

Figure S1. Streptavidin-HRP dot blots of biotinylated RNA. RNA shown in this figure were used for RNA immunoprecipitation shown in Figures 3, 5, 7, and 8.

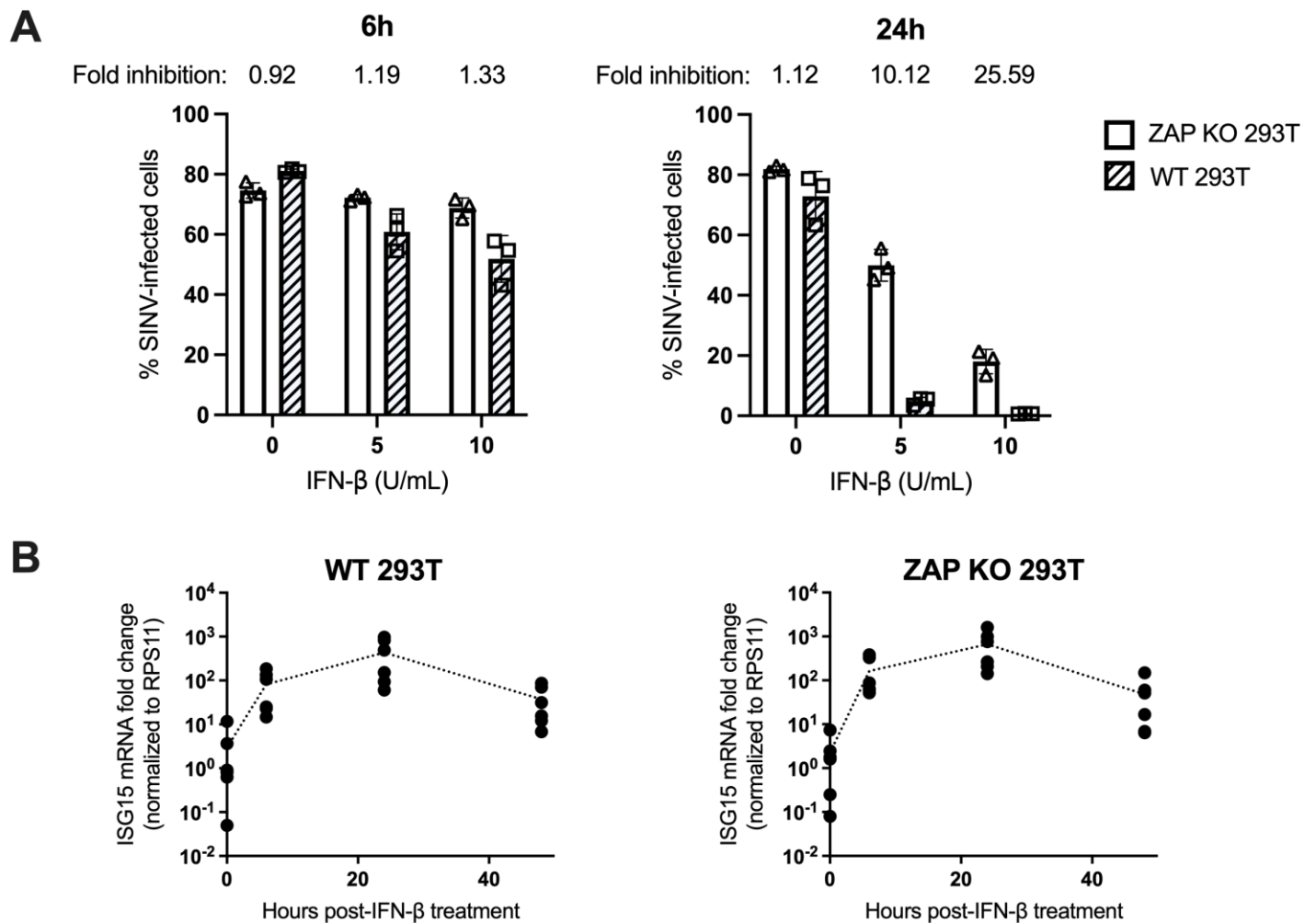


Figure S2. Pre-treatment of WT and ZAP KO 293T cells with IFN prior to SINV infection. **(A)** Following treatment with 0, 5, or 10 U/mL IFN- β for 6 or 24 h, ZAP KO and WT 293T cells were infected with GFP-expressing SINV (MOI = 1 PFU/cell). Cells were harvested and fixed at 24 h.p.i., and their percent infection was determined by flow cytometry. Fold inhibition of SINV in WT 293T was calculated relative to ZAP KO 293T for each IFN- β treatment condition to indicate the antiviral effects of endogenous ZAP. **(B)** WT and ZAP KO 293T cells were treated with 5 U/mL IFN- β , and total RNA was extracted at 0, 6, 24, and 48 h post-IFN-treatment for RT-qPCR analysis of ISG15 and RPS11 mRNA levels.

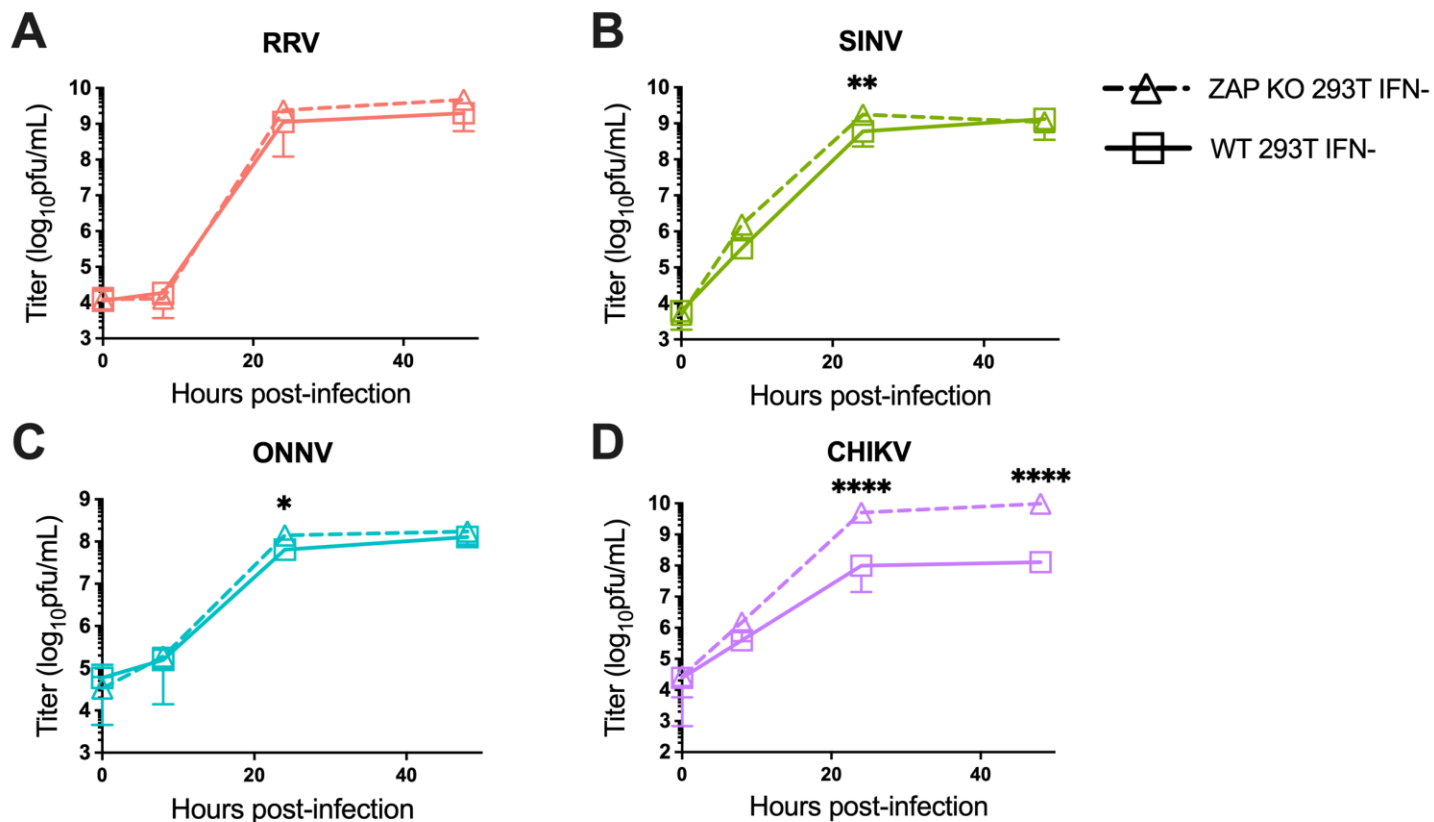


Figure S3. Alphavirus production shows differential sensitivity to basal endogenous ZAP in 293T cells. ZAP KO and WT 293T untreated with IFN- β were infected with RRV (A), SINV (B), ONNV (C), and the CHIKV vaccine strain (181/clone 25) (D) at MOI = 0.1 PFU/cell. Media overlaying the cells were harvested at 0, 8, 24, and 48 h.p.i., and the viral titer was determined by infection of BHK-21 cells in standard plaque assays. Mean values from four biological replicates across two independent experiments are plotted, and error bars represent the SD. Asterisks indicate statistically significant differences (two-way ANOVA with Bonferroni posttest: *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$).

Window 1

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SINV_window_1 CAUACCUAAAGGAAACUUUCAGGCUACUAUAGAGGACUGGGAAGCUGAACACAAGGGAAU
RRV_window_1 -----GGGCGAUUUCAGCGCUACGUUGGAGGAGUGGCAAGAGGAGCAUGACAACAU
ONNV_window_1 -----CAAAAGGGAACUUUAAGGCAACAAUCAAGGAGUGGGAAGCGGAACACGCCUCCAU
CHIKV_window_1 -----GGAAACUUCAAAGCAACUAUUAAGGAGUGGGAGUGGAGCAGCAUCGAU
                ** * **      ** ** *   *** ** * * ** **      **

SINV_window_1 AAUUGCUGCAAUAAACAGCCCCACUCCCCGUGCCAAUCCGUUCAGCUGCAAGACCAACGU
RRV_window_1 CAUGAACGCCCCUUCGCGAGAGGUCGACAGCAGUAGACCCGUUCCAGAACAAAGCCAAGU
ONNV_window_1 UAUGGCAGGAAUAUGCAAUCACCAGAUGGCUUUUGACACAUUUCAGAACAAAGCUAAUGU
CHIKV_window_1 AAUGGCGGGCAUCUGCAGUCACCAAGUGACC UUUGACACAUUCCAAAAACAAAGCCAACGU
                **      * * *      *      * * **      *** * ** **

SINV_window_1 UUGCUGGGCGAAAGCAUUGGAACCGAUACUAGCCACGGCCGGUAUCGUACUUAACCGGUUG
RRV_window_1 CUGCUGGGCAAAGUCCUCGUGCAGGUCCUAGAAACGGCUGGGAUACGCAUGACGGCAGA
ONNV_window_1 AUGCUGGGCUAAAUGCCUGGUCCCUAUUCUUGACACCGCUGGAAUCAAACUAAGUGACAG
CHIKV_window_1 UUGCUGGGCUAAGAGCUUGGUCCCUAUCCUCGAAACAGCGGGGAUAAAACUAAAUGAUAG
                ***** **      * * *      * ** *   ** ** ** ** * * *

SINV_window_1 CCAGUGGAGCGAACUGUUCCACAGUUUGCGGAUGACAAACCACAUUCGGCCAUUUACGC
RRV_window_1 GGAGU---GGGAUACAGUGUUGGCUUUCGCGAAGACAGGGCGUACUCACCCGAAGUGGC
ONNV_window_1 GCAGUGGUCUCAGAUAGUGCAAGCUUUUAAAGAAGAUAGGGCCUACUCUCCAGAAGUUGC
CHIKV_window_1 GCAGUGGUCCCAGAUAAUUAAGCCUUCAAAGAAGACAAAGCAUACUCACCCGAAGUAGC
                ***      *      *      **      ** ** *   * * ** *      **

SINV_window_1 CUUAGACGUAAUUUGCAUUAAGUUUUUCGGAUGGACUUGACAAGCGGACUGUUUUCUAA
RRV_window_1 UCUGAACGAGAUCUGUACCAAGUAUUACGGCGUUGACUUGACAGCGGAUUGUUCUCCGC
ONNV_window_1 ACUGAAUGAAAUAUGUACCCGCAUAUAUGGGGUAAGUUGGACAGCGGACUAUUUUCAAA
CHIKV_window_1 CCUGAAUGAAAUAUGCAGCGCGAUGUAUGGGGUGGAUCUAGACAGUGGGCUAUUCUCUAA
                * * *   ** ** *      * ** *   ** ** *   ** ** *

SINV_window_1 ACAGAGCAUCCACUAACGUACCAUCCCGCCGAUUCAGCGAGGCCGGUAGCUCAUUGGGA
RRV_window_1 CCAAUCCGGUCUCAUU---GUACUAUGA-----AAAC-AACCACUGGGA
ONNV_window_1 GCCGCGUAUAUCCGU---CUACUAUGC-----AGAC-AACCACUGGGA
CHIKV_window_1 ACCGUUGGUAUCUGU---GUAUUACGC-----GGAU-AACCAUUGGGA
                *      * * *   ** *      **      ** *****

SINV_window_1 CAACAGCCCAGGAACCCGCAAGUAUGGGUACGAUCACGCCAUUGCCGCCGAACUCUCCCG
RRV_window_1 CAAUAGACCGGGCGGCCGGAUGUAUGGAUUAACCGCGAAGUCGCCCGUAAGUUUGAGCA
ONNV_window_1 CAAUAGACCAGGAGGAAAAAUGUUCGGGUUCAACCCUGAGGUGGCACUUAUGCUUGAAAA
CHIKV_window_1 UAAUAGGCCGGGAGGAAAGAUGUUCGGAUUAACCCUGAGGCAGCGUCCAUUCUAGAAAG
                ** ** ** **      * **      ** *   *   **      *

SINV_window_1 UAGAUUUCGGUG-UUCCAGCUAGCUGGGA--AGGGCACACAACUUGAUUUGCAGA-CGG
RRV_window_1 ACGCUACCCAUUCCUGAGAGGCAAGAUGGACUCGGGGCUACAAGUUAUGU----UCCAG
ONNV_window_1 GAAAUAUCCCUUUACAAAAGGUAAGUGGAACAU----CAACAAGCAGAUUAUGUAUAACUA
CHIKV_window_1 AAAGUACCCAUUUACAAAAGGAAAGUGGAACAU----CAACAAGCAGAUUCGCGUGACUA
                * ** *      ** *      ** *      ****      ** *   *

SINV_window_1 GGAGAACC-----AGAGU-UAUCUCUGC-----ACAG-----
RRV_window_1 AGAGAAAAGUACAGCCAUUCAAUGCGGAAUGUAUAUAUUAACCAUCAAAACAGGCGAC
ONNV_window_1 CCAGAAAGGUUGACGAAUUUAACCCCGAAACCAACAUAUAUACCGGCAAC-----
CHIKV_window_1 CCAGGAGGAUAGAAGACUUAACCCUACCACCAACAUAUAUACCGGUCAACAGG----
                ** *      * * *      **      **
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SINV_window_2	UUUGCAUUAAGUUUUUCGGCAUGGACUUGACAGCGGACUGUUUUUUAACACAGAGCAUCC
RRV_window_2	-----UAUUACGGCGUUGACUUGACAGCGGAAUGUUCUCCGCCCAAUCGGUCU
ONNV_window_2	----UACCCGCAUAUAUGGGGUAGAUCUGGACAGCGGACUAUUUUCAAAGCCGCUGAUAU
CHIKV_window_2	-----GCGCAUGUAUGGGGUGGAUCUAGACAGUGGGCUAUUCUCUAAACCGUUGGUAU * ** * * * ** ** * * * * *
SINV_window_2	CACUAACGUACCAUCCCGCCGAUUCAGCGAGGCCGGUAGCUCAUUGGGACAACAGCCCAG
RRV_window_2	CAUU---GUACUAUGA-----AAC-AACCACUGGGACAAUAGACCGG
ONNV_window_2	CCGU---CUACUAUGC-----AGAC-AACCACUGGGACAAUAGACCAG
CHIKV_window_2	CUGU---GUAUUACGC-----GGAU-AACCAUUGGGAAUAUAGGCCGG * * ** * ** ***** ** * * *
SINV_window_2	GAACCCGCAAGUAUGGGUACGAUACGCCAUUGCCGCCGAACUCUCCCGUAGAUUUCGG
RRV_window_2	GCGGCCGGAUGUAUGGAUUAACCCGCGAAGUCGCCCGUAAGUUUGAGCAACGCUACCCAU
ONNV_window_2	GAGGAAAAAUGUUCGGGUUAACCCUGAGGUGGCACUUAUGCUUGAAAAGAAUAUCCCU
CHIKV_window_2	GAGGAAAGAUGUUCGGAUUAACCCUGAGGCAGCGUCCAUUCUAGAAAGAAAGUACCCAU * * ** ** * * * * ** * * **
SINV_window_2	UG-UUCCAGCUAGCUGGGA--AGGGCACACAACUUGAUUUGCAG-ACGGGGAGAAC---
RRV_window_2	UCCUGAGAGGCAAGAUGGACUCGGGGCUACAAGUUAUUGU----UCCAGAGAGAAAAGUA
ONNV_window_2	UUACAAAAGGUAAGUGGAACAU---CAACAAGCAGAUUGUAUAACUACCAGAAAGGUU
CHIKV_window_2	UUACAAAAGGAAAGUGGAACAU---CAACAAGCAGAUUCGCGUGACUACCAGGAGGAUA * ** * * ***** ** * * ** *
SINV_window_2	-CAGAGUUAUCUCUGCACAGCAUAACCUUGUCCCGGUGAACCGCAAUCUCCUCACGCCU
RRV_window_2	CAGCCAUUC--AAUGCGAAUGUAAUUAUUAUACCAUCAAACAGGCGACUCCGCACGCCC
ONNV_window_2	GACGAAUUU--AACCCGAAACCAACAUAUUAACCGCCAACCGAAGACUGCCGCACUCAC
CHIKV_window_2	GAAGACUUC--AACCCUACCACCAACAUAUUAACCGGUCAACAGGAGACUACCACACUCAU ** * ** * * * *** * ** ** ** * *
SINV_window_2	UAGUCCCCGAGUACAAG-GAGAAGCAACCCGGCCCGGUCAAAAAAUUCUUGAACCCAGUUC
RRV_window_2	UCGUCACCAGCUACCAG-CAGUGCCAGGGCGAGAGGUAGAUAUGGCUUUUGAAGAGCUU
ONNV_window_2	UCGUGGCGAAGAACCAUACAGUGAGA-GGGGAAAGAAUGGAAUGGCUUGGUAACAAAAUC
CHIKV_window_2	UAGUGGCCGAACACCGCCAGUAAAA-GGGGAAAGAAUGGAAUGGCGUGGUAAACAAGAU * * * ** ** * * * ** * * * * *
SINV_window_2	AAACACCACUCAGUACUUGUGGUAUCAGAGGAAAAAAUUGAAGCUCCCCGUAAGAGAAUC
RRV_window_2	CCCGGAUACCAUUUAUUGCUGGUAAGCGAGUACAACUCGGCGUGCCCCAUAAAAGAGUC
ONNV_window_2	AACGGUCACCACAUGUUGUUGGUUAGUGGUUAUAUAUCUUAUAUAUACCAACAAAAGAGUC
CHIKV_window_2	AACGGACACCAGUACUUGGUUAGCGGCUAUAACCUUGCACUGGUACUAAAGAGAGUC ** * * * ***** * * * * ** ** ** ** *
SINV_window_2	GAAUGGAUCGCCCCGAUUGGCAUAGCCGGUGCAGAUAGAACUACAACCUGGCUUUCGGG
RRV_window_2	UUUUGGAUUGCACCACCCAUGUGUCUGGUGCAGAUUCGUAUUUAUGAUUCUUGACCUAGGA
ONNV_window_2	ACCUGGGUAGCACCGUUAGGCACCAGAGGUGCAGACUACACAUUAUACCUGGAACUUGGU
CHIKV_window_2	ACCUGGUAGCGCCACUAGGUGUCCGCGAGCGGACUAUAACUACAACCUAGAGCUGGGU *** * ** ** ** ** * ** * * ** *
SINV_window_2	UUUCCGCCGCAG---GCACGGUACGACCUGGUG-----
RRV_window_2	UUACCCUGAAUGCAGGCCGUUACGACUUGGUUUUGGAACAUACACACUGAGUACAG
ONNV_window_2	CUACCAGCCACACUAGGCAGAUUAGACCUGGUAGUUUAUCAAUAUCCAUAUCUC-----
CHIKV_window_2	CUACCAGCAACGCUUGGAGGUAGGACCUAGUGGUCAUAAACAUCCACACACCUU---- * ** * * * * * * * * ** * * *

Window 3

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SINV_window_3      GGAGACAGGCACCGCAAGAAUGACUGUGCCUAGGAAAGAAAGUGAUCCACGCGGUCGG
RRV_window_3       -----GGCCA-----A-----CGGUAGA---AUGUCAUCCACGCGGUAGG
ONNV_window_3      -----CA-----AUCAUGUGCGGUCAAUACCCCGUCAUCCACGCGGUAGG
CHIKV_window_3     -----GUGCGGUACGUAUCCAGUAAUCCACGCCGUAGG
                                *                ** ***** ** **

SINV_window_3      CCCUGAUUUCCGGAAGCACCCAGAAGCAGAAGCCUU----GAAAUUGCUACAAAACGCCU
RRV_window_3       CCCGAAUUUUCUCCACGGUGACCGAGGCAGAGGGCGACAGAGAGUUGGCC----GCCGCAU
ONNV_window_3      CCCAAACUUCUCAAAACUAUUCUGAGGCUGAAGGGGAUAGGGAAUUGGCU----UCAGUGU
CHIKV_window_3     ACCAAACUUCUCAAAAUAAUUCGGAGUCUGAAGGGGACCCGGAAUUGGCG----GCUGCCU
                                ** * *** *      * ** * * * *      ** * **      * *

SINV_window_3      ACCAUGCAGUGGCAGACUUAAGUAAAU--GAACAUAACAUAAGUCUGUCGCCAUUCCACU
RRV_window_3       ACCGUGCCGUGGCG--GGUAAUUAUCAAUGCUAGUAACAUAUAGAGUGUAGCCAUCCUCU
ONNV_window_3      AUAGAGAAGUGGCGAAAGAAGUGUCUAGGCUAGGAG--UGAGCAGUGUAGCCAUCCUUU
CHIKV_window_3     AUCGAGAAGUCGCAAGGAAGUAACUAGACUGGGAG--UAAAUAGCGUAGCUAUACCUCU
                                *      * ** **      * *      * * *      ** ** ** ** ** **

SINV_window_3      GCUAUCUACAGGCAUUUACGCAGCCGGAAGACCGCCUUGAAGUAUCACUUAACUGCUU
RRV_window_3       GUUGUCGACGGGAGUGUUCUCCGGAGGUAAGAUAGAGUCAUGCAGUCACUAAUACCCU
ONNV_window_3      GCUUUCAACCGGUGUGUACUCAGGAGGCAAAGAUAGACUGCUGCAUACUAAACCAUCU
CHIKV_window_3     CCUCUCCACAGGUGUAUACUCAGGAGGGAAGACAGGCUAACCCAGUCACUGAACCACCU
                                * ** ** ** * * * * * ** ***** * *      ***** **      *

SINV_window_3      GACAACCGCGCUAGACAGAACUGACGCGGACGUAACCAUCUAUUGCCUGGAUAAGAAGUG
RRV_window_3       GUUUUACCGCAAUGGACACCACGGACGCUAGUAGUACUUAUUGCCGCGACAAAGCCUG
ONNV_window_3      UUUACACGCGAUGGAUUCGACAGAUGCAGAUUGUUGUACUUAUUGCAGGGUAAGGAAUG
CHIKV_window_3     CUUUACAGCCAUGGACUCGACGGAUGCAGACGUGGUACUACUGCCGAGACAAGGAAUG
                                ** ** * **      ** ** * * * *      ***** **      ** ** **

SINV_window_3      GAAGGAAAGAAUCGACGCGGCACUCCAACUUAAGGAGUCUGUAACAGAGCUGAAGGAUGA
RRV_window_3       GGAGAAGAAAAUCCAGGAGGCUAUCGAUCGCCG---CACCGCCGUGGAAUUGGUUUCUGA
ONNV_window_3      GGAGAAGAAGAUACUGAAGCCAUUAUUAAG---AUCCAGGUAGAACUACUAGAUGA
CHIKV_window_3     GGAGAAGAAAAUUCUGAGGCCAUACAGAUGCG---GACCCAAGUGGAGCUGCUGGAUGA
                                * ** * * * *      * ** *      *      ** *      ***

SINV_window_3      AGAUUUGGAGAUAGCAGAUAGAGUUAUGAUUGGAUUAUCCAGACAGUUGCUUGAAGGGAAG
RRV_window_3       AGACAUCUCACUCGAGUCUGACUUGAUACGGGUACACCCAGAUAGUUGCUUGGUAGGCAG
ONNV_window_3      UCACAUCUCAGUGGAUUGCGACAUUGUACGCGUUAUCCAGACAGCAGCUUGGCAGGCCG
CHIKV_window_3     GCACAUCUCCAUGACUGCGAUGUACUUCGCGUGCACCCUGACAGUAGCUUGGCAGGCAG
                                * **      * **      ** * * * * * * * * * * ***** ** *

SINV_window_3      AAAGGGAUUCAGUACUACAAAAGGAAAAUUGUAUUCGUACUUCGAAGGCACCAAUCCA
RRV_window_3       AAAAGGUUACAGCAUAACAGAUGGGAAGCUGCAUUAUACCUGGAAGGUACCCGCUUUA
ONNV_window_3      AAAAGGGUACAGCACAGUAGAGGGGACACUCUACUCGUACCUAGAGGGAACAAGAUCCA
CHIKV_window_3     AAAAGGAUACAGCACACCGGAAGGCGCACUGUAUUAUUAUAGAGGGACACGUUUUA
                                *** ** * ** *      * **      * * * * * * * * * * ** **

SINV_window_3      UCAAGCAGCAAAAGACAUGGCGGAG-----
RRV_window_3       UCAGACUGCGGUGGACAUGGCUGAGAUACUACCUUGUGGCCGAAACUUCAGGAC
ONNV_window_3      CCAAACUGCUGUAGAUUUGGCAGAAAUAUAUACCAUGUGGCCAAAACAAAC-----
CHIKV_window_3     CCAGACGGCAGUGGAUUGGCAGAGAUUAACACUUGUGGCCAAAGCAAACAGAG
                                ** * **      ** ***** **

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Figure S4. Alignments of CpG-correlated windows. Sequences of CpG-correlated windows identified in Figure 6 were aligned across alphaviruses using Clustal Omega.

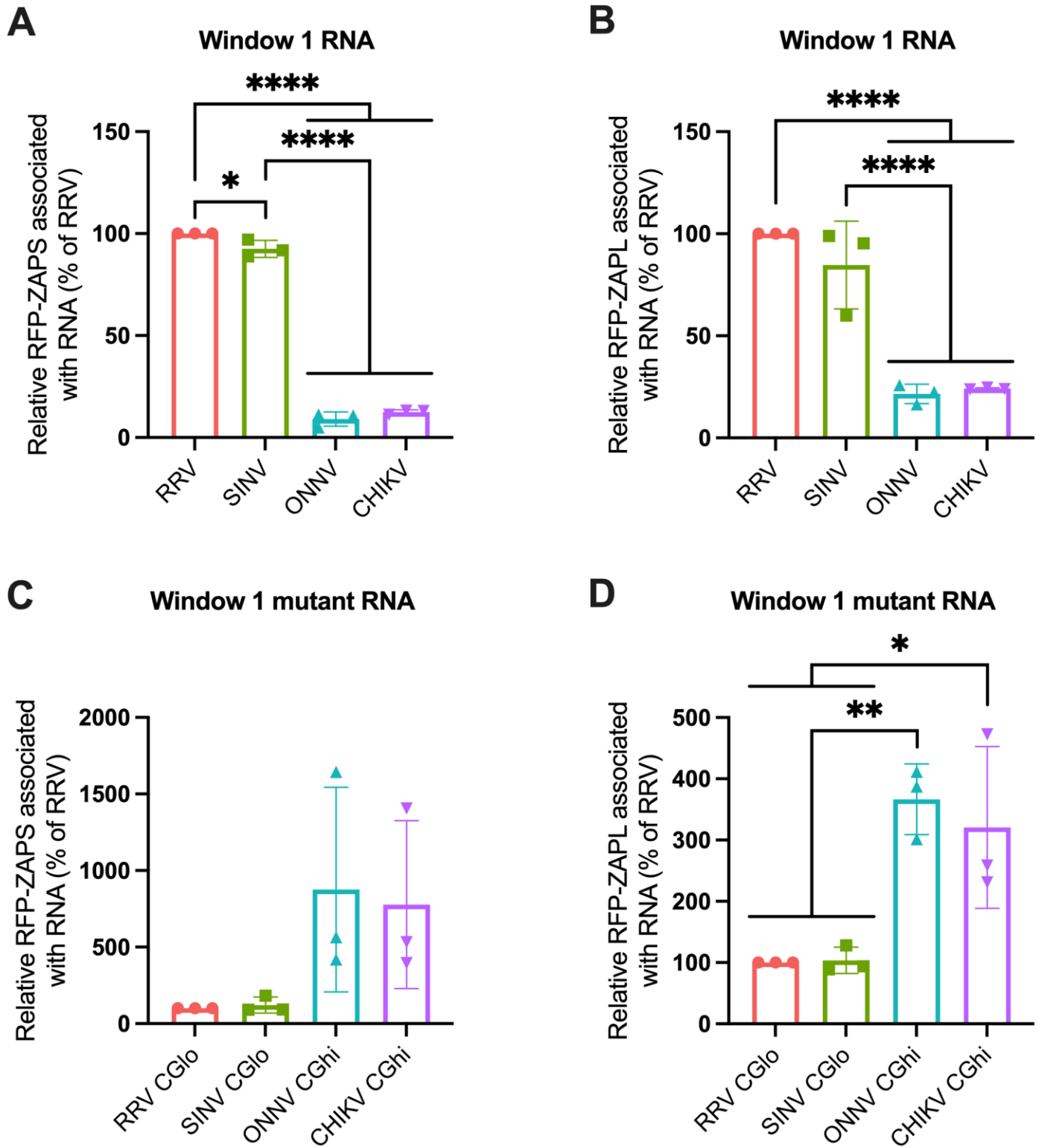


Figure S5. Quantifications of ZAPS and ZAPL binding to CpG-correlated window 1 RNA and CpG-correlated window 1 mutant RNA. ImageJ quantifications are shown of ZAP RNA pull-down assays for CpG-correlated window 1 RNA with RFP-ZAPS (A) or RFP-ZAPL (B) and for window 1 mutant RNA with RFP-ZAPS (C) or RFP-ZAPL (D). Data are combined from three independent experiments. Error bars represent the SD. Asterisks indicate statistically significant differences (one-way ANOVA and Tukey's multiple comparisons test: *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$).

Supplementary Tables

Table S1. Primers for generation of EGFP-expressing CHIKV vaccine strain 181/clone 25.

Fragment	Origin	Sequence (5'-3')	Forward (F) / Reverse (R)
Pre-GFP	CHIKV vaccine strain 181/clone 25	AGAGTGGCGGACCCGCTAAAAAGGTTATTTAAATTGGGCAAACCG	F
		TTAGCGG	
		TCACCATGGCGCGCCTGATTGGTATTTAGGTACCTGCTG	R
EGFP	ONNV-GFP	TACCTAAATACCAATCAGGCGCGCCATGGTGAG	F
		CAAAGGTGGCCATGGACTAGTTTACTTGTACAGCTCGTCCATGCC	R
Post-GFP	CHIKV vaccine strain 181/clone 25	TACAAGTAACTAGTCCATGGCCACCTTTGC	F
		GTTAAGGATGCTTGTAGCAGCTGATAGTACCCGGGGCTCATGACGT	
		TGTCTT	R

Table S2. Primers to generate CpG-correlated window DNA templates for T7 transcription.

Virus	Window	Sequence (5'-3', T7 promoter sequence in lowercase)	Forward (F) / Reverse (R)
RRV	window 1	taatacgactcactataggGGGCGATTTACGCGCTAC	F
		GTGCCTGTTTGATGGTAATATAT	R
	window 2	taatacgactcactataggTATTACGGCGTTGACTTAGAC	F
		CTGTACTCAGTGTGTATGTTT	R
	window 3	taatacgactcactataggGGCCAACGGTATGAATGTC	F
		GTCCTGAAGTTTCGGCCAC	R
	window 1 CGlo	taatacgactcactataggGGGAGGTTTCAGGGCTAC	F
		GTGGCCTGTTTGATGGTA	R
	window 1	taatacgactcactataggCATACCTAAAGGAACTTTCAGGC	F
		CTGTGCAGAGATAACTCTGGTTC	R
SINV	window 2	taatacgactcactataggTTGCATTAAGTTTTTCGGCATGGAC	F
		CACCAGGTCGTACCGTGCC	R
	window 3	taatacgactcactataggGGAGACAGGCACCGCAAG	F
		CTCCGCCATGTCITTTGC	R
	window 1 CGlo Same primers as for SINV window 1		
ONNV	window 1	taatacgactcactataggCAAAAGGGAACTTTAAGGCAAC	F
		GTTGGCCGGTATTATGTTGG	R
	window 2	taatacgactcactataggTACCCGCATATATGGGGTAG	F
		GAGTATGGATATTGATAACTACCAGG	R
	window 3	taatacgactcactataggCAATCATGTGCGGTCAATACCC	F
		GTTTGTTTTGGCCACATGG	R
	window 1 CGhi	taatacgactcactataggCAAAAGGGAACTTTAACGC	F
		GTTGGCGGGTATTATGTTG	R
CHIKV	window 1	taatacgactcactataggGGAACCTTCAAAGCAACT	F
		CCTGTTGACCGGTATAATG	R
	window 2	taatacgactcactataggGCGCATGTATGGGGTGGATC	F
		AAGGTGTGTGGATGTTTATGACCACTAG	R
	window 3	taatacgactcactataggGTGCGTACGTATCCAGTAATCC	F
		CTCTGTTTGCTTTGGCCACATAG	R
	window 1 CGhi	Same forward primer as for CHIKV window 1	F
		CCTGTTGACCGGTATAATG	R

Table S3. qPCR primers.

Target	Sequence (5'-3')	Forward (F) / Reverse (R)
ISG15	GGCTGGGAGCTGACGGTGAAG	F
	GCTCCGCCCCGCCAGGCTCTGT	R
RPS11	GCCGAGACTATCTGCACTAC	F
	ATGTCCAGCCTCAGAACTTC	R

Table S4. CpG and UpA dinucleotide contents of CpG-correlated windows.

Virus	Window	CpG content (observed/expected)	UpA content (observed/expected)
RRV	window 1	0.970	0.838
	window 2	0.859	0.964
	window 3	0.906	0.967
	window 1 CGlo	0.357	0.880
SINV	window 1	0.864	0.867
	window 2	0.893	0.727
	window 3	0.828	0.744
	window 1 CGlo	0.446	0.838
ONNV	window 1	0.455	0.882
	window 2	0.523	1.092
	window 3	0.469	0.881
	window 1 CGhi	1.285	0.882
CHIKV	window 1	0.626	0.899
	window 2	0.639	1.269
	window 3	0.684	1.047
	window 1 CGhi	1.437	0.891