Supplementary Figures

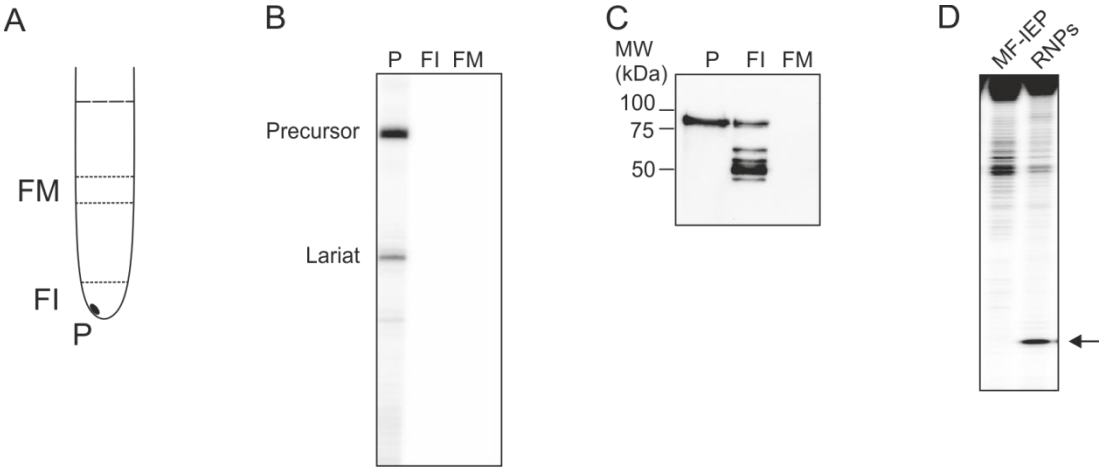

Figure S1. Detection of intron RNA and MF-IEP protein during RNP preparation. (A) Fractions taken along the tube during RNP preparations. FM (in the middle), FI (at the bottom) and P (pellet). (B) Primer extension to determine the presence of intron RNA in the different fractions during the RNP preparations. (C) Detection of MF-IEP protein in the fractions was carried out by western blot using antibodies anti-Flag. (D) Endonuclease assay of reconstituted RNPs. Cleavage of substrate was observed only in lane when RNPs were used (RNPs lane arrow) and not when the protein was used.

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RmInt1-IEP      MTSE------------STTDKPF-----------------RIEKRRVYEA 21
5HHL-Eu.re.I2   MDT------------SNLME------------------QILSSDNLNRA 19
5HHJ-Ro.in.IEP  MVKSSGTERKERMDTSSLME------------------QILSNDNLNRA 31
6AR1-Ge.st.IEP  M---------------ALLE------------------RILARDNLITA 16
5G2X-Ll.LtrA    MKPT-----------MAILERISKNSQENIDEVFTRLYRYLLRPDIYYVA 39
                *                :                     :      *

RmInt1-IEP      YKAVKANRGAAGVDGQTLEIFEKDLAANLYKIWNRMSSGTYFPPPVRAVS 71
5HHL-Eu.re.I2   YLQVVRNKGAEGVDGMKYTELKEHLAKNGETIKGQLRTRKYKPQPARRVE 69
5HHJ-Ro.in.IEP  YLQVVRNKGAEGVDGMKYTELKEYLAKNGEIIKEQLRIRKYKPQPVRRVE 81
6AR1-Ge.st.IEP  LKRVEANQGAPGIDGVSTDQLRDYIRAHWSTIHAQLLAGTYRPAPVRRVE 66
5G2X-Ll.LtrA    YQNLYSNKGAST-KGILDDTADGFSEEKIKKIIQSLKDGTYYPQPVRRMY 88
                   :  *:**   .*           :   *   :   .* * *.* :

RmInt1-IEP      IPKKAG-GERVLGVPTVSDRIAQMVVKQMIEPDLDSLFLPDSYGYRPGKS 120
5HHL-Eu.re.I2   IPKPDG-GVRNLGVPTVTDRFIQQAIAQVLTPIYEEQFHDHSYGFRPNRC 118
5HHJ-Ro.in.IEP  IPKPDG-GVRNLGVPTVTDRFIQQAIAQVLTPIYEEQFHDHSYGFRPNRC 130
6AR1-Ge.st.IEP  IPKPGG-GTRQLGIPTVVDRLIQQAILQELTPIFDPDFSSSSFGFRPGRN 115
5G2X-Ll.LtrA    IAKKNSKKMRPLGIPTFTDKLIQEAVRIILESIYEPVFEDVSHGFRPQRS 138
                *.*  .   * **:**. *:: * .:   : .  :  *   *.*:** :

RmInt1-IEP      ALDAVGVTRQR-CWKYDWVLEFDIKGLFDNLPHDLLLKAVRKDVKCNWAL 169
5HHL-Eu.re.I2   AQQAILTALNIMNDGNDWIVDIDLEKFFDTVNHDKLMTLIGRTIKDGDVI 168
5HHJ-Ro.in.IEP  AQQAILTALDMMNDGNDWIVDIDLEKFFDTVNHDKLMTIIGRTIKDGDVI 180
6AR1-Ge.st.IEP  AHDAVRQAQGYIQEGYRYVVDMDLEKFFDRVNHDILMSRVARKVKDKRVL 165
5G2X-Ll.LtrA    CHTALKTIKRE-FGGARWFVEGDIKGCFDNIDHVTLIGLINLKIKDMKMS 187
                .  *:           :.:: *::  ** : *  *:  :   :*

RmInt1-IEP      LYIERWLTAPMEKNGEVIERSRGTPQGGVVSPILANLFLHYAFDLWM--- 216
5HHL-Eu.re.I2   SIVRKYLVSGIMIDDEYEDSIVGTPQGGNLSPLLANIMLNE-LDKEM--- 214
5HHJ-Ro.in.IEP  SIVRKYLVSGIMIDDEYEDSIVGTPQGGNLSPLLANIMLNE-LDKEM--- 226
6AR1-Ge.st.IEP  KLIRAYLQAGVMIEGVKVQTEEGTPQGGPLSPLLANILLDD-LDKEL--- 211
5G2X-Ll.LtrA    QLIYKFLKAGYLENWQYHKTYSGTPQGGILSPLLANIYLHE-LDKFVLQL 236
                  :  :* :    :    .   ****** :**:***: *.  :*  :

RmInt1-IEP      -------------------------------------------------- 216
5HHL-Eu.re.I2   -------------------------------------------------- 214
5HHJ-Ro.in.IEP  -------------------------------------------------- 226
6AR1-Ge.st.IEP  -------------------------------------------------- 211
5G2X-Ll.LtrA    KMKFDRESPERITPEYRELHNEIKRISHRLKKLEGEEKAKVLLEYQEKRK 286


RmInt1-IEP      --------TRTHPDLPWCRYADDGLVHCQ-SEQQAEALRVELSSRLA-AC 256
5HHL-Eu.re.I2   ----------EKRGLNFVRYADDCIIMVG-SEMSANRVMRNISRFIEEKL 253
5HHJ-Ro.in.IEP  ----------EKRGLNFVRYADDCIIMVG-SEMSANRVMRNISRFIEEKL 265
6AR1-Ge.st.IEP  ----------EKRGLKFCRYADDCNIYVK-SLRAGQRVKQSIQRFLEKTL 250
5G2X-Ll.LtrA    RLPTLPCTSQTNKVLKYVRYADDFIISVKGSKEDCQWIKEQLKLFIHNKL 336
                          :  * : *****  :    *    : :  .:.  :

RmInt1-IEP      GLQMHPTKTKIVYCKDQRRREAYPNVTFDFLGYQFRPRRVANTQRDEFFC 306
5HHL-Eu.re.I2   GLKVNMTKSKVDR----------PS-GLKYLGFGFYFDPRAH-------- 284
5HHJ-Ro.in.IEP  GLKVNMTKSKVDR----------PR-GIKYLGFGFYYDTSAQ-------- 296
6AR1-Ge.st.IEP  KLKVNEEKSAVDR----------PW-KRAFLGFSFTPERKARIRLAPRS- 288
5G2X-Ll.LtrA    KMELSEEKTLITH----------SSQPARFLGYDIRVRRSGTIKRSGKVK 376
                 :::   *: :            .     :**: :     .
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RmInt1-IEP      GYTPAVSPTALKSMRATIKSLNIPRQT------------------PGTLA 338
5HHL-Eu.re.I2   -------------------------------------------------- 284
5HHJ-Ro.in.IEP  -------------------------------------------------- 296
6AR1-Ge.st.IEP  ----------IQRLKQRIRQLTNPNWS-------------------ISMP 309
5G2X-Ll.LtrA    KRTLNGSVELLIPLQDKIRQFIFDKKIAIQKKDSSWFPVHRKYLIRSTDL 426

RmInt1-IEP      EIAKQLNPLLRGWIAYYGRYS-------------RSALSTLADYVNQKLR 375
5HHL-Eu.re.I2   -------------------------------------------------- 284
5HHJ-Ro.in.IEP  -------------------------------------------------- 296
6AR1-Ge.st.IEP  ERIHRVNQYVMGWIGYFRLVET------------PSVLQTIEGWIRRRLR 347
5G2X-Ll.LtrA    EIITIYNSELRGICNYYGLASNFNQLNYFAYLMEYSCLKTIASKHKGTLS 476

RmInt1-IEP      A---------------------WIRRKFKRFQSHK--------------- 389
5HHL-Eu.re.I2   -------------------------------------------------- 284
5HHJ-Ro.in.IEP  -------------------------------------------------- 296
6AR1-Ge.st.IEP  LCQ-------------------WLQW--KRVRT----------------- 359
5G2X-Ll.LtrA    KTISMFKDGSGSWGIPYEIKQGKQRRYFANFSECKSPYQFTDEISQAPVL 526

RmInt1-IEP      -------------------------------------------------- 389
5HHL-Eu.re.I2   -------------------------------------------------- 284
5HHJ-Ro.in.IEP  -------------------------------------------------- 296
6AR1-Ge.st.IEP  --------------RIRELRALGLKETAVMEIANTRKGAWRTTKTPQLHQ 395
5G2X-Ll.LtrA    YGYARNTLENRLKAKCCELCGTSDENTS-YEI----------HHVNKVKN 565

RmInt1-IEP      --TRASLFLRKLARENPGLFV--HWKAFGTNT-FT 419
5HHL-Eu.re.I2   --------QFK------------------AKP-HA 292
5HHJ-Ro.in.IEP  --------QFK------------------AKPHAK 305
6AR1-Ge.st.IEP  ALGKTYWTAQGLKSLTQRYFEL-RQGHHHHHH-HH 428
5G2X-Ll.LtrA    LKGKEKWEMAMIAKQRKTLVVCFHCHRHVIHK-HK 599
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Figure S2. S**tructure-based sequence alignment of IEPs.** The sequence of RmInt1 IEP is aligned to the sequences of Eu.re.I2 (from PDB: 5HHL), Ro.in.IEP (from PDB: 5HHJ), Ge.st.IEP (from PDB: 6AR1) and LtrA (from PDB: 5G2X). The QMxxxxLxxLFL motif is highlighted in yellow

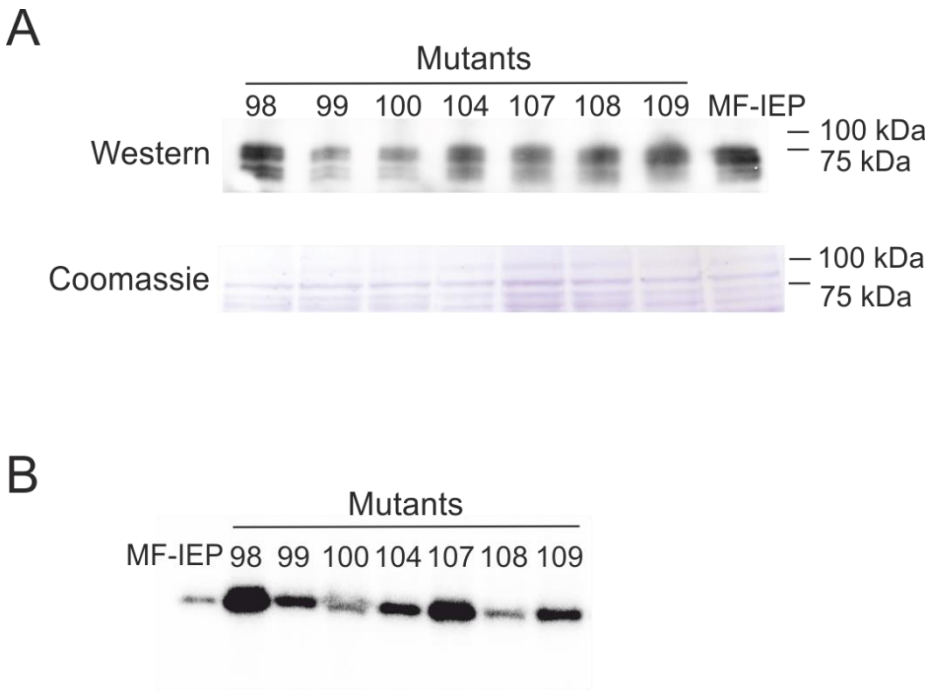

Figure S3. (A). Western analysis using anti-Flag antibodies of mutants done in MF-IEP. Protein loaded in each lane is shown in the panel labelled as Coomassie (B) Primer extension of mutants of MF-IEP protein.

Supplementary material and methods

Primer extension

15 µg RNA samples were annealed with 300,000 c.p.m. 5'-labeled P primer (5'-TGAAAGCCGATCCCGGAG-3') in 10 mM Pipes (pH 7.5) and 400 mM NaCl by heating at 85°C for 5 minutes and fast cooling to 60°C followed by slow chilling to 45°C. The cDNA was synthesized using AMV Reverse Transcriptase (Roche Diagnostics) in the presence of 50 mM Tris–HCl pH 8.0; 60 mM NaCl; 10 mM DTT; 6 mM MgOAc;1 mM dNTPs; 60 µg/ml actinomycin D (Sigma-Aldrich) and 0.4 U RNase Inhibitor (NEB) at 42°C for 60 minutes. The reactions were ethanol precipitated and the products were separated by denaturing 6% polyacrylamide gel electrophoresis

Endonuclease assays.

For DNA cleavage assays, 5' end-labeled gel-purified 70-mer single-stranded DNA oligonucleotide (300,000 cpm) was incubated with reconstituted RNP s(1.5 µg) or MF-IEP protein (1.5 µg) at 37°C for 2 h in reaction buffer [50 mM Tris-HCl pH 7.5, 10 mM KCl, 25 mM $MgCl_2$, 5 mM DTT]. The reactions were extracted with phenol-chloroform-isoamyl alcohol (25:24:1) followed by ethanol precipitation, and the products were separated by denaturing 7 M urea- 6% (w/v) polyacrylamide gel electrophoresis. Bands were revealed by dry gel exposition to digital imaging plates (Fuji), which were scanned using Quantity One software (Bio-Rad).