Supplementary Data

Engineering Human PrimPol into an efficient RNA-dependent-DNA primase/polymerase

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SUPPLEMENTARY MATERIAL AND METHODS

Primase-polymerase assays on RNA heteropolymeric templates

Priming assays using *Hs*PrimPol in the presence of heteropolymeric oligonucleotides were performed as described for GTCC or *GUCC* oligonucleotides using radiolabeled [α-^{32}P]GTP (16 nM). The specific oligonucleotide template and concentration of additional dNTPs are indicated in the corresponding figure legends.

SUPPLEMENTARY TABLE AND FIGURES LEGENDS

Table S1. Primers used for saturation mutagenesis an enzymatic characterization of *Hs*PrimPol in the present work.

Name	Sequence (5'→3')[1]	Amplicon[2]
Sp1C[3]	gatcacagtgagtac	N.A.
T13T[3]	agaagtgtatcttgtactcactgtgatc	N.A.
T13U[3]	*agaaguguaucuuguacucacugugauc*[3]	N.A.
88/89-NBC/NDT-Fw	tatgctgaattttggnbcndttataaatccagaaaaaatctcttac	N.A.
88/89-NBC/NDT-rv	tctggatttataahngvnccaaaattcagcataggttgtc	N.A.
GTCC	ttttttttttttttttttttttttttttttttttttcctgttttttttttttttttttt*t*t[4]	N.A.
ATCC	ttttttttttttttttttttttttttttttttttttcctattttttttttttttttttt*t*t	N.A.
GUCC	ttttttttttttttttttttttttttttccugttttttttttttttt	N.A.
NS3-F1	agctgatggctacacctccaag	937 nt
NS3-R3	aatggaggtagcatccacagcgtggc	
NS5A-F2	actaccttctccagagtttttc	1051 nt
NS5A-R3	cagcgagttactcaaagggttgattggcaac	
GAPDH-Fw	ctcccactcttccaccttcg	77 nt
GAPDH-rv	cataccaggaaatgagcttgacaa	
BACT-Fw	ctaaggccaaccgtgaaaag	104 nt
BACT-rv	accagaggcatacagggaca	
18S-Fw	ctcaacacgggaaacctcac	110 nt
18S-rv	cgctccaccaactaagaacg	
PPIA-Fw	acgccactgtcgcttttc	111 nt
PPIA-rv	gcaaacagctcgaaggagac	
HPRT1-Fw	tcctcctcagaccgctttt	90 nt

HPRT1-rv	gctggttcatcatcgctaatc	
ARBP-Fw	gatgcccagggaagacag	66 nt
ARBP-rv	tccaaaagttggatgatcttga	
Polγ-Fw	gcggctggtggaagagcgtt	227 nt
Polγ-rv	gagatggccatgtgcatgctc	
TFAM-Fw	caggaggcaaaggatgattc	144 nt
TFAM-rv	ccaagacttcatttcattgtcg	

[1] Nucleotide sequences indicated in italics are composed of ribonucleotides.

[2] Nucleotide (nt) length of the PCR product amplified using the corresponding pair of primers in a PCR reaction. N.A.: not applicable.

[3] Primers provided by the manufacturer already purified by PAGE.

[4] Asterisks indicate phosphorothioate bonds. Underlined sequences highlight the priming site.

Table S2. List of *Hs*PrimPol variants that showed primase-polymerase activity in the fluorometric screening assay.

Variant
Y89C
Y89G
Y89R
F88A-Y89L
F88A-Y89N
F88A-Y89R
F88C-Y89S
F88G-Y89D
F88G-Y89G
F88G-Y89S
F88I-Y89R
F88L-Y89S
F88P-Y89R
F88R-Y89S
F88S-Y89C
F88S-Y89H
F88T-Y89H
F88V-Y89N

Purified variants whose primase activity was confirmed by *in vitro* assays on GTCC and *GUCC* oligonucleotide template (as described in Materials and Methods) are shown in bold.

Figure S1. Fluorometric detection of DNA primase-polymerase activity using crude cell extracts overexpressing different *Hs*PrimPol variants. Primase-polymerase activity was measured as the fluorescence increase produced by tight binding of SYBR® green I dye to nascent dsDNA. **A**) fluorescence kinetics of five independent replicates, assayed with supernatants from cells overexpressing WT *Hs*PrimPol in the presence of the GTCC template, dGTP, dATP and $MnCl_2$. **B**) Same as (A), but using supernatants from cells overexpressing the catalytically inactive AxA *Hs*PrimPol mutant (1). Fluorescence increase was observed only when a catalytic-competent *Hs*PrimPol was present. Experimental conditions used for screening are described in Materials and Methods. **C**) Fluorescence kinetics shown by different supernatants *from Hs*PrimPol variants in a representative RdDP activity screening assay using *GUCC* as template. Sequence of mutants displaying RdDP activity is shown.

Figure S2. *Hs*PrimPol priming on heteropolymeric ssRNA templates. Primase activity of WT *Hs*PrimPol or its Y89R variant was determined using either T13U (1 μM; 28 nt) or HCV RNA (150 ng; ≈9600 nt) as template, 16 nM [α-^{32}P]dGTP, 10 μM dGTP, and 100 μM dATP, dCTP, dTTP. “nt”, control experiment of *Hs*PrimPol reaction without added RNA as template. Arrowheads indicate specific primase products. “*”, adducts produced by *Hs*PrimPol in the absence of exogenously added template. Reaction conditions are described in Supplementary Materials and Methods.

1. Garcia-Gomez, S., Reyes, A., Martinez-Jimenez, M.I., Chocron, E.S., Mouron, S., Terrados, G., Powell, C., Salido, E., Mendez, J., Holt, I.J. *et al.* PrimPol, an archaic primase/polymerase operating in human cells. *Mol Cell*, **52**, 541-553.

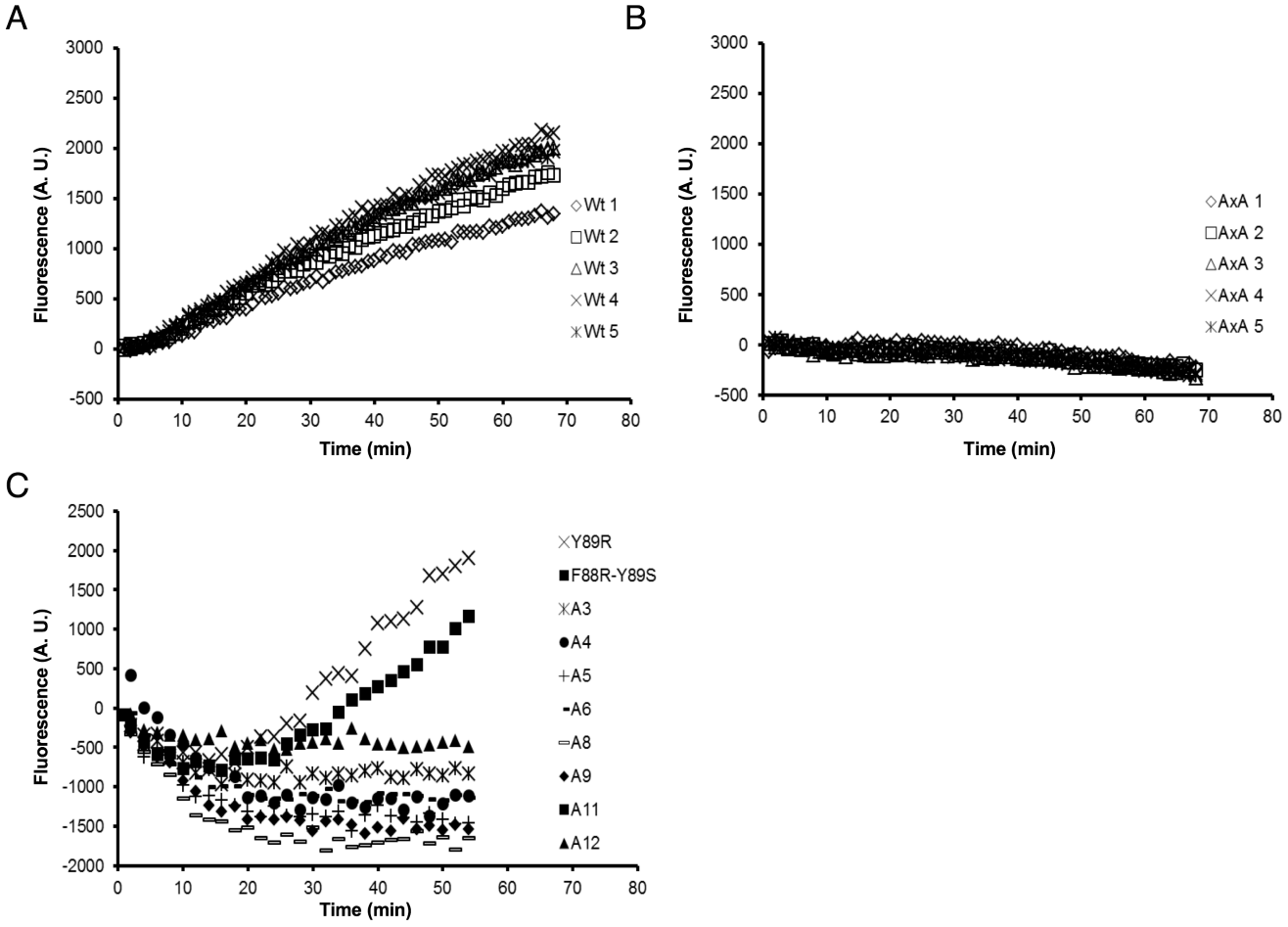

Figure S1 Agudo et al.

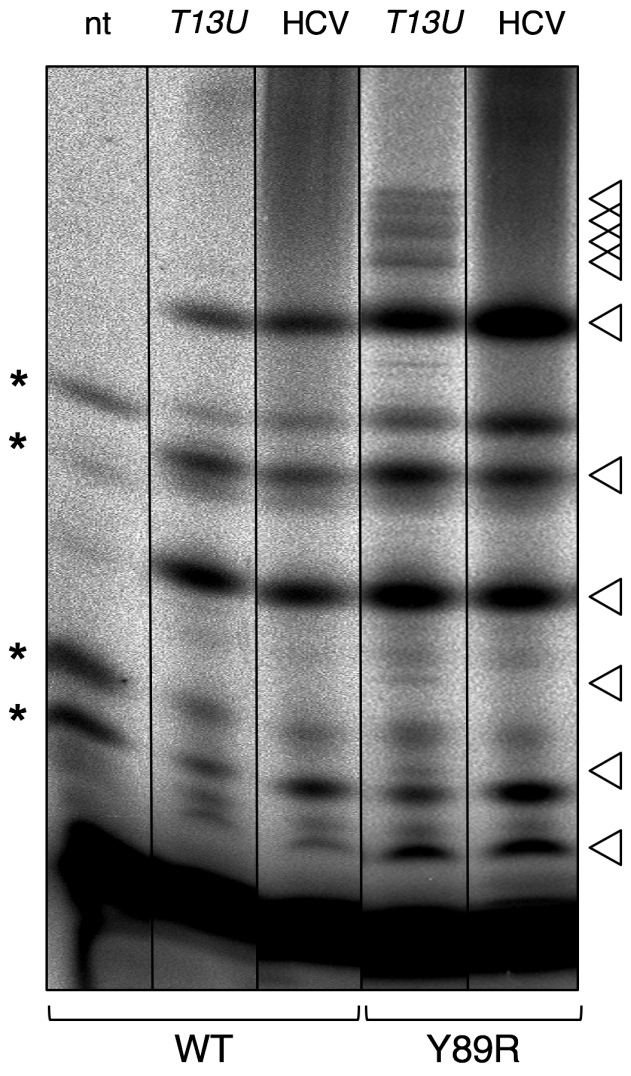

Figure S2 Agudo et al.