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RNA Polymerase III Detects Cytosolic DNA and Induces Type I Interferons through the RIG-I Pathway

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Supplemental Experimental Procedures

Plasmids, Stable cell lines and Antibodies

Human RIG-I, POLR3D and POLR3F coding sequences were amplified by PCR and then cloned into mammalian expression vector pEF-IRES-Puro in frame with a C- (for RIG-I) or N-terminal (for POLR3) FLAG tag. pEF-Renilla-IRES-puro was constructed by subcloning the Renilla coding sequence into pEF-IRES-puro under the control of EF1α promoter. Plasmids for IFN-β-luciferase (Luc) and pCMV-LacZ have been described previously (Deng et al., 2000; Seth et al., 2005). Stable cell lines expressing FLAG-tagged RIG-I, POLR3D or POLR3F were generated by transfecting pEF-RIG-I-FLAG-IRES-Puro, pEF-FLAG-POLR3D-IRES-Puro or pEF-FLAG-POLR3F-IRES-Puro into HEK293 cells followed by selection with puromycin (1μg/ml). Single cell clones were picked and the expression of FLAG-tagged proteins was verified by immunoblotting. The HEK293-IFNβ-Luc reporter cell line was generated by co-transfecting HEK293 cells with IFN-β-Luc and pEF-Renilla-IRES-puro followed by selection with puromycin (1μg/ml). The antibodies for human IRF3, POLR3F, POLR3G, and TBP were purchased from Santa Cruz Biotechnology, Inc. The antibody for FLAG (M2) was purchased from Sigma-Aldrich.

Cell culture, Transfection, Luciferase Reporter Assay and IRF3 Dimerization Assay

HEK293 and Raw264.7 cells were cultured in DMEM supplemented with 10% calf serum or fetal bovine serum (FBS), respectively. Isolation and culture of wild-type and Mavs-deficient mouse embryonic fibroblasts (MEFs) and bone marrow-derived macrophages (BMDMs) have been described previously (Sun et al., 2006). B95-8 cells were cultured in RPMI supplemented with 15% FBS, 1% sodium pyruvate, and antibiotics. Transfection of DNA or RNA into HEK293 or MEF cells was carried out using Lipofectamine 2000 (Invitrogen). Luciferase reporter assay were done in HEK293-IFNβ-luciferase reporter cells or HEK293 cells transiently transfected with IFN-β-Luc (25 ng/ml) and pCMV-LacZ (50ng/ml). Cells were harvested 16 hours after transfection and lysed in the passive lysis buffer (Promega). Luciferase and Renilla activities were measured with a luminometer (BMG LABTECH, FLUOstar OPTIMA) using luciferin (Promega) and coelenterazine (Biotium) as substrates, respectively. β-galactosidase activity was measured with a Thermo Labsystems microplate reader at the wavelength of 405 nm using o-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate. Cells were lysed either in the passive lysis buffer or in the TBS lysis buffer (20 mM Tris, pH7.5, 150 mM NaCl and 0.5% NP-40) for IRF3 dimerization assay described previously (Seth et al., 2005).

RNAi

siRNA oligos at a final concentration of 20 nM were transfected into HEK293 cells using the calcium phosphate precipitation method. The transfection procedure was repeated on the next day. On the third day, cells were transfected with 1μg/ml of poly(dA-dT)·poly(dA-dT) using Lipofectamine 2000 (Invitrogen) or infected with 80 hemagglutinating (HA) units/ml of Sendai virus (Cantell strain; Charles River laboratories). Cells were harvested on the fourth day for analysis unless indicated otherwise. The sequences of the siRNA oligos are as follows (only the sense strands are shown): GFP (471-489), GCAGAAGAACGGCAUCAAG; RIG-I a (297-315), GUUGGAGGAGUAUAGAUUA; RIG-I b (376-394), GAUCUGUCUGAAUGUUUAA; MAVS a (899-917), CCACCUUGAUGCCUGUGAA; MAVS b (1364-1382), CAGAGGAGAAUGAGUAUAA; POLR3F a (740-758), AAGUGGAGAUGACGAUUAU; POLR3F b (930-948), UGACAGAGUGGCUCGAAUU. These RNA oligos were synthesized at the UT Southwestern Center for Biomedical Invention (CBI) facility or purchased from Dharmacon.

Enzyme Treatments of Nucleic Acids

1μg of nucleic acids was treated with DNase I (0.2 U/μl) or RNase A (0.1 mg/ml) (Ambion) at 37^{o}C for 1 hour. For RNase experiments, 1μg of RNA was digested with RNase A, RNase III, and RNase T1 (Ambion) at indicated concentration at 37^{o}C for 1 hour. The undiluted (1X) concentrations of RNase used in the reactions were 0.1 mg/ml (RNase A), 0.1 U/μl (RNase III) and 100 U/μl (RNase T1). Unit of RNase is defined by manufacturer. 1μg of RNA was treated with shrimp alkaline phosphatase (0.1U/μl, SAP; Roche Applied Science), T4 Polynucleotide kinase (1U/μl, PNK; Ambion) or Terminator Exonuclease (0.1U/μl, Ter Ex; Epicentre biotechnologies) at 37^{o}C for 1 hour. SAP-treated RNA was incubated at 65 ^{o}C for 15 min for heat inactivation before further treatment with PNK. Enzyme-treated DNAs or RNAs were precipitated with isopropanol before transfection. Small molecule fractions were incubated at 37 ^{o}C for 1 hour with proteinase K (0.02, 0.2 or 2 mg/ml), DNase I (0.2 U/μl) or RNase If (5 U/μl; Ambion), followed by 75 ^{o}C incubation for 20 min to inactivate the enzymes.

Purification of small molecules

HeLa S100 was applied to a HiTrap Q column (10 ml bed volume, GE Healthcare) pre-equilibrated with Buffer A and eluted with 0.3 M NaCl in the same buffer. The eluate was subjected to filtration (5kDa cut-off filter) and the filtrate was heated at 75 ^{o}C for 15 min, followed by proteinase K (2mg/ml) treatment at 37 ^{o}C for 1 hour. The samples were heated again at 75 ^{o}C for 20 min followed by ethyl acetate extraction. The water soluble fraction was loaded onto a Superdex-75 gel filtration column (24 ml bed volume, GE Healthcare) pre-equilibrated with Buffer A. The active fractions were applied to a HiTrap Q column (1 ml bed volume, GE Healthcare). Bound molecules were eluted with a 12 ml linear gradient of KCl (0-300 mM), and the active fractions (100-125 mM KCl) were applied to a Superdex-peptide gel filtration column (24 ml bed volume, GE Healthcare). After elution with Buffer A, active fractions were applied to a MonoQ column (0.1 ml) using the SMART micropurification system (GE Healthcare). The column was eluted with a 3 ml linear gradient (0-300 mM) of KCl, and the active fractions were further separated on a

Superdex-peptide column (0.1 ml bed volume, GE Healthcare). Finally, the activity was eluted with deionized water and analyzed by LC-MS and NMR.

NMR and LC-MS Analysis

All NMR experiments were performed on a Varian NMR System at 600 MHz for ^{1}H and at 150 MHz for ^{13}C equipped with a 5mm triple resonance probe with triple axis gradients. All experiments were run at a constant temperature of 298 ºK in D_2O. All low resolution mass spectroscopy was performed on an Agilent Model 6130 Single Quadrupole MS equipped with an Agilent Model 1200 LC and diode array detector. HPLC analysis was carried out using a Phenomenex Luna C_{18} (2) analytical column (5µ, 4.6 x 150 mm) at a flow rate of 0.7 mL/min under isocratic conditions of 98% H_2O (0.5% formic acid) and 2% CH_3CN for 10 minutes (condition 1) or isocratic conditions of 99% H_2O (0.1 M NH_4OAc) and 1% CH_3CN for 10 minutes (condition 2). Authentic standards of UDP and UTP were used to verify the retention time of the detected metabolites.

Affinity Purification of Pol-III complex

Pol-III complex was purified by two-step affinity purification. HEK293 cells stably expressing FLAG-POLR3F were lysed in Buffer A by douncing and centrifuged at 100,000xg for 30 min to obtain S100. S100 (3 mg protein) was incubated with the anti-FLAG M2 affinity gel (0.25 ml; Sigma-Aldrich) at 4 ^{o}C for 2 hours followed by extensive washing with Buffer A. The Pol-III complex was eluted with FLAG peptide (0.25 mg/ml; Sigma-Aldrich) and then incubated with rabbit IgG or POLR3G-specific antibody (2 µg) at 4 ^{o}C for 1 hour and protein A/G (Thermo Scientific) for another hour. After wash with Buffer A, bound proteins were eluted with SDS sample buffer (10mM Tris, pH6.8, 0.8% SDS, 10% glycerol, and 0.05% bromophenol blue) at 95 ^{o}C for 5 min and then analyzed by SDS-PAGE and silver staining. Twelve unique bands comparing to IgG control were excised for in-gel digest with trypsin. Peptide masses were acquired by nano-HPLC/MS/MS.

Protein Identification by Nano-electrospray Mass Spectrometry

Following separation of proteins by SDS-PAGE and silver staining, protein bands of interest were excised and digested with trypsin in situ at 37^{o}C for 16 hours. The tryptic peptides were extracted in 50% acetonitrile (ACN), 5% Formic acid (FA), lyophilized and then resuspended in 10 µl of 2% ACN and 0.1% FA. 5 µl of the peptide sample was loaded via an autosampler (Finnigan Micro AS) onto a trap column (New Objective; 5 µm PROTEOPEP™ II C18, 300 Å, 100 µm x 25 mm;) using an HPLC system (Eksigent NanoLC 2D) at 5 µl/min for 2.6 min in 2% ACN, 0.1% FA. Peptides were eluted from the trap column and directly separated on an analytical column with an electrospray tip packed in house (Michrom, 3 µm Magic C18, 100 Å; 76 µm x 105 mm) at a flow rate of 200 nl/min. A 60 min gradient of buffer A (water + 0.1% FA) and buffer B (100% ACN + 0.1% FA) was used according to the following program: 2% B, 0-5 min; 2-40% B, 5-40 min; 40% B, 40-45 min; 80% B, 45-55 min; 2% B, 55-60 min. Eluted peptide ions were scanned by an LTQ XL mass spectrometer (Thermo) with a spray voltage of 2kV. MS/MS spectra were acquired in a data-dependent mode with dynamic exclusion whereby the top six most abundant parent ions were subjected to further fragmentation by collision-induced

dissociation (CID). The spectra were used to search the human RefSeq protein database using Sequest (Thermo).

Northern Blotting and In vitro Transcription

RNA samples were separated on 6% denaturing polyacrylamide gel containing 8 M of urea and electro-transferred to nylon membrane in 0.5X TBE at 2.5 mA/cm^2 for 40 min. After UV cross-linking at 0.15 J/cm^2, the membrane was blocked in 6X SSC, 7% SDS at 68 ^oC for 16 hours in the hybridization chamber. The RNA probes were prepared by MEGAscript T7 kit (Ambion) using DNA templates (Invitrogen) according to manufacturer's instruction and labeled with PNK and γ-^{32}P-ATP (Perkin Elmer) after SAP treatment. The RNA probes were further purified on illustra MicroSpin G-25 Columns (GE Healthcare) and added to the hybridization chamber at $6X10^5$ cpm/ml for hybridization at 42 ^oC for 24 hours. The membrane was washed three times with 3X SSC, 0.1% SDS at 42 ^oC for 20 min and then twice with 1XSSC, 0.1% SDS at 42 ^oC for 15 min and analyzed by phosphoimager (Molecular Dynamics).

The sequences of DNA templates for in vitro RNA synthesis using T7 RNA polymerase are as follows (only the sense strands are shown): T7-dAdT: GGCTTAATACGACTCACTATAGGGAGATATATATATATATATATATATATATAT ATATATATATATATATATATATATATATATATATATATATATATATAT; T7-dGdCdAdT: GGCTTAATACGACTCACTATAGGGAGAGCATGCATGCATGCATGCATGCATGCA TGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCAT; T7-Random: GGCTTAATACGACTCACTATAGGGAGACCATCTGCTACTCGATCGATATCCTAG CATATCGTACGCTCGAGGTCACTTCTGACTGGTACCTAGCATAATC.

Real Time RT-PCR

Reverse transcription and real-time PCR reactions were carried out using iScript cDNA synthesis kit and iQ SYBR Green Supermix (Bio-Rad) according to manufacturer's instructions. The sequences of DNA primers for real-time PCR are as follows: Mouse IFN-β, sense: TCCGAGCAGAGATCTTCAGGAA, anti-sense: TGCAACCACCACTCATTCTGAG; Mouse β-actin, sense: TGACGTTGACATCCGTAAAGACC, anti-sense: AAGGGTGTAAAACGCAGCTCA; Human IFN-β, sense: AGGACAGGATGAACTTTGAC, anti-sense: TGATAGACATTAGCCAGGAG; Human GAPDH, sense: ATGACATCAAGAAGGTGGTG, anti-sense: CATACCAGGAAATGAGCTTG. Human α-actin, sense: ATCTCACCGACTACCTGATG, anti-sense: GATCTCGCGCTCAGCT; EBER1, sense: AGGACCTACGCTGCCCTAGA, anti-sense: AAAACATGCGGACCAGC; EBER2, sense: AGGGACAGCCGTTGCCCTAGTGGTTTCGGA; anti-sense: AAAACAGCGGACAAGCCGAATACC.

Bacterial Replication Assay

Raw264.7 cells were pre-treated with ML-60218 in the presence or absence of mouse IFN-β (Sigma) for 10 hours and then infected with *Legionella pneumophila* at MOI = 50 for 24 hours. Cells were washed with PBS and then lysed in 0.1 % saponin for 5 min. Cell lysates

were diluted with PBS by 40,000 Fold and then spread on buffered charcoal yeast extract (BCYE) agar plates (Opitz et al., 2006). After incubation at 37^{o}C for 2 days, colonies were counted to determine colony-forming units (CFU).

Supplemental References

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AT100: ATATATATATATATATATATATATATATATATATATATATATATATAT
ATATATATATATATATATATATATATATATATATATATATATATATAT

GCAT100: GCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGC
ATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCAT

AT50: ATATATATATATATATATATATATATATATATATATATATATATAT

GC50: GCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC

AT20GC10AT20: ATATATATATATATATATATGCGCGCGCATATATATATATATATATAT

AT10GC20AT10: ATATATATATGCGCGCGCGCGCGCGCATATATATAT

AT50%: ATCCGTGCTACTCGATCGATATCCTAGCATATCGTACGCTCGAGGTCACT

AT80%: ATCTATACTACCATTACATTATCGATTTATTCATATAATATCTAATTCTA

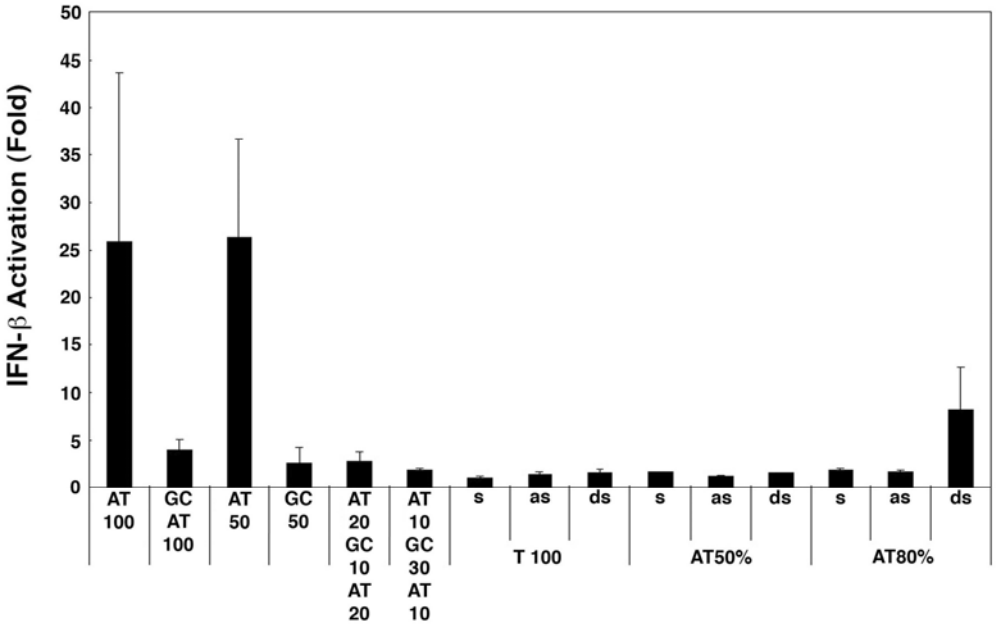


Figure S1. AT-rich DNA duplexes Induce IFN-β

Synthetic DNA oligos of different compositions were transfected into HEK293-IFNβ-Luc reporter cells followed by luciferase assays. Palindromic sequences (e.g, AT100 or GCAT100), which form double-stranded DNA were transfected directly, whereas non-palindromic sequences (e.g, T100 or AT50%) were transfected as sense (s) or antisense (as) single-stranded DNA or annealed to double-stranded DNA (ds) before transfection. Error bars represent the variation range of duplicate experiments.

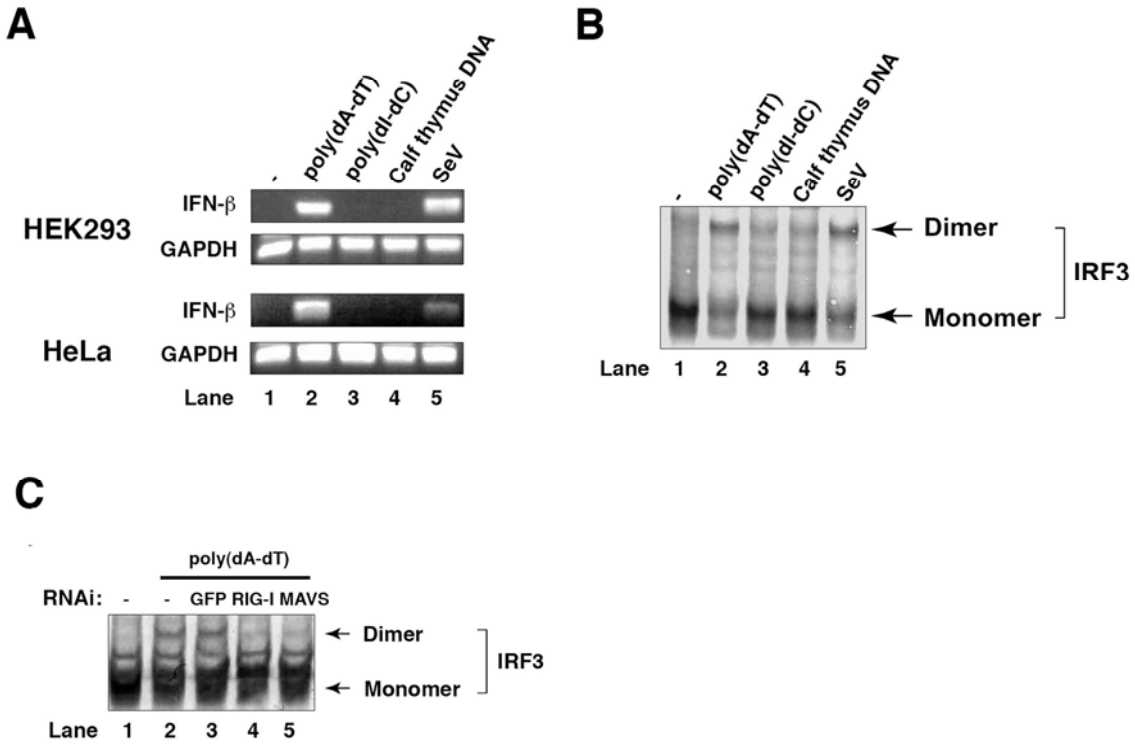


Figure S2. Poly(dA-dT) activates RIG-I- and MAVS-dependent IFN-β production in HeLa cells.

(A) HEK293 and HeLa cells were transfected with the indicated DNA (1 μg/ml) or infected with Sendai virus (SeV) for 16 hours. RNAs were extracted from the transfected or infected cells for RT-PCR with *IFN-β* and *GAPDH* specific primers. (B) HeLa cells were treated as described in (A) and protein extracts were prepared for IRF3 dimerization assays using native gel electrophoresis. (C) HeLa cells were transfected with siRNAs against GFP, RIG-I or MAVS and subsequently transfected with poly(dA-dT). Cell extracts were prepared for IRF3 dimerization assays.

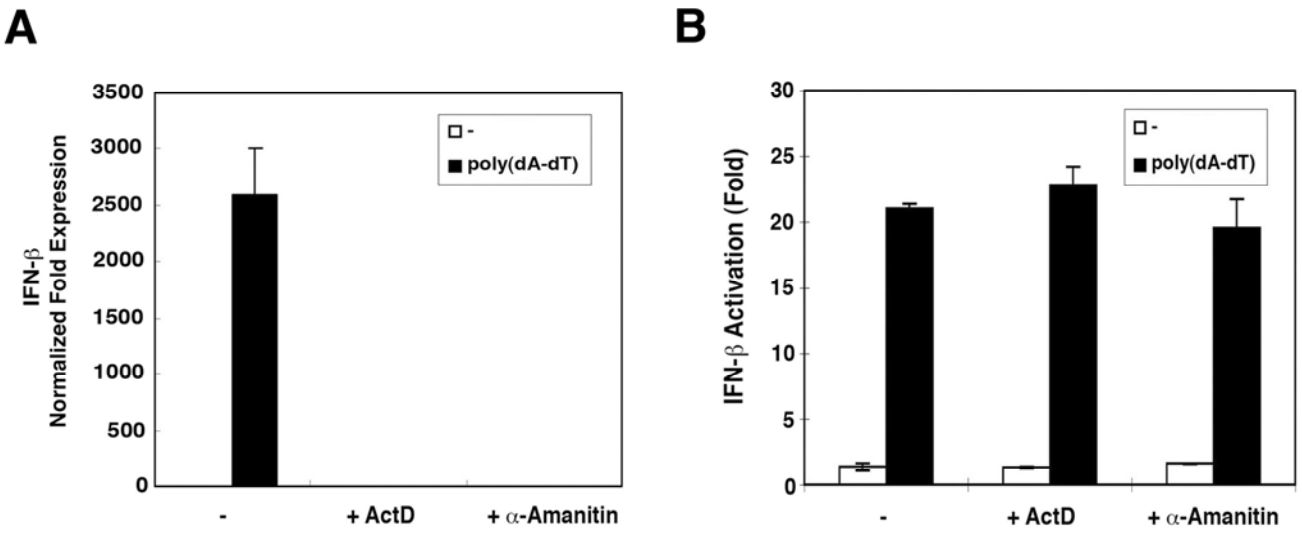


Figure S3. RNA polymerase II is not involved in generating IFN-inducing RNA from poly(dA-dT)

(A) HEK293 cells were pre-treated with Actinomycin D (2.5 μM) or α-amanitin (10 μg/ml) for 30 min and then transfected with or without poly(dA-dT). Total RNAs were extracted and analyzed by real-time PCR using *IFN-β* specific primers. (B) RNAs prepared as described in (A) were transfected into HEK293 IFN-β-Luc reporter cells followed by luciferase activity assays. Error bars represent the variation range of duplicate (B) or triplicate (A) experiments.

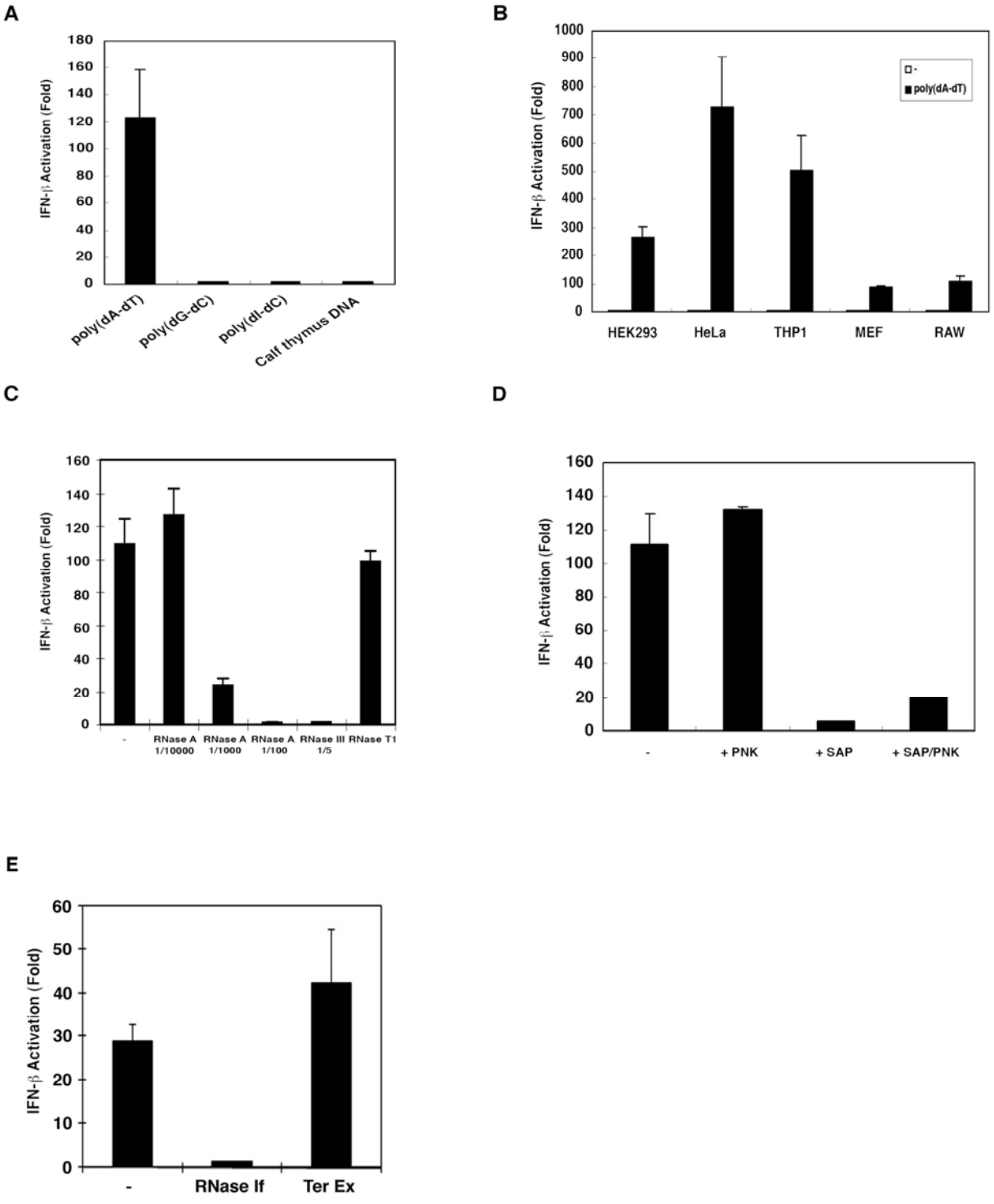


Figure S4. In vitro generation of IFN-inducing RNA

(A) HeLa cell lysates (S100) were incubated with the indicated DNA (1 μg/ml) in the presence of ATP, and then RNA was extracted for IFN-β reporter assays. (B) Cell lysates (S100) from the indicated cell lines were incubated with poly(dA-dT) and ATP before RNA was extracted for IFN-β reporter assays. (C) RNAs extracted from the in vitro reaction

containing HeLa S100 and poly(dA-dT) were digested with various amounts of RNAse A, RNase III or RNase T1 and then tested in IFN-β reporter assays. (D) RNAs extracted from the in vitro reaction were treated with PNK or SAP at 37^{o}C for 1 hour. An aliquot of SAP-treated RNA was further treated with PNK and ATP at 37^{o}C for 1 hour. RNAs were precipitated by ethanol precipitation after the enzyme treatments and then tested in IFN-β reporter assays. (E) RNAs extracted from the in vitro reactions were treated with RNase If or Ter Ex at 37^{o}C for 1 hour and then tested in IFN-β reporter assays. Error bars represent the variation range of duplicate experiments.

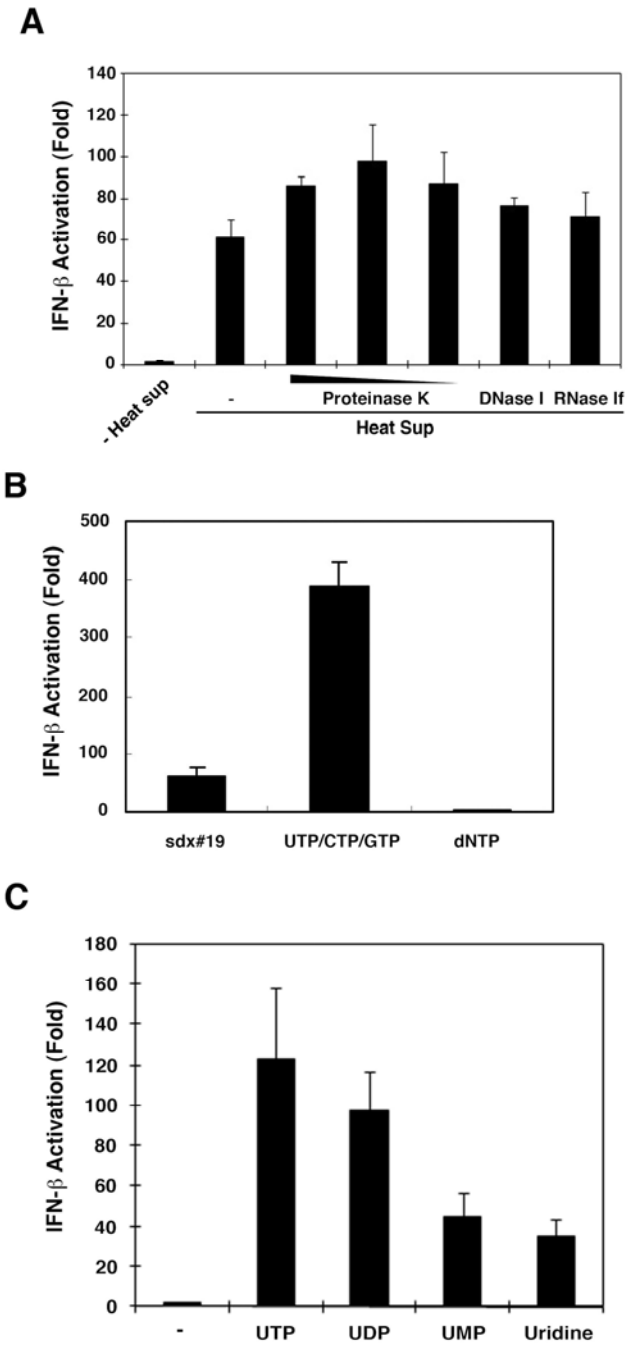


Figure S5. UTP and its analogues support the generation of IFN-inducing RNA in vitro

(A) Heat-resistant supernatant from HeLa S100 was treated with proteinase K (2, 0.2, 0.02 mg/ml), DNase I (0.2 U/μl) or RNase If (5 U/μl) at 37^{o}C for 1 hour and then at 75 ^{o}C for 20 min to inactivate the enzymes. The treated supernatant ("heat sup") was incubated with dialyzed 40% ammonium sulfate precipitate, poly(dA-dT) and ATP, and then RNA was extracted from the reactions for IFN-β reporter assays. (B) A mixture of UTP, CTP and GTP (1.5mM) or dNTP (1.5 mM) was used in place of the "heat sup" in the assay as described in (A). Sdex#19 is the partially purified fraction from "heat sup" as shown in Figure 3D. (C) Similar to (B), except that UTP, UDP, UMP or uridine (0.2 mM) was used in the in vitro reactions. Error bars represent the variation range of duplicate experiments.

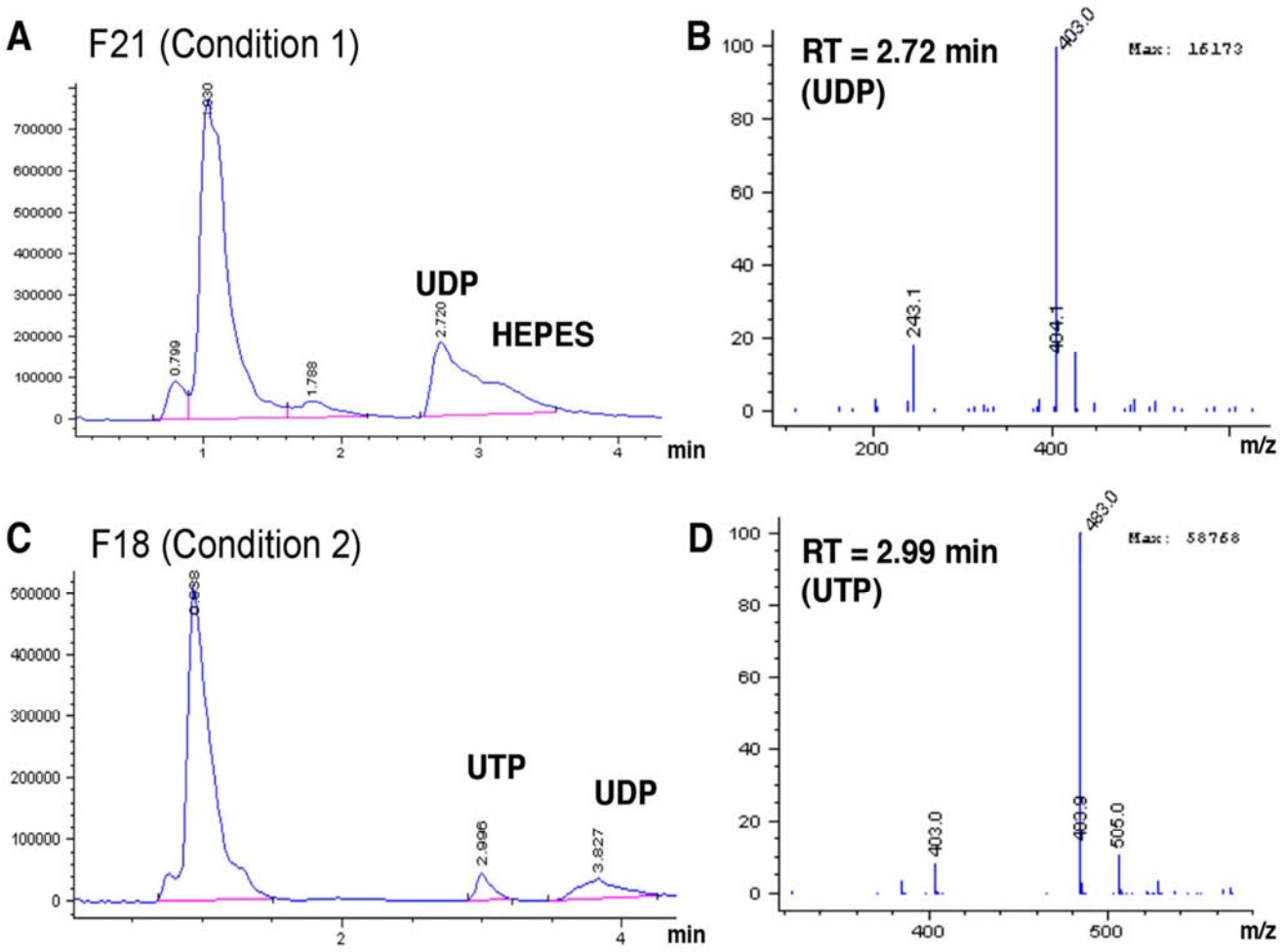


Figure S6. LC-MS profile for fractions 21 and 18 from Superdex peptide column
(A) LC trace of fraction 21 using LC condition 1 (TFA buffer). UDP was found with a retention time of 2.7 min. (B) MS spectrum of UDP from F21 (*m/z* = 403.0 $[M - H]^+$). (C) LC trace of fraction 18 using LC condition 2 (0.1 M ammonium acetate buffer). Under these conditions UDP has a retention time of 3.83 min while UTP has a retention time of 2.99 min (*m/z* = 483.0 $[M - H]^+$). Retention times were verified with authentic standards of UDP and UTP. (D) MS spectrum of UTP from F18 (*m/z* = 483.0 $[M - H]^+$).

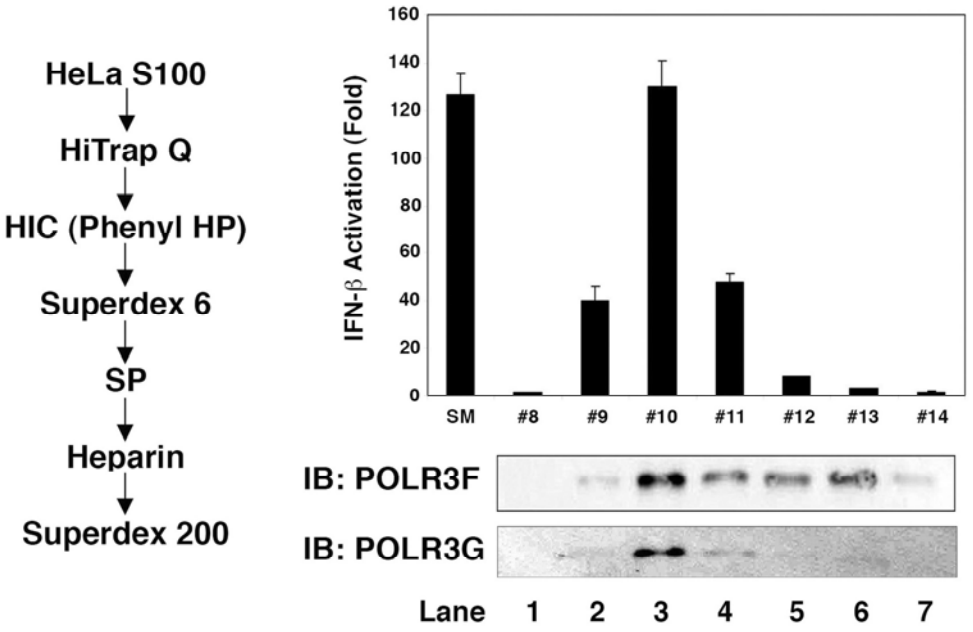
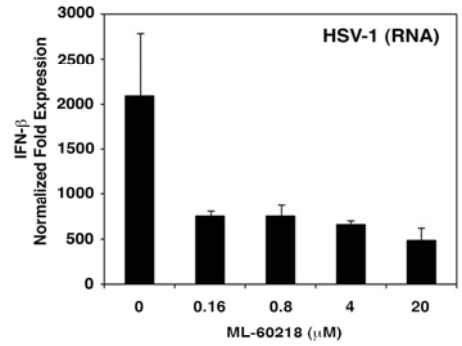


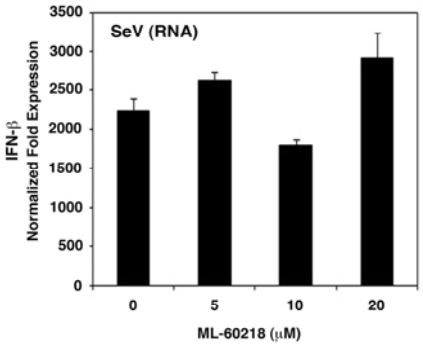
Figure S7. Pol-III subunits co-purify with the activity that generates IFN-inducing RNA

HeLa S100 was fractionated as described in the purification scheme, and the fractions from the last Superdex 200 column were assayed for the generation of IFN-inducing RNA. Aliquots of the fractions were analyzed by immunoblotting with an antibody against POLR3F or POLR3G. Error bars represent the variation range of duplicate experiments.

A



B



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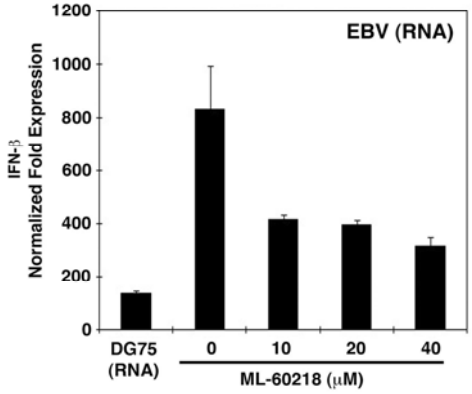

Figure S8. Pol-III is required for the generation of IFN-inducing RNA by DNA viruses

(A & B) Raw264.7 cells were treated with the indicated concentrations of ML-60218 for 10 hours and then infected with HSV-1 (A) or Sendai virus (SeV; B) for 8 and 14 hours, respectively. Total RNAs were extracted with Trizol and then transfected into Raw264.7 cells. After 8 hours of transfection, RNAs were isolated for qPCR of *IFN-β* and *β-actin*. (C) RNAs extracted from EBV-transformed B95-8 cells, which were pretreated with the indicated concentrations of ML-60218, were transfected into Raw264.7 cells for 8 hours. As a control, the RNA from EBV-negative DG75 B cells was also transfected to Raw264.7 cells. RNAs from the transfected Raw264.7 cells were analyzed by qPCR for expression of *IFN-β* and *β-actin*. Error bars represent standard deviations of triplicate experiments.

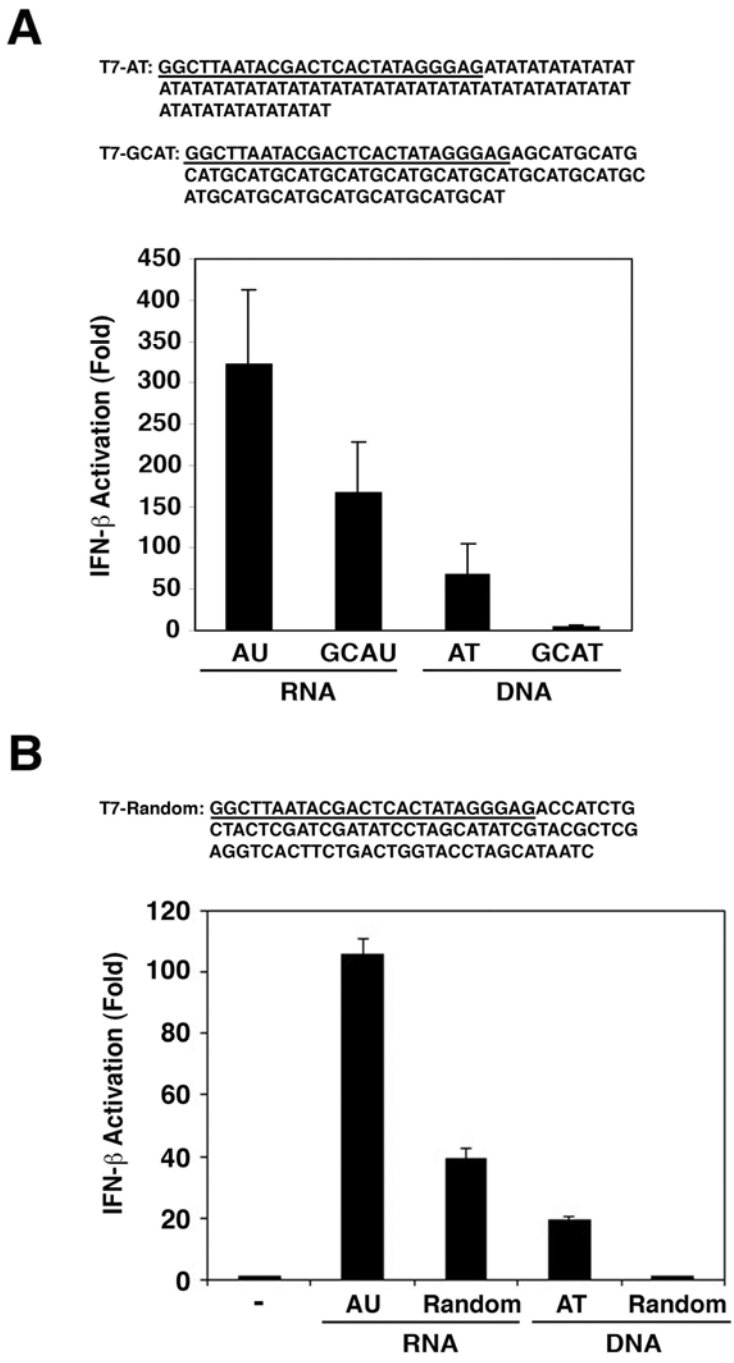

Figure S9. RNAs lacking AU-rich sequences are potent inducers of IFN-β
RNA containing poly-AU, GCAU (A), or a random (B) sequence was produced by in vitro transcription using T7 RNA polymerase, and then transfected into HEK293 IFNβ-Luc reporter cells. In parallel experiments, DNA containing the same sequences were directly transfected into the reporter cells to measure IFN-β induction. Error bars represent the variation range of duplicate experiments.

Table S1. Identification of RNA polymerase III subunits by nano-electrospray tandem mass spectrometry.

Protein	Accession No.	Peptide
POLR3A	NP_008986	QQAHIQVVSK, QFLDYLK, EVEPLLGR, PSVVSDLK, SGTNEDDLTMK, GFVQR, RVDFSGR, TVISPDPNLR, IDEVAVPVHVAK, ILTFPEK, ANINFLR, MAQELK, YGDIVER, AEALVLMGTK, ANLVTPR, LPPPTILK, DWGQNEAADAMSR, LAPVYLSNR, YELLNAGYK, ELSVIR, VPDGFENR, TAETGYMQR, VLDNIK, NELILTTESIMK, DKYGINDNGTTEPR, YGINDNGTTEPR, VLYQLDR, EIINASK, LEVNAETVR, VVVQGIPEVSR, AVIHIDEQSGK, LLVEGDNLR, AVMATHGVK, TTSNNTYEVEK, TLGIEAAR
POLR3B	NP_060552	LLPAFLK, VTSDADPMWYLK, NALPIGR, MPIMLR, TPAEFAK, VILIQEQLSK, IIVEADR, IIVEADRK, GAVGASVTSSTHEK, AQIFTQMQALK, MWGGGPK, AKCIYTAVMVR, VILAQGDNK, VILAQGDNKVDDR, VDDRDYYGNK, KFNSEMK, FNSEMK, KIADQVIPK, IADQVIPK, AAQFDVVK, QGVTQVLSR, LSYISALGMMTR, ISSQFEK, KLVNTFR, LVNTFR, HMEELAQGYR, QAMGTIGYNQR, TIELIEFEK, RYTNQTFDK, YTNQTFDK, VMGPMLDAATR, QVLVNK, DVPITYK, GATDSYIEK, RPEIGDK, LIELLAGK, FHYGTAFGGSK, LGEMER, SSCHVSSLR
TRM-1 like	NP_112196	HISIQR, VSVEFEGYR, RTDMLGHVR, SSYIAASTAK, SSYIAASTAKPPK, TDSYFNPK, SDDILEEGEK, MDANVLMHLR, RHYGCNIVR, IVVAAVAR, VLRGPTSADETAK, DGNMVEENPYR, QEENGVFIK, TTDDTTTDNYIAQGK, KSNEMITNLGK, SNEMITNLGK, THFDPMGVR, TDAPLMQFK
POLR3E	NP_060589	YAAALYR, FSRPESEQAR, VQSYEFLQK, VAMLVQGNWVVK, SDILYPK, DFVMWK, KEVATVTK, VYNLVK, AQQNHALLER, VPAVPPGVR, LANGLPLGR, AFVEATFQR, QFVLTLSELK, QVLLEIFSK, NMIQSR
POLR3C	NP_006459	MTQAEIK, IGVHLIR, VIAHDTGTSLDQVK, GVVEYEAQCSR, YIYTTK, LTMSAVVK, LTETMEDGK, TMDYAEVSNTFVR, LADTHFVQR, DMYLVPK, LSLLGK, DQAIVSAVNR, MDQTSSEIVR, SLPVGYNISK, SGDSGGGMYVINLHK, ALASLATATLESVVQER, QVEDFAMIPAK, DMLYK, MLSENFMSLQEIPK, SIANLIER
POLR3D	NP_001713	PLLTGAR, RPAPPLTPGR, DLTLGGVK, IKEEPKEEVTVK, ETDEETKQILR, VKEEPRDEEEEAK, DVSVAELLR, PIKTEVQGEDGQVVLIK, TEVQGEDGQVVLIK, DREAKLAENACTLADLTEGQVGK, VQLLLGK
POLR1C	NP_976035	VVLGEFGVR, ILLAEVPTMAVEK, NQGDEEGTEIDTLQFR, FSPVATASYR, LDTFSR, FLDELDAVQMD
POLR3F	NP_006457	VQPPDADPVEIENR, AVAINR, LLSMGQLDLLR, SNTGLLYR, LVYQIIEDAGNK, SNLPLTEINK, SVAASK, FLQSK, EGTVGSVDGHMK, AVNPIIPPTGLVK
POLR3F	NP_006457	LVYQIIEDAGNK, SVAASK
POLR3H	NP_612211	IPPWQFER, KLNDSIAEELNK, KLNDSIAEELNKK
POLR3G	NP_006458	AAYTFNIEAVGFSK, TGEGEEYMLALK, MPYFIETPEER, GTPLTNTEDVLK, GTPLTNTEDVLKK, KMEELEK, KMEELEKR, MEELEK, MEELEKR
POLR3GL	NP_115681	KTTEDKEETIQKL
RPS4	NP_000998	VAAPK, YALTGDEVK, TGENFR, LIYDTK, ITPEEAK, KIFVGTK, GIPHLVTHDAR, TIRYPDPLIK, VPDPLIK, VNDTIQIDLETGK, ITDFIK, IGVITNR, HPGSFDVVHVK, DANGNSFATR, LSNIFVIGK, PWISLPR, LTIAEER
CGRP-RCP	NP_001035737	HQSPEIVR, EFLTALK, KNTNSNVAMDEEDPA, NTNSNVAMDEEDPA