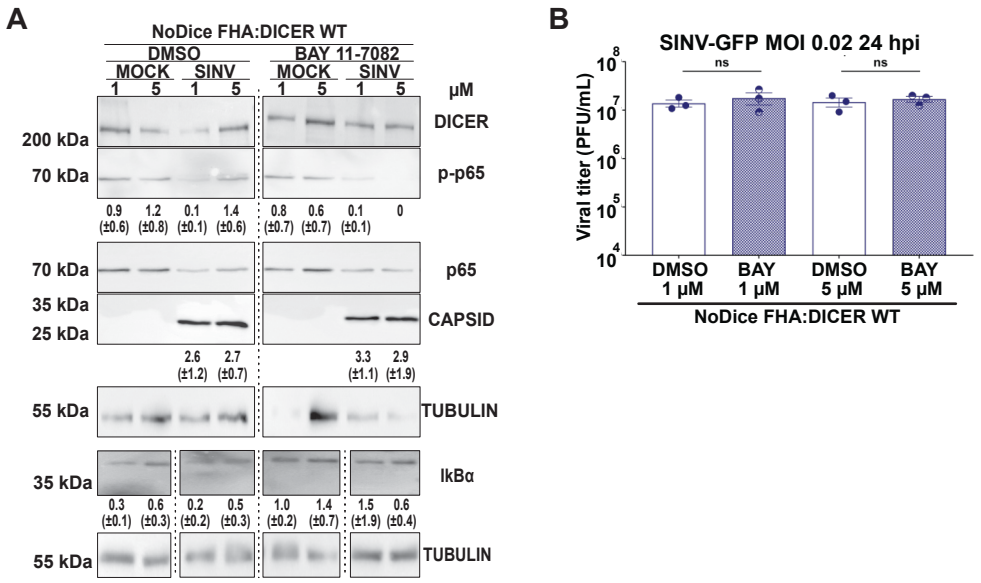
Appendix Figure S2. Dicer N1 enhances SARS-CoV-2 replication.
A. Immunofluorescence analysis on NoDice FHA:DICER WT and N1 cells in SARS-CoV-2 infected cells at an MOI of 0.001 for 48h. J2 antibody (red) was used to detect dsRNA upon infection. DAPI was used to stain the nuclei (blue). Scale bar = 100 µm, (n = 3 biological replicates).
B. RT-qPCR on SARS-CoV-2 genome (ORF1A and SPIKE) in NoDice FHA:DICER WT and N1 cells infected with SARS-CoV-2 at an MOI of 0.001 for 48h. Mean (+/-SEM); n = 3 biological replicates. Unpaired t-test. *: $p<0.05$; ns: non-significant.



Appendix Figure S3. Dicer N1 cells do not accumulate more virus-derived small RNAs than Dicer WT cells.

A. Representative histograms of the distribution of viral reads (in percent) per RNA length upon small RNA sequencing for replicates 2 and 3 in NoDice FHA:DICER WT (left) and N1 (right) cells infected with SINV-GFP at an MOI of 0.02 for 24h. Blue: positive strand; red: negative strand.

B. Representative graphs of the mapping of 22-nt-reads on SINV-GFP genome for replicates 2 and 3 in NoDice FHA:DICER WT (left) and N1 (right) cells.



Appendix Figure S4. NF-kB/p65 is not involved in the infection outcome in Dicer WT cells.
A. Western blot analysis of DICER, p-p65, p65, CAPSID and IkBα expression in SINV-GFP infected NoDice FHA:DICER WT cells at an MOI of 0.02 for 24h. Before infection, cells were treated with the NF-kB/p65 inhibitor, BAY 11-7082 or the vehicle (DMSO) at the indicated concentrations for 1 h. Alpha-Tubulin was used as loading control. Mean (+/- SD) of bands quantification of three independent replicates normalized to Tubulin are represented under the corresponding lane; p-p65 = p-p65/p65 quantification.
B. Mean (+/- SEM) of SINV-GFP viral titers in the same samples as in 1, infected at an MOI of 0.02 for 24h (n = 3 biological replicates) from plaque assay quantification. Ordinary one-way ANOVA test with Sidak's correction. ns: non-significant.

Appendix Table S1. List of oligonucleotides used in this study.

Type	Target	Fw	Rev
PCR primers for mutagenesis	PKR K296R	CGTTATTAGACGTGTTAAATATAATAACGAGAAGG	ACACGTCTAATAACGTAAGTCTTTCCG
	PKR T451A	TAAGGGAGCATTGCGATACATGAGCCCAGAAC	CGCAATGCTCCCTTACTCCTTGTTCGCTTTCC
	MYC EMPTY	AGGATCTGACCCAGCTTTCTTGTACAAAGTGG	GCTGGGTCAGATCCTCTTCTGAGATGAGTTT
	N1-CM	ACCAATTCAGTCGACATGGAAGCAGAATTC	GAATTCTGCTTCCATGTCGACTGAATTGGT
	ΔHEL1	CGCCAAGAAAATATCAGGTTAGTAATGCTGAAACTGC	GTTGCAGTTTCAGCATTACTAACCTGATATTTTCTTG
	ΔHEL2i	TTAGACAGAAATTTTCCTTCTCCTTTTACCAAC	AAAATTTCTGTCTAAGACCACCAGGTCAG
	ΔHEL2	CAGAGACACCTGTCATGGATGATGATCACG	TGACAGGTGTCTCTGGCTTCTCTTTTTCTTC
RT-PCR primers	RT specific SINV	ATTGACGGCGTAGTACACACTATTGAATCAAACAGCC GACCA	/
	PCR SINV antigenome	TAGACGTAGACCCCCAGAGTC	CATTCTACGAGCCGGTGCGC
qPCR primers	SINV genome	CCACTACGCAAGCAGAGACG	AGTGCCCAGGGCCTGTGTCCG
	GAPDH	CTTTGGTATCGTGGAAGGACT	CCAGTGAGCTTCCCGTTCAG
	OAS3	TGCTGCCAGCCTTTGACGCC	TCGCCCGCATTGCTGTAGCTG
	PTGS2	ACCCACTCCAAACACAGTGC	GCTTCCCAGCTTTTGTAGCC
	APOBEC3B	GACCCTTTGGTCCTTCGAC	GCACAGCCCCAGGAGAAG
	MX1	AAGCTGATCCGCCTCCACTT	TGCAATGCACCCCTGTATACC
	IFN ß	AAGGCCAAGGAGTACAGTC	ATCTTCAGTTTVGGAGGTAA
	IFIT3	ATGAGTGAGGTCACCAAGAATTCCC	GGCTGCCTCGTTGTTACCAT
Northern blot probes	miR-16	CGCCAATATTTACGTGCTGCTA	
	U6	GCAGGGGCCATGCTAATCTTCTCTGTATCG	