

Supplementary Information for

Recognition of 5'-triphosphate by RIG-I requires short blunt double stranded RNA as contained in panhandle of negative strand virus

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Supplementary Table, Figures, Legends and Literature

Suppl. Table 1: Sequences

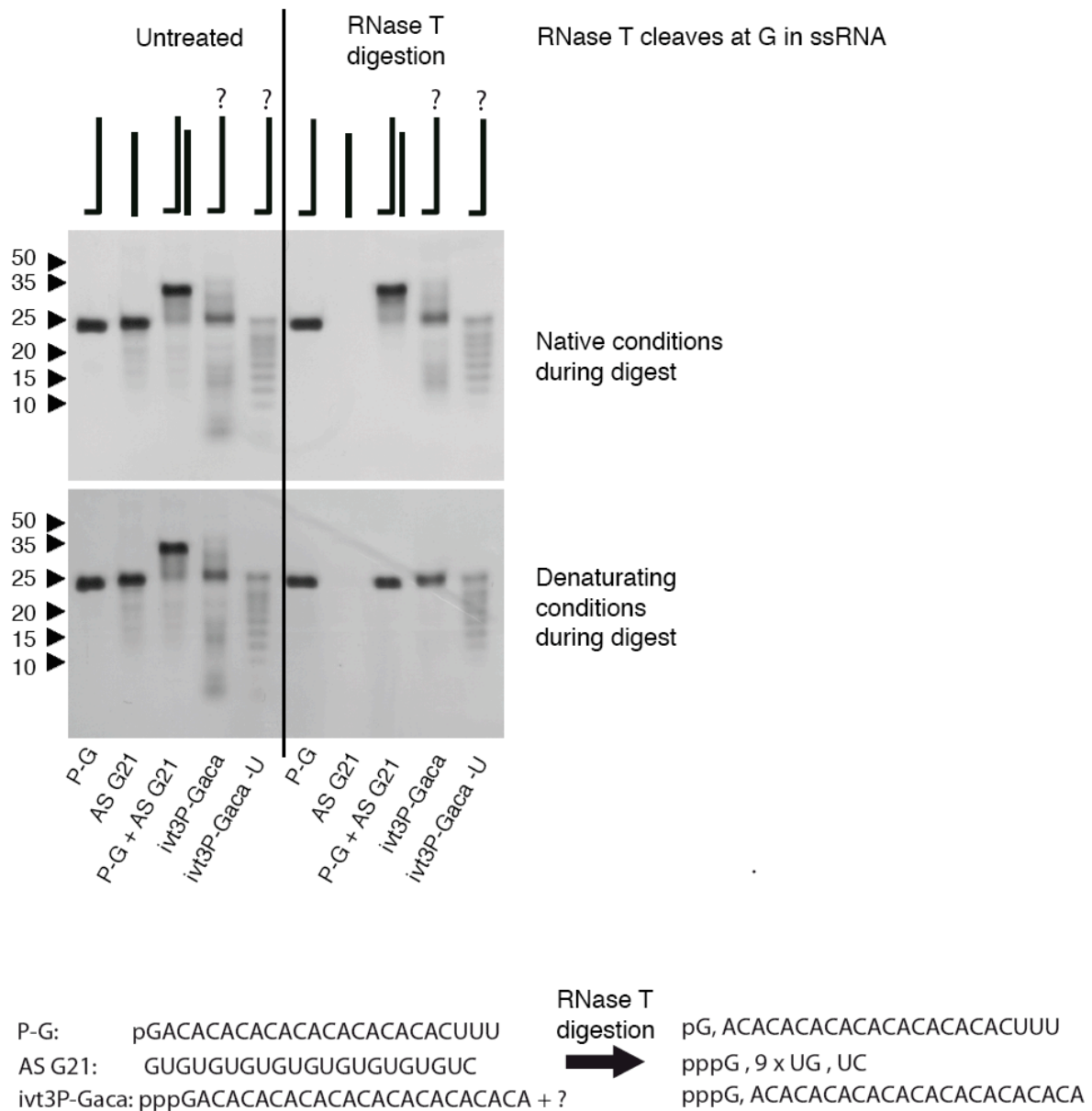
Name	Sequence	5' end	Type	Source
3P-A	<u>A</u> ACACACACACACACACACACUUU	3P	RNA	see methods
ivt3P-G	<u>G</u> ACACACACACACACACACACUUU	3P	RNA	IVT
ivt3P-Gaca	<u>G</u> ACACACACACACACACACACACA	3P	RNA	IVT
3P-G	<u>G</u> ACACACACACACACACACACUUU	3P	RNA	see methods
3P-C	<u>C</u> ACACACACACACACACACACUUU	3P	RNA	see methods
3P-U	<u>U</u> ACACACACACACACACACACUUU	3P	RNA	see methods
HO-A	<u>A</u> ACACACACACACACACACACUUU	OH	RNA	Biomers
P-A	<u>A</u> ACACACACACACACACACACUUU	P	RNA	Metabion
P-G	<u>G</u> ACACACACACACACACACACUUU	P	RNA	Biomers
P-C	<u>C</u> ACACACACACACACACACACUUU	P	RNA	Biomers
P-U	<u>U</u> ACACACACACACACACACACUUU	P	RNA	Biomers
AS A34	AAAGUGUGUGUGUGUGUGUGUGUGUGU <u>U</u> GUGUGUGUGU	OH	RNA	Biomers
AS A26	AAAGUGUGUGUGUGUGUGUGUGUGUGU <u>U</u> GU	OH	RNA	Biomers
AS A25	AAAGUGUGUGUGUGUGUGUGUGUGUGU <u>U</u> G	OH	RNA	Biomers
AS A24+2A	AAAAAGUGUGUGUGUGUGUGUGUGUGU <u>U</u>	OH	RNA	Biomers
AS A24+A	AAAAGUGUGUGUGUGUGUGUGUGUGU <u>U</u>	OH	RNA	Biomers
AS A24	AAAGUGUGUGUGUGUGUGUGUGUGU <u>U</u>	OH*	RNA	Biomers
AS A23	AAGUGUGUGUGUGUGUGUGUGUGU <u>U</u>	OH	RNA	Biomers
AS A21	GUGUGUGUGUGUGUGUGUGUGU <u>U</u>	OH	RNA	Biomers
AS A20	UGUGUGUGUGUGUGUGUGUGU <u>U</u>	OH*	RNA	Biomers
AS A19	GUGUGUGUGUGUGUGUGUGU <u>U</u>	OH	RNA	Biomers
AS G26	AAAGUGUGUGUGUGUGUGUGUGUGU <u>C</u> GU	OH	RNA	Biomers
AS G25	AAAGUGUGUGUGUGUGUGUGUGUGU <u>C</u> G	OH	RNA	Biomers
AS G24+2A	AAAAAGUGUGUGUGUGUGUGUGUGU <u>C</u>	OH	RNA	Biomers
AS G24+A	AAAAGUGUGUGUGUGUGUGUGUGU <u>C</u>	OH	RNA	Biomers
AS G24	AAAGUGUGUGUGUGUGUGUGUGU <u>C</u>	OH	RNA	Biomers
AS G23	AAGUGUGUGUGUGUGUGUGUGU <u>C</u>	OH	RNA	Biomers
AS G21	GUGUGUGUGUGUGUGUGUGU <u>C</u>	OH	RNA	Biomers
AS G20	UGUGUGUGUGUGUGUGUGU <u>C</u>	OH	RNA	Biomers
AS G19	GUGUGUGUGUGUGUGUGU <u>C</u>	OH	RNA	Biomers
AS G17	GUGUGUGUGUGUGUGU <u>C</u>	OH	RNA	Biomers
AS G15	GUGUGUGUGUGUGU <u>C</u>	OH	RNA	Biomers
AS G13	GUGUGUGUGUGU <u>C</u>	OH	RNA	Biomers
AS C26	AAAGUGUGUGUGUGUGUGUGUGUGU <u>G</u> GU	OH	RNA	Biomers
AS C24	AAAGUGUGUGUGUGUGUGUGUGU <u>G</u>	OH	RNA	Biomers

AS U26	AAAGUGUGUGUGUGUGUGUGUGU <u>AGU</u>	OH	RNA	Biomers
AS U24	AAAGUGUGUGUGUGUGUGUGUGU <u>A</u>	OH	RNA	Biomers
AS23	AAAGUGUGUGUGUGUGUGUGUGU	OH*	RNA	Biomers
AS21	AAAGUGUGUGUGUGUGUGUGU	OH	RNA	Biomers
AS19	AAAGUGUGUGUGUGUGUGU	OH	RNA	Biomers
IVT2	GACGACGACGACGACGACGACGACGACGAC	3P	RNA	IVT
dAdT	(AT) ₂₀₀₋₄₀₀₀	P	DNA	Sigma
ASGFP2 24	AAGAUGAACUUCAGGGUCAGCGUC	OH	RNA	Biomers
ASGFP2 2+5'24	AAGAUGAACUUCAGGGUCAGCGUCAA	OH	RNA	Biomers
ASGFP2 3'23	AAGAUGAACUUCAGGGUCAGCGU	OH	RNA	Biomers
ASGFP2 3'21	AAGAUGAACUUCAGGGUCAGC	OH	RNA	Biomers
ASGFP2 3'19	AAGAUGAACUUCAGGGUCA	OH	RNA	Biomers
ASGFP2 5'21	AUGAACUUCAGGGUCAGCGUC	OH	RNA	Biomers
ASGFP2 5'20	UGAACUUCAGGGUCAGCGUC	OH	RNA	Biomers
ASGFP2 5'19	GAACUUCAGGGUCAGCGUC	OH	RNA	Biomers
ASGFP2 5'18	AACUUCAGGGUCAGCGUC	OH	RNA	Biomers
ASGFP2 5'16	CUUCAGGGUCAGCGUC	OH	RNA	Biomers
ASGFP2 5'14	UCAGGGUCAGCGUC	OH	RNA	Biomers
ASGFP2 5'9	GUCAGCGUC	OH	RNA	Biomers
AS GFP2 C>U1	AAGAUGAACUUCAGGGUCAGCGU <u>U</u>	OH	RNA	Biomers
AS GFP2 C>A1	AAGAUGAACUUCAGGGUCAGCGUA	OH	RNA	Biomers
AS GFP2 U>A2	AAGAUGAACUUCAGGGUCAGCGAC	OH	RNA	Biomers
AS GFP2 8b3	AAGAUGAACUUCAGAGCCAGCGUC	OH	RNA	Biomers
HO-VH1-bio**	ACACACACACACACACACACAAAACC	OH	RNA	Dharmacon
3P-VH1-bio**	ACACACACACACACACACACAAAACC	OH	RNA	see methods
AS-VH1	GGUUUUGUGUGUGUGUGUGUGUGUGU	OH	RNA	Dharmacon
3P-GFP1	GGGGCUGACCCUGAAGUUCAUCUU	3P	RNA	see methods
3P-GFP2	GACGCUGACCCUGAAGUUCAUCUU	3P	RNA	see methods
3P-GFP3	GGGGCGCUGACGCCUGAAGUUCA	3P	RNA	see methods
Rabies Panhandle match	GACGCUUAACAAAUAAACAACAAAAAUGAGAAAA ACAAUCAUAUGUCUGUUUUUUCUUUGAUCUGGUU GUUAAGCGUC	3P	RNA	IVT
Rabies Panhandle mismatch	GACGCUUAACAAAUAAACAACAAAAAUGAGAAAA ACAAUCAUAUGUCUGUUUUUUCUUUGAUCUGGUU GUUAAGCGAUAUUUUAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAA	3P	RNA	IVT

RNA and DNA stimuli. 3P = triphosphate, P = monophosphate, IVT = in vitro transcription

* Oligos used for alpha screen were labeled with biotin at the 5' end.

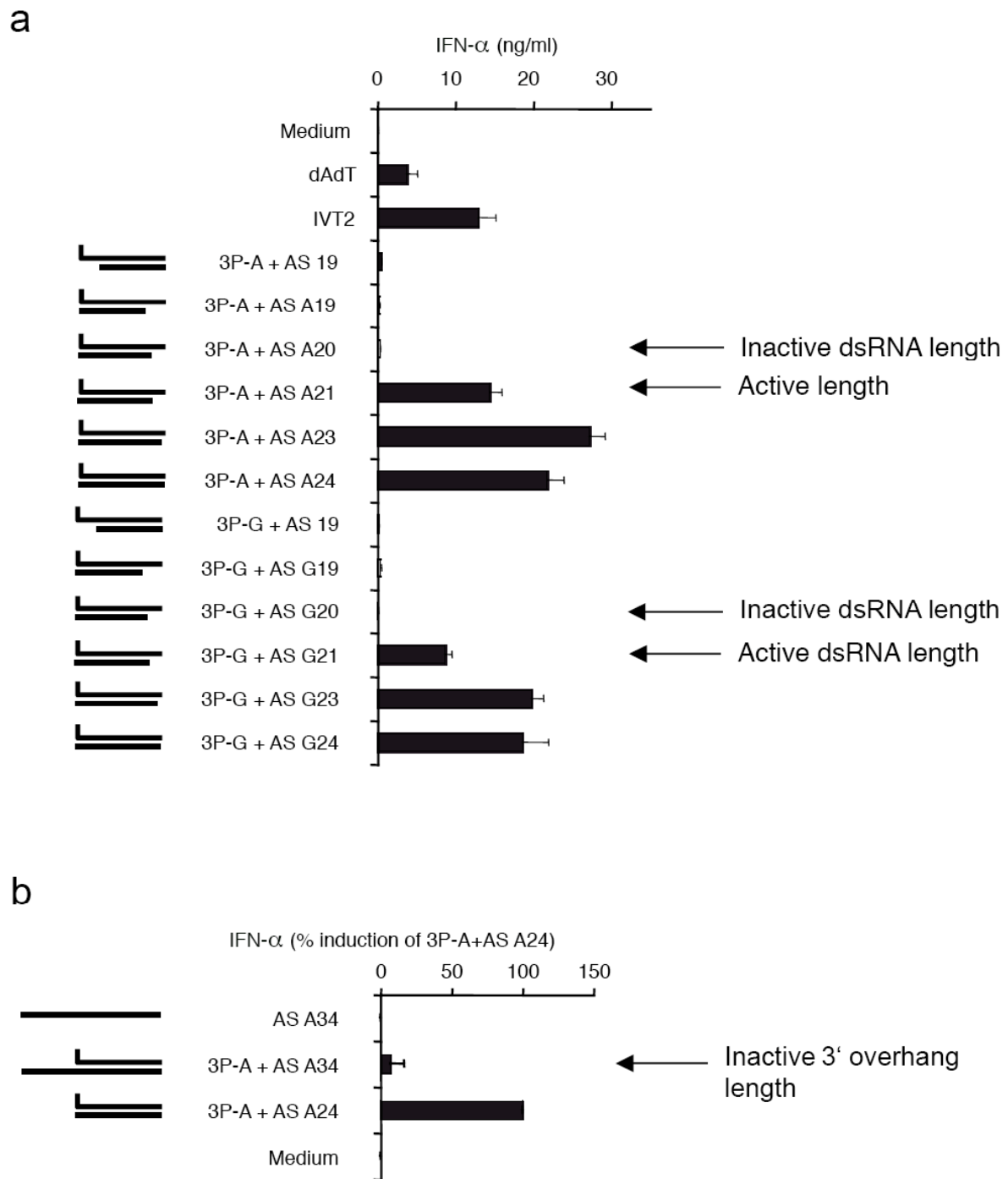
** VH1-bio and 3P-VH1-bio were labeled with c6-biotin at the 3' end.



Suppl. Fig. 1

T7 polymerase in vitro transcription mix contains high amounts of sequences not encoded by the DNA-template.

Indicated ssRNA and dsRNA oligonucleotides and in vitro-transcribed RNA (ivt3P-Gaca) were digested by RNaseT under native conditions (37°C, upper panel) or denaturing conditions ((Nallagatla et al., 2007), 6M urea, 50°C, lower panel). After digestion, the RNA was separated by urea polyacrylamide gelelectrophoresis and stained with Methylene Blue. The RNA length marker is denoted as number of nt.



Suppl. Fig. 3: Length and 5' and 3' overhang impact on IFN- α stimulating activity of short double strand RNA.

Purified monocytes were stimulated with the indicated single strand or double strand synthetic RNA oligonucleotides. IFN- α production was analyzed 24 hours after stimulation. a: 3P-G and 3P-A hybridized with antisense strands of different lengths and binding positions are compared. b: 3P-A hybridized with an antisense strand resulting in a double strand RNA with a 10 base long 3' overhang at the triphosphate end.

References

Nallagatla, S.R., Hwang, J., Toroney, R., Zheng, X., Cameron, C.E., and Bevilacqua, P.C. (2007). 5'-triphosphate-dependent activation of PKR by RNAs with short stem-loops. *Science* 318, 1455-1458.

Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* 31, 3406-3415.