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Supplementary Data

863 **Self assembling short immunostimulatory duplex RNAs with broad spectrum antiviral**
864 **activity**

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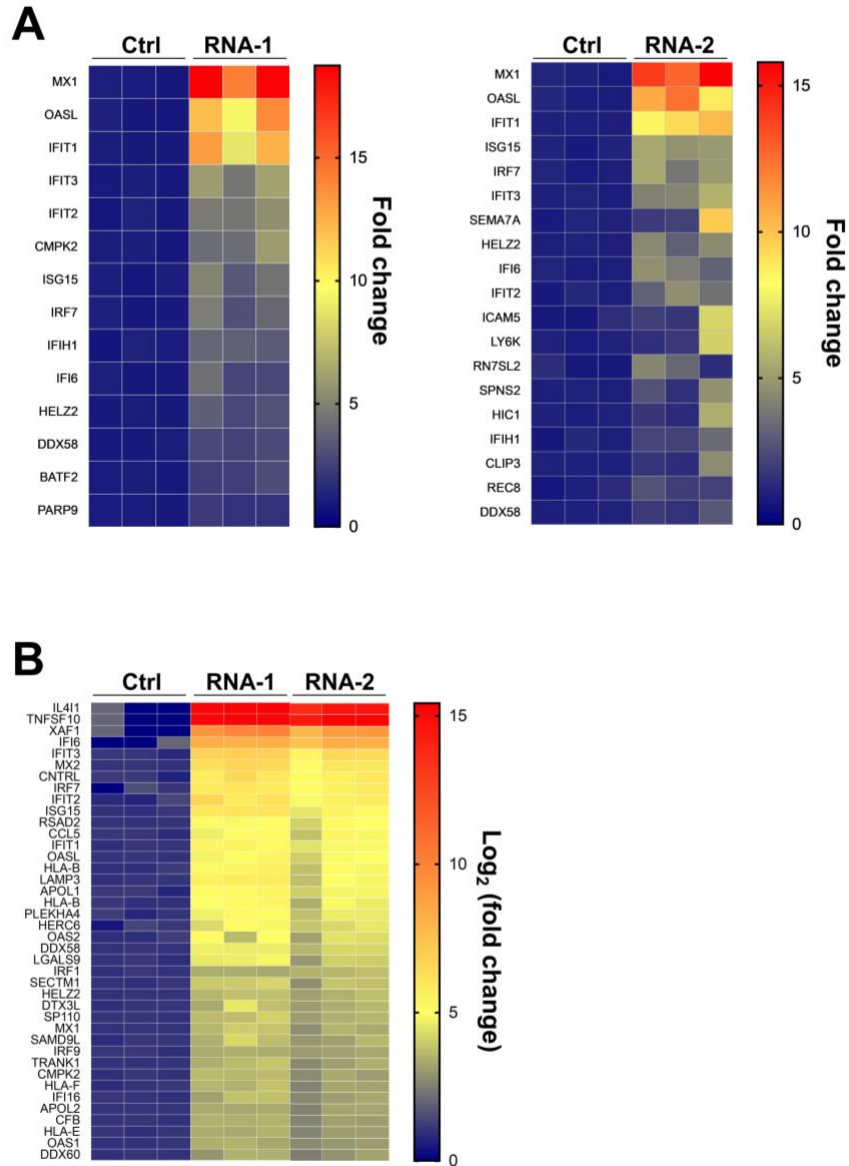
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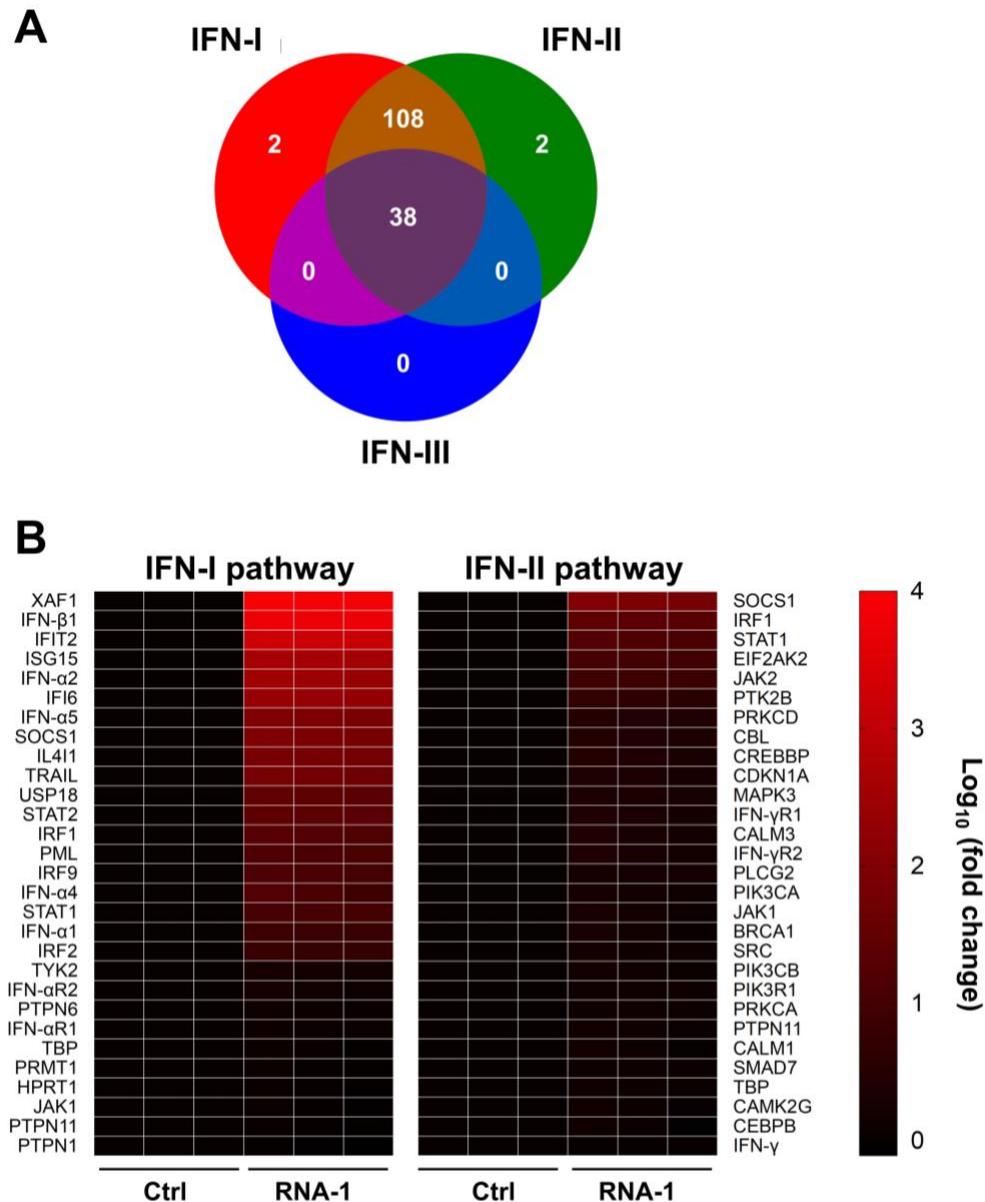
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905 **Figure S3. RNA-induced gene expression associated with type I interferon pathway. (A)**

906 Venn diagram showing differentially expressed ISGs from TMT Mass Spec by RNA-1 belong to

907 type I or type II interferon stimulated genes. **(B)** Heat map of qPCR results showing RNA-I

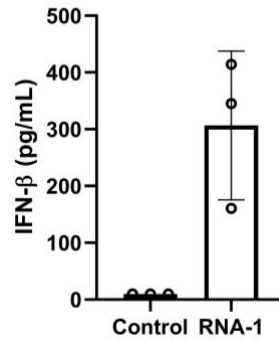
908 preferentially activates type I interferon pathway. A549 cells were transfected with RNA-1 or

909 scrambled dsRNA control, collected at 48 hr and analyzed by qPCR (expression levels were

910 normalized to GAPDH; gene levels induced by the RNA control were set as 1; N = 3).

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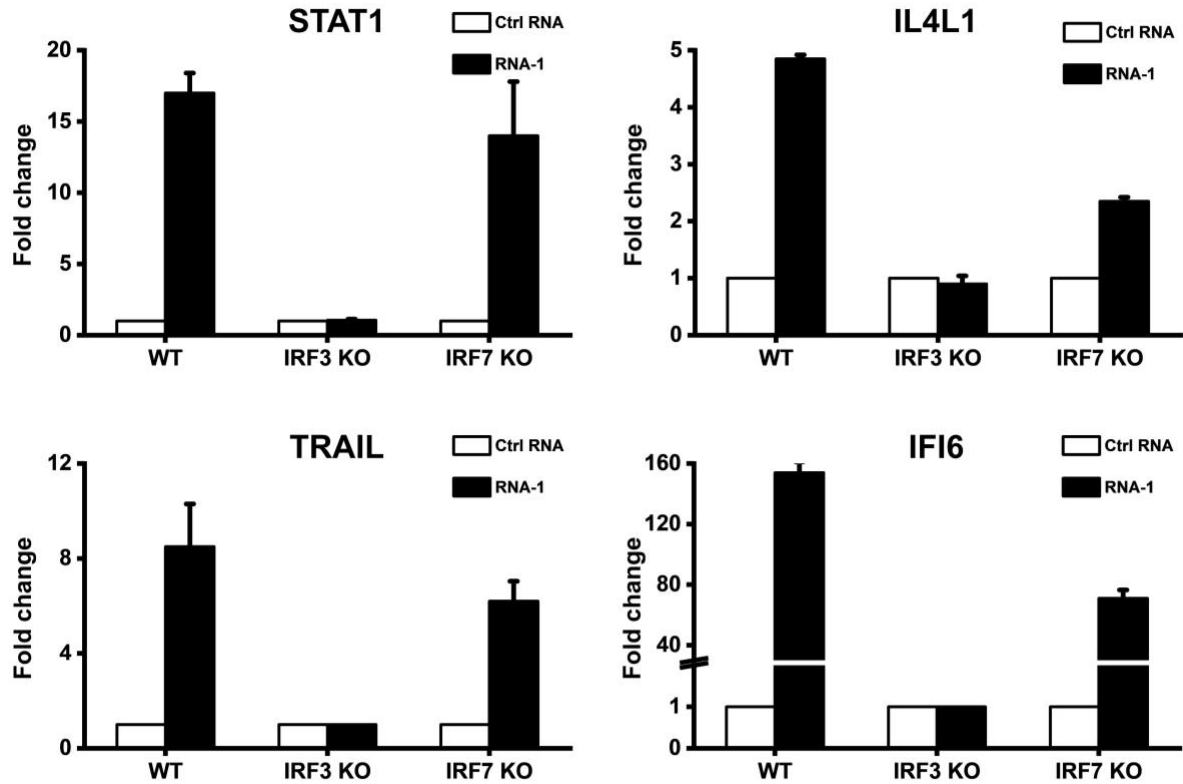


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914 **Figure S4. The levels of IFN- β protein induced by RNA-1.** A549 cells were transfected with
915 RNA-1 (34 nM) for 48 h, and then supernatants were collected for detection of IFN- β using
916 ELISA. Scrambled RNA control NC-1 is used as negative (N = 3).

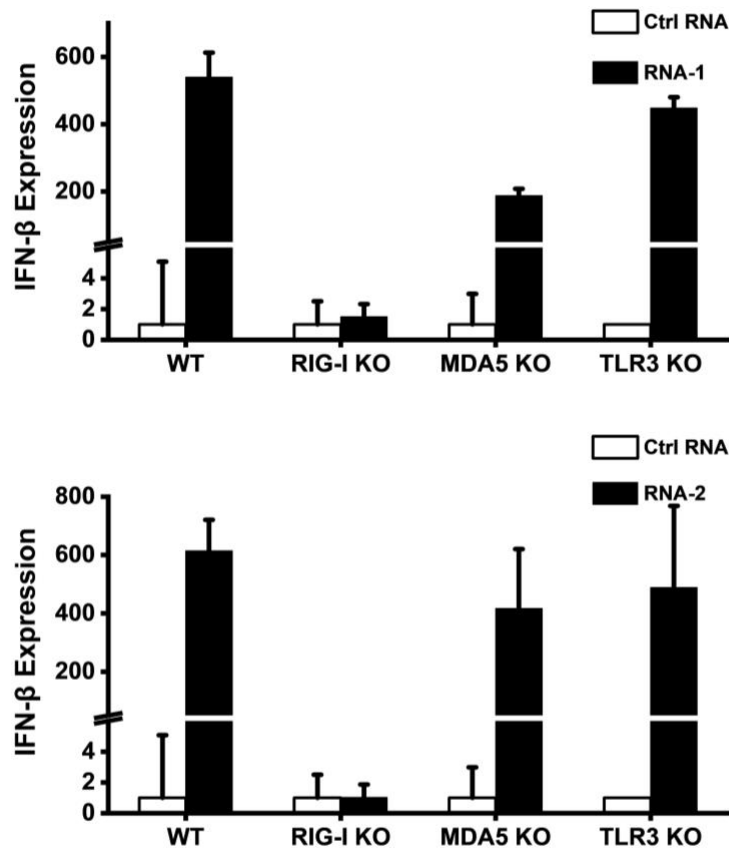
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920 **Figure S5. IRF3 knockout abolished the ability of immunostimulatory RNAs to induce**
921 **IFN-I pathway associated genes.** Wild-type (WT) HAP1 cells, IRF3 knockout HAP1 cells, or
922 IRF7 knockout HAP1 cells were transfected with RNA-1 or a scrambled RNA control and
923 STAT1, IL4L1, TRAIL, and IFI6 mRNA levels were quantified by qPCR at 48 h post transfection.
924 Data are presented as fold change relative to RNA control (N = 3).

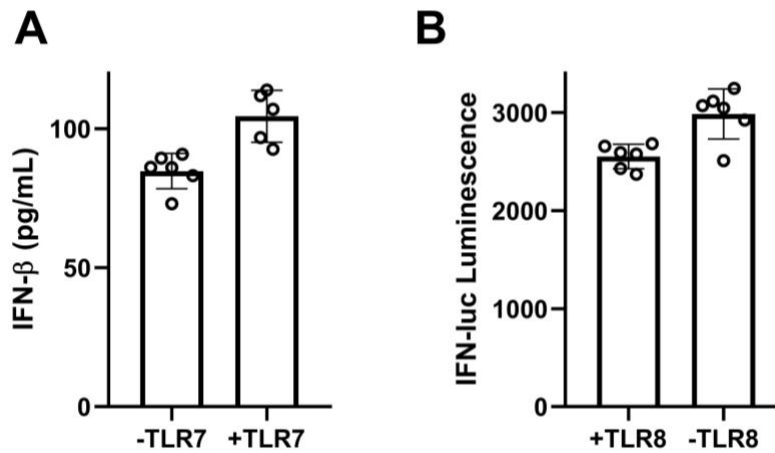


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926 **Figure S6. RIG-I knockout abolished the induction effects of the immunostimulatory**
927 **RNAs on IFN-β.** Wild-type (WT) A549-Dual cells, RIG-I knockout A549-Dual cells, MDA5
928 knockout A549-Dual cells, or TLR3 knockout A549 cells were transfected with RNA-1, RNA-2,
929 or a scramble RNA control and IFN-β mRNA levels were detected by Quanti-Luc assay in WT,
930 RIG-I KO, and MDA5 KO A549-Dual cells or qPCR in TLR3 KO A549 cells at 48 h post
931 transfection. Data are shown as fold change relative to the scrambled RNA control (N = 6).

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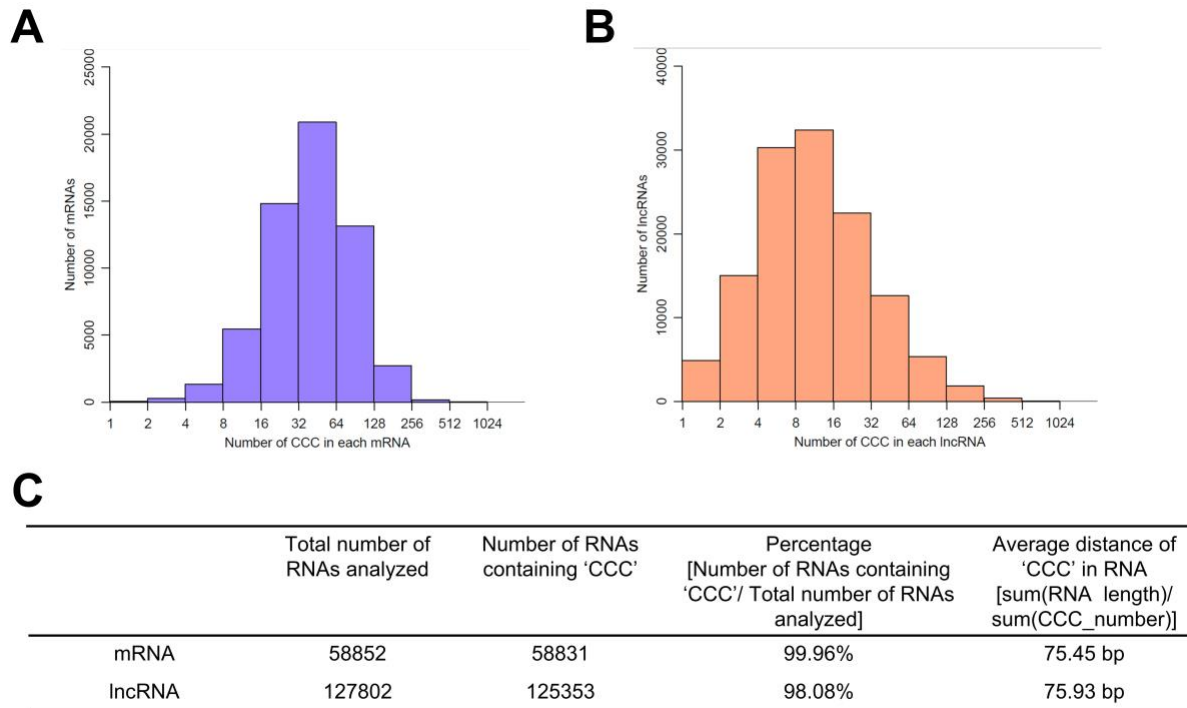


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935 **Figure S7. TLR7/8 knockout or overexpression did not have effect on the**
936 **immunostimulatory activity of RNA-1. (A)** Graph showing that the overexpression of TLR7 in
937 HEK cells had no effect on production of IFN-β induced by RNA-1. **(B)** Graph showing that the
938 knockout of TLR8 in THP1 cells had no effect on IFN production induced by RNA1. These cell
939 lines are commercial and could be purchased from InvivoGen.

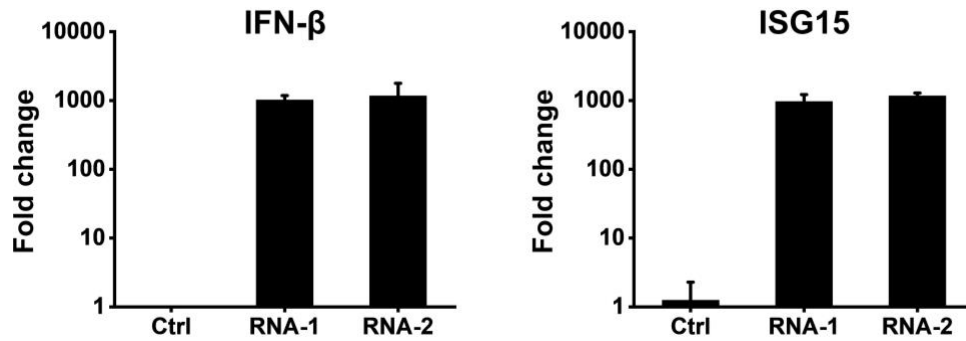
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943 **Figure S8. 'CCC' motif is widely distributed in human genome.** (A) Graph showing the
 944 distribution of the number of CCC sequences in human mRNAs (retrieved from UCSC hg38
 945 refGene with prefix NM). (B) Graph showing the distribution of the number of CCC sequences in
 946 human lncRNAs (retrieved from Incipedia). (C) Table showing the percentage of human mRNAs
 947 and lncRNAs containing the CCC motif and their average density.



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949 **Figure S9. Immunostimulatory RNA-mediated production of IFN in ACE2-overexpressing**
950 **A549 cells.** IFN- β and ISG15 levels were detected in cells transfected with RNA-1, RNA-2, or
951 scramble dsRNA control by qPCR at 48 h post-transfection. The IFN- β or ISG15 level induced
952 by the scramble dsRNA control was set as 1. Data are shown as fold change relative to the
953 control (N = 3).

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960 **Table S1. Summary of characteristics of reported immunostimulatory RNAs.**

Characteristic	Signaling pathway	Cytokines
5'-UGUGU-3' motif	Toll-like receptor (TLR)8	IFN-alfa
5'-GUCCUCAA-3' motif	TLR7/8	IFN-alfa
GU or AU rich	TLR7/8	IFN-alfa, TNF-alfa
Uracil repeats	TLR7	IFN-alfa, IL-6, TNF-alfa
Blunt ended dsRNA	RIG-I	Type I IFN, p56
5'-triphosphate; 5'-diphosphate	RIG-I	IFN-alfa, IFN-beta
MicroRNA-like siRNA	TLR7/8	IFN-alfa, TNF-alfa
Long dsRNA	MDA5	Type I IFN
Long dsRNA	TLR3	Type I IFN
Single stranded (ss)RNA	TLR7	Type I IFN

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