Supplementary Information for

The cryo-EM structure of the UPF-EJC complex shows UPF1 poised towards the RNA 3′ end

Roberto Melero, Gretel Buchwald, Raquel Castaño, Monika Raabe, David Gil, Melisa Lázaro, Henning Urlaub, Elena Conti and Oscar Llorca

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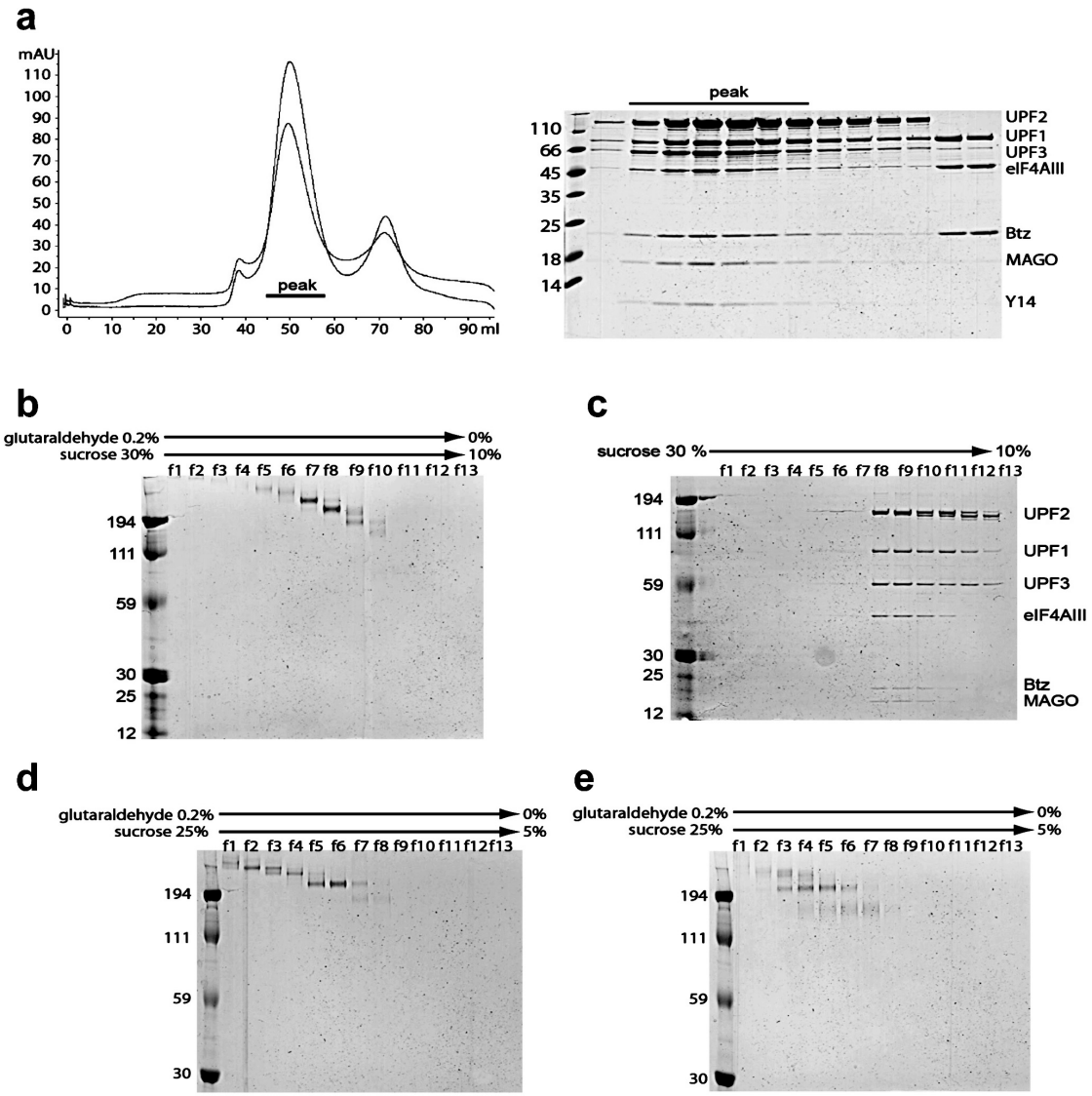
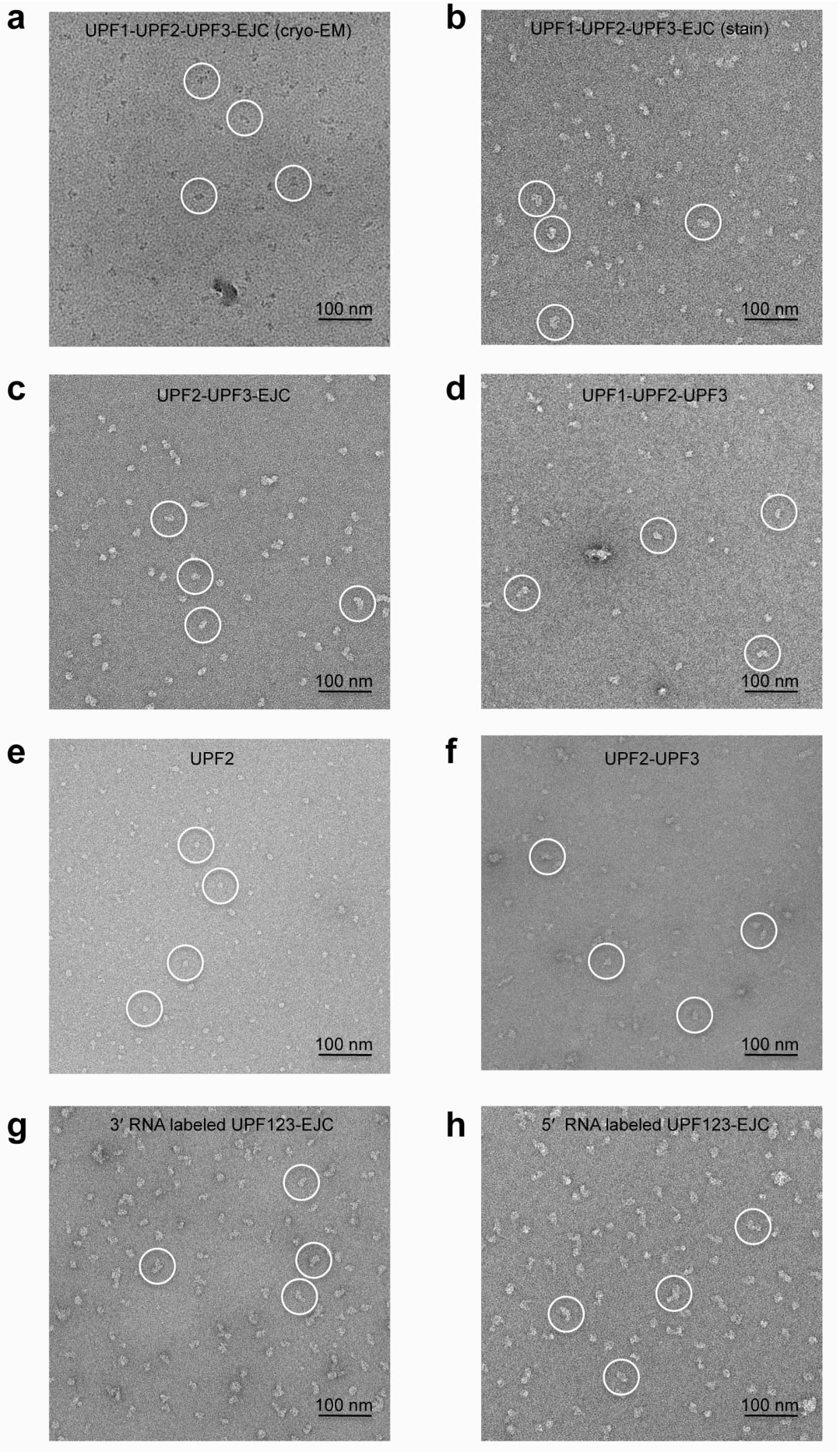
SUPPLEMENTARY FIGURES AND LEGENDS

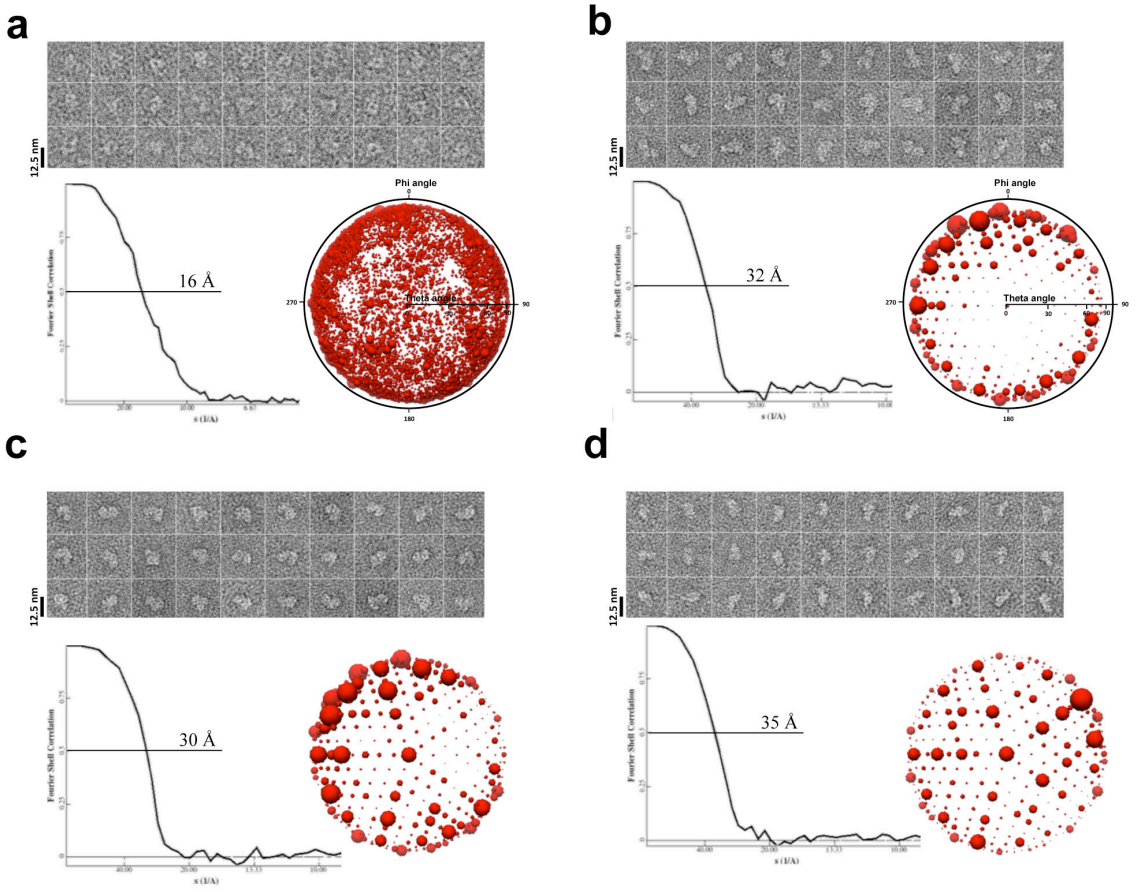


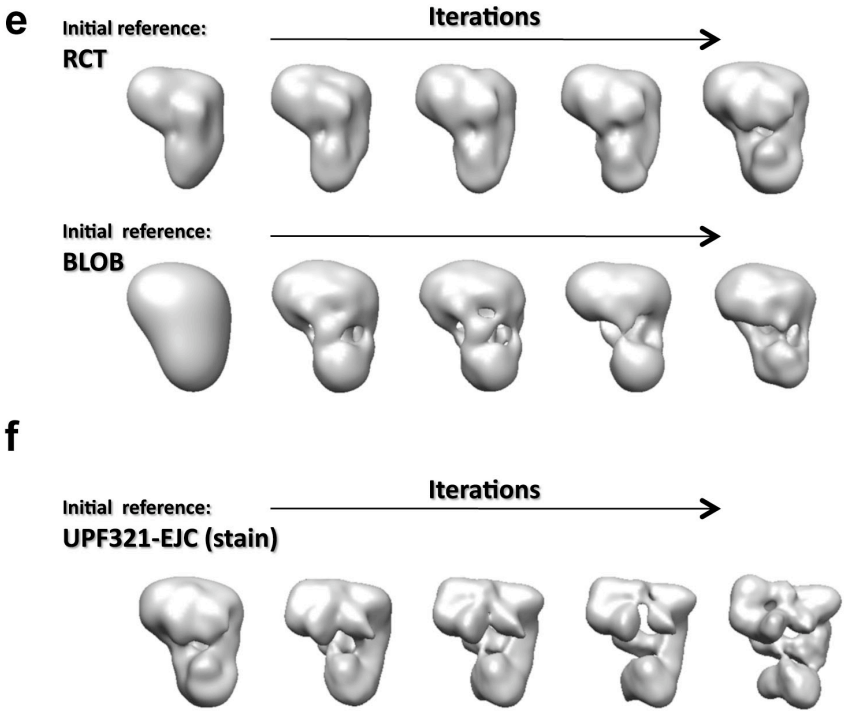
Supplementary Figure 1. Purification and crosslinking of the UPF-EJC complexes. (**a**) The left panel shows the chromatogram of the gel filtration using a Sepharose S200 (16/60) column with running buffer (40 mM Tris-HCl pH 7.5, 300 mM NaCl, 3 mM $MgCl_2$, 10 % glycerol, 2 mM DTT (dithiothreitol)). Peak I contains all protein components of the full complex, whereas the second peak has the excess of proteins used for complex formation. The two traces shown correspond to two wavelengths used during the experiment. Right panel: The peak fractions were separated on a 15 %

(wt/vol) acrylamide SDS-PAGE gel together with a protein marker (left lane), and stained with coomassie. The corresponding protein constructs were labeled on the right. (**b**) Fractions of the crosslinked UPF1-UPF2-UPF3-EJC complex were separated on a 4-12 % gradient gel (Nupage®, Invitrogen) and stained with coomassie. Fraction f7 was selected for EM analysis and mass spectrometry (see below). (**c**) Fractions from a control gradient that lacked glutaraldehyde were separated on a 4-12 % gradient gel (Nupage®, Invitrogen) together with the protein marker on the left lane. The UPF1-UPF2-UPF3-EJC complex appeared in the middle of the sucrose gradient in fraction f8 and f9. (**d**) Fractions of the crosslinked UPF2-UPF3-EJC complex were separated on a gradient gel using the same conditions. Fraction f6 was selected for EM analysis and mass spectrometry (see below). (**e**) Fractions of crosslinked UPF1-UPF2-UPF3b$_{RRM}$ were separated using the same conditions as mentioned above. Fraction f5 was selected for EM analysis as well as mass spectrometry (see below). In experiments “b” and “c”, small shifts in the fractions obtained in the presence or absence of cross-linker can occur due to small error during fractionation of the gradients. But in all cases the complex migrates roughly in the middle of the gradient.


a
UPF1-UPF2-UPF3-EJC (cryo-EM)
100 nm
b
UPF1-UPF2-UPF3-EJC (stain)
100 nm
c
UPF2-UPF3-EJC
100 nm
d
UPF1-UPF2-UPF3
100 nm
e
UPF2
100 nm
f
UPF2-UPF3
100 nm
g
3′ RNA labeled UPF123-EJC
100 nm
h
5′ RNA labeled UPF123-EJC
100 nm

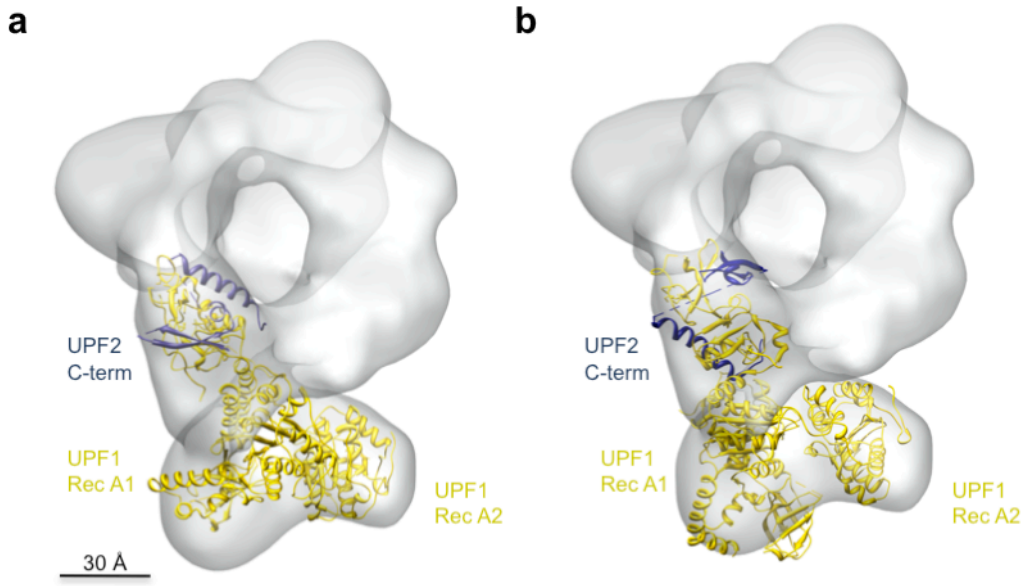
Supplementary Figure 2. Electron microscopy of UPF-EJC complexes. Representative Electron Microscopy fields for (**a**) UPF1-UPF2-UPF3-EJC (cryo-EM) (for clarity, we show a micrograph taken at defocus > 4 μm and with the protein density in black, to help visualizing the particles), (**b**) UPF1-UPF2-UPF3-EJC (negative staining), (**c**) UPF2-UPF3-EJC, (**d**) UPF1-UPF2-UPF3$_{RRM}$, (**e**) UPF2, (**f**) UPF2-UPF3, (**g**) 3′ RNA-labeled UPF1-UPF2-UPF3-EJC and (**h**) 5′ RNA-labeled UPF1-UPF2-UPF3-EJC. Some particles within the micrographs have been highlighted within circles. Scale bar represents 100 nm.


a
12.5 nm
16 Å
Phi angle
Theta angle
b
12.5 nm
32 Å
Phi angle
Theta angle
c
12.5 nm
30 Å
d
12.5 nm
35 Å


e
Initial reference:
RCT
Iterations
Initial reference:
BLOB
f
Initial reference:
UPF321-EJC (stain)
Iterations

Supplementary Figure 3. Image processing of UPF-EJC complexes. Image processing details for (**a**) UPF1-UPF2-UPF3-EJC (cryo-EM), (**b**) UPF1-UPF2-UPF3-EJC (negative staining), (**c**) UPF2-UPF3-EJC and (**d**) UPF1-UPF2-UPF3$_{RRM}$, including raw particles, Fourier Shell Correlation plots for estimation of the resolution and plots showing the angular distribution of the particles in the final step of refinement (Euler angles). Scale bar represents 12.5 nm.

Image processing was performed using several templates as initial reference for refinement, including a random conical tilt (RCT) structure. All these strategies converged to similar structures, validating the absence of significant bias from the reference used. (**e**) Refinement of UPF1-UPF2-UPF3-EJC (negative staining) was performed using an *ab initio* structure obtained by the RCT method (top left) or a featureless Gaussian blob constructed using EMAN (bottom left). (**f**) Refinement of the cryo-EM data for the UPF1-UPF2-UPF3-EJC complex was performed using the negative stain structure of the same complex as initial reference.



Supplementary Figure 4. Docking of UPF1 into the UPF-EJC complex. Docking of the crystal structure of UPF1 bound to the C-terminal domain of UPF2 (UPF1-UPF2$_{Cterm}$) into the "foot" of the UPF1-UPF2-UPF3-EJC complex was performed initially as a rigid body (**a**). The two modules of the helicase region in the crystal structure fitted within the two lobes of the cryo-EM density. The CH domain and UPF2 C_{term} fitted into the density of the ring-like structure.

The linker connecting the CH domain with the helicase region of UPF1 has been shown to be flexible. Thus, the fitting of the two CH and helicase regions in UPF1 was refined interpedently to improve the scores (cross-correlation coefficients) and fit most of the protein within the cryo-EM density. The result of these rearrangements is shown in (**b**).

Supplementary Figure 5

1. GraFix-treated UPF1-UPF2-UPF3(RRM)

UPF2 – f5 sample (see below)

```
   1                            MPAE RKKPASMEEK DSLPNNKEKD
  25 CSERRTVSSK ERPKDDIKLT AKKEVSKAPE DKKKRLEDDK RKKEDKERKK
  75 KDEEKVKAEE ESKKKEEEEK KKHQEEERKK QEEQAKRQQE EEAAAQMKEK
 125 EESIQLHQEA WERHHLRKEL RSKNQNAPDS RPEENFFSRL DSSLKKNTAF
 175 VKKLKTITEQ QRDSLSHDFN GLNLSKYIAE AVASIVEAKL KISDVNCAVH
 225 LCSLFHQRYA DFAPSLLQVW KKHFEARKEE KTPNITKLRT DLRFIAELTI
 275 VGIFTDKEGL SLIYEQLKNI INADRESHTH VSVVISFCRH CGDDIAGLVP
 325 RKVKSAAEKF NLSFPPSEII SPEKQQPFQN LLKEYFTSLT KHLKRDHREL
 375 QNTERQNRRI LHSKGELSED RHKQYEEFAM SYQKLLANSQ SLADLLDENM
 425 PDLPQDKPTP EEHGPGIDIF TPGKPGEYDL EGGIWEDEDA RNFYENLIDL
 475 KAFVPAILFK DNEKSCQNKE SNKDDTKEAK ESKENKEVSS PDDLELELEN
 525 LEINDDTLEL EGGDEAEDLT KKLLDEQEQE DEEASTGSHL KLIVDAFLQQ
 575 LPNCVNRDLI DKAAMDFCMN MNTKANRKKL VRALFIVPRQ RLDLLPFYAR
 625 LVATLHPCMS DVAEDLCSML RGDFRFHVRK KDQINIETKN KTVRFIGELT
 675 KFKMFTKNDT LHCLKMLLSD FSHHHIEMAC TLLETCGRFL FRSPESHLRT
 725 SVLLEQMMRK KQAMHLDARY VTMVENAYYY CNPPPAEKTV KKKRPPLQEY
 775 VRKLLYKDLS KVTTEKVLRQ MRKLPWQDQE VKDYVICCMI NIWNVKYNSI
 825 HCVANLLAGL VLYQEDVGIQ VVDGVLEDIR LGMEVNQPKF NQRRISSAKF
 875 LGELYNYRMV ESAVIFRTLY SFTSFGVNPD GSPSSLDPPE HLFRIRLVCT
 925 ILDTCGQYFD RGSSKRKLDC FLVYFQRYVW WKKSLEVWTK DHPFSIDIDY
 975 MISDTLELLR PKIKLCNSLE ESIRQVQDLE REFLIKLGLV NDKDSKDSMT
1025 EGENLEEDEE EEEGGAETEE QSGNESEVNE PEEEEGSDND DDEGEEEEEE
1075 NTDYLTDSNK ENETDEENTE VMIKGGGLKH VPCVEDEDFI QALDKMMLEN
1125 LQQRSGESVK VHQLDVAIPL HLKSQLRKGP PLGGGEGEAE SADTMPFVML
1175 TRKGNKQQFK ILNVPMSSQL AANHWNQQQA EQEERMRMKK LTLDINERQE
1225 QEDYQEMLQS LAQRPAPANT NRERRPRYQH PKGAPNADLI FKTGGRRR
```

UPF3(RRM) - f5 sample (see below)

```
   1 MKEEKEHRPK EKRVTLLTPA GATGSGGGTS GDSSKGEDKQ DRNKEKKEAL
  51 SKVVIRRLPP TLTKEQLQEH LQPMPEHDYF EFFSNDTSLY PHMYARAYIN
 101 FKNQEDIILF RDRFDGYVFL DNKGQEYPAI VEFAPFQKAA KKKTKKRDTK
 151 VGTIDDDPEY RKFLESYATD NEKMTSTPET LLEEIEAKNR ELIAKKTTPL
 201 LSFLKNKQRM REEKREERRR REIERKRQRE EERRKWKEEE KRKRKDIEKL
 251 KKIDRIPERD KLKDEPKIKL LKKPEKGDEK ELDKREKAKK LDKENLSDER
 301 ASGQSCTLPK RSDSELKDEK PKRPEDESGR DYREREREYE RDQERILRER
 351 ERLKRQEEER RRQKERYEKE KTFKRKEEEM KKEKDTLRDK GKKAESTESI
 401 GSSEKTEKKE EVVKRDRIRN KDRPAMQLYQ PGARSRNRLC PPDDSTKSGD
 451 SAAERKQESG ISHRKEGGEE
```

2. GraFix-treated UPF1-UPF2-UPF3-EJC samples

UPF2 - Sample f7 (see below)

```
   1                            MPAE RKKPASMEEK DSLPNNKEKD
  25 CSERRTVSSK ERPKDDIKLT AKKEVSKAPE DKKKRLEDDK RKKEDKERKK
  75 KDEEKVKAEE ESKKKEEEEK KKHQEEERKK QEEQAKRQQE EEAAAQMKEK
 125 EESIQLHQEA WERHHLRKEL RSKNQNAPDS RPEENFFSRL DSSLKKNTAF
 175 VKKLKTITEQ QRDSLSHDFN GLNLSKYIAE AVASIVEAKL KISDVNCAVH
 225 LCSLFHQRYA DFAPSLLQVW KKHFEARKEE KTPNITKLRT DLRFIAELTI
 275 VGIFTDKEGL SLIYEQLKNI INADRESHTH VSVVISFCRH CGDDIAGLVP
 325 RKVKSAAEKF NLSFPPSEII SPEKQQPFQN LLKEYFTSLT KHLKRDHREL
 375 QNTERQNRRI LHSKGELSED RHKQYEEFAM SYQKLLANSQ SLADLLDENM
 425 PDLPQDKPTP EEHGPGIDIF TPGKPGEYDL EGGIWEDEDA RNFYENLIDL
 475 KAFVPAILFK DNEKSCQNKE SNKDDTKEAK ESKENKEVSS PDDLELELEN
 525 LEINDDTLEL EGGDEAEDLT KKLLDEQEQE DEEASTGSHL KLIVDAFLQQ
 575 LPNCVNRDLI DKAAMDFCMN MNTKANRKKL VRALFIVPRQ RLDLLPFYAR
 625 LVATLHPCMS DVAEDLCSML RGDFRFHVRK KDQINIETKN KTVRFIGELT
 675 KFKMFTKNDT LHCLKMLLSD FSHHHIEMAC TLLETCGRFL FRSPESHLRT
 725 SVLLEQMMRK KQAMHLDARY VTMVENAYYY CNPPPAEKTV KKKRPPLQEY
 775 VRKLLYKDLS KVTTEKVLRQ MRKLPWQDQE VKDYVICCMI NIWNVKYNSI
 825 HCVANLLAGL VLYQEDVGIQ VVDGVLEDIR LGMEVNQPKF NQRRISSAKF
 875 LGELYNYRMV ESAVIFRTLY SFTSFGVNPD GSPSSLDPPE HLFRIRLVCT
 925 ILDTCGQYFD RGSSKRKLDC FLVYFQRYVW WKKSLEVWTK DHPFSIDIDY
 975 MISDTLELLR PKIKLCNSLE ESIRQVQDLE REFLIKLGLV NDKDSKDSMT
1025 EGENLEEDEE EEEGGAETEE QSGNESEVNE PEEEEGSDND DDEGEEEEEE
1075 NTDYLTDSNK ENETDEENTE VMIKGGGLKH VPCVEDEDFI QALDKMMLEN
1125 LQQRSGESVK VHQLDVAIPL HLKSQLRKGP PLGGGEGEAE SADTMPFVML
1175 TRKGNKQQFK ILNVPMSSQL AANHWNQQQA EQEERMRMKK LTLDINERQE
1225 QEDYQEMLQS LAQRPAPANT NRERRPRYQH PKGAPNADLI FKTGGRRR
```

UPF3(full length) - Sample f7 (see below)

```
   1 MKEEKEHRPK EKRVTLLTPA GATGSGGGTS GDSSKGEDKQ DRNKEKKEAL
  51 SKVVIRRLPP TLTKEQLQEH LQPMPEHDYF EFFSNDTSLY PHMYARAYIN
 101 FKNQEDIILF RDRFDGYVFL DNKGQEYPAI VEFAPFQKAA KKKTKKRDTK
 151 VGTIDDDPEY RKFLESYATD NEKMTSTPET LLEEIEAKNR ELIAKKTTPL
 201 LSFLKNKQRM REEKREERRR REIERKRQRE EERRKWKEEE KRKRKDIEKL
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```
251 KKIDRIPERD KLKDEPKIKL LKKPEKGDEK ELDKREKAKK LDKENLSDER
301 ASGQSCTLPK RSDSELKDEK PKRPEDESGR DYRERE REYE RDQERILRER
351 ERLKRQEEER RRQKERYEKE KTFKRKEEEM KKEKDTLRDK GKKAESTESI
401 GSSEKTEKKE EVVKRDRIRN KDRPAMQLYQ PGARSRNRLC PPDDSTKSGD
451 SAAERKQESG ISHRKEGGEE
```

3. GraFix-treated UPF2-UPF3-EJC samples

UPF2 - Sample f6 (see below)

```
  1                          MPAE RKKPASMEEK DSLPNNKEKD
 25 CSERRTVSSK ERPKDDIKLT AKKEVSKAPE DKKKRLEDDK RKKEDKERKK
 75 KDEEKVKAEE ESKKKEEEEK KKHQEEERKK QEEQAKRQQE EEAAAQMKEK
125 EESIQLHQEA WERHHLRKEL RSKNQNAPDS RPEENFFSRL DSSLKKNTAF
175 VKKLKTITEQ QRDSLSHDFN GLNLSKYIAE AVASIVEAKL KISDVNCAVH
225 LCSLFHQRYA DFAPSLLQVW KKHFEARKEE KTPNITKLRT DLRFIAELTI
275 VGIFTDKEGL SLIYEQLKNI INADRESHTH VSVVISFCRH CGDDIAGLVP
325 RKVKSAAEKF NLSFPPSEII SPEKQQPFQN LLKEYFTSLT KHLKRDHREL
375 QNTERQNRRI LHSKGELSED RHKQYEEFAM SYQKLLANSQ SLADLLDENM
425 PDLPQDKPTP EEHGPGIDIF TPGKPGEYDL EGGIWEDEDA RNFYENLIDL
475 KAFVPAILFK DNEKSCQNKE SNKDDTKEAK ESKENKEVSS PDDLELELEN
525 LEINDDTLEL EGGDEAEDLT KKLLDEQEQE DEEASTGSHL KLIVDAFLQQ
575 LPNCVNRDLI DKAAMDFCMN MNTKANRKKL VRALFIVPRQ RLDLLPFYAR
625 LVATLHPCMS DVAEDLCSML RGDFRFHVRK KDQINIETKN KTVRFIGELT
675 KFKMFTKNDT LHCLKMLLSD FSHHHIEMAC TLLETCGRFL FRSPESHLRT
725 SVLLEQMMRK KQAMHLDARY VTMVENAYYY CNPPPAEKTV KKKRPPLQEY
775 VRKLLYKDLS KVTTEKVLRQ MRKLPWQDQE VKDYVICCMI NIWNVKYNSI
825 HCVANLLAGL VLYQEDVGIQ VVDGVLEDIR LGMEVNQPKF NQRRISSAKF
875 LGELYNYRMV ESAVIFRTLY SFTSFGVNPD GSPSSLDPPE HLFRIRLVCT
925 ILDTCGQYFD RGSSKRKLDC FLVYFQRYVW WKKSLEVWTK DHPFSIDIDY
975 MISDTLELLR PKIKLCNSLE ESIRQVQDLE REFLIKLGLV NDKDSKDSMT
1025 EGENLEEDEE EEEGGAETEE QSGNESEVNE PEEEEGSDND DDEGEEEEEE
1075 NTDYLTDSNK ENETDEENTE VMIKGGGLKH VPCVEDEDFI QALDKMMLEN
1125 LQQRSGESVK VHQLDVAIPL HLKSQLRKGP PLGGGEGEAE SADTMPFVML
1175 TRKGNKQQFK ILNVPMSSQL AANHWNQQQA EQEERMRMKK LTLDINERQE
1225 QEDYQEMLQS LAQRPAPANT NRERRPRYQH PKGAPNADLI FKTGGRRR
```

UPF3(full length) - Sample f6 (see below)

```
  1 MKEEKEHRPK EKRVTLLTPA GATGSGGGTS GDSSKGEDKQ DRNKEKKEAL
 51 SKVVIRRLPP TLTKEQLQEH LQPMPEHDYF EFFSNDTSLY PHMYARAYIN
101 FKNQEDIILF RDRFDGYVFL DNKGQEYPAI VEFAPFQKAA KKKTKKRDTK
151 VGTIDDDPEY RKFLESYATD NEKMTSTPET LLEEIEAKNR ELIAKKTTPL
201 LSFLKNKQRM REEKREERRR REIERKRQRE EERRKWKEEE KRKRKDIEKL
251 KKIDRIPERD KLKDEPKIKL LKKPEKGDEK ELDKREKAKK LDKENLSDER
301 ASGQSCTLPK RSDSELKDEK PKRPEDESGR DYRERE REYE RDQERILRER
351 ERLKRQEEER RRQKERYEKE KTFKRKEEEM KKEKDTLRDK GKKAESTESI
401 GSSEKTEKKE EVVKRDRIRN KDRPAMQLYQ PGARSRNRLC PPDDSTKSGD
451 SAAERKQESG ISHRKEGGEE
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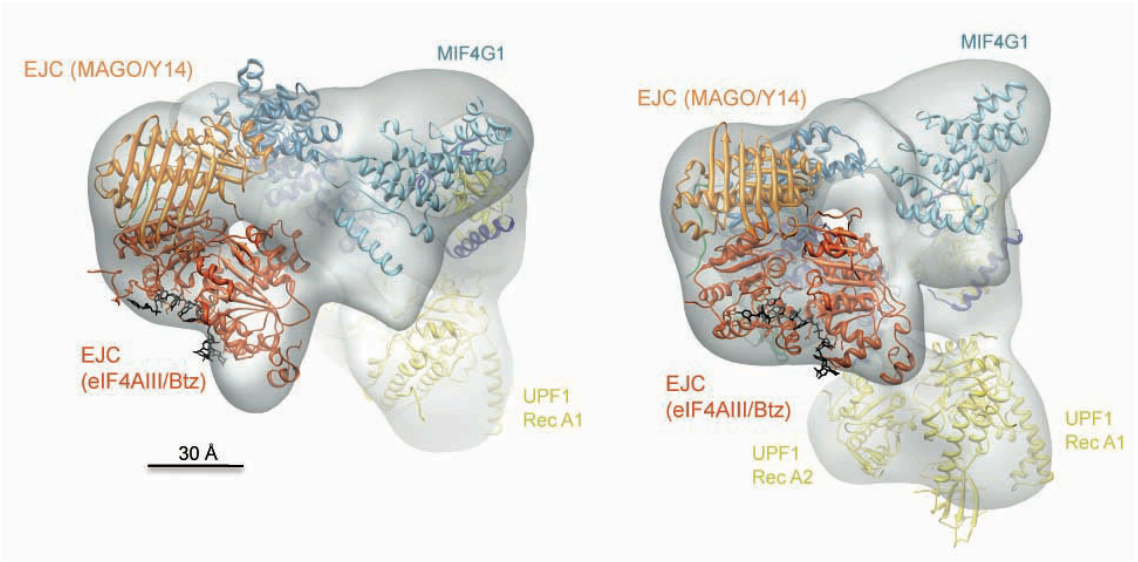
Supplementary Figure 5. MS-based detection of peptides derived from GraFix-treated samples. Amino acid sequences of proteins UPF2 and UPF3 as identified by mass spectrometry from GraFix-treated complexes after digestion of the complexes with endoproteinase trypsin. Samples analyzed are exactly the same used for all electron microscopy studies: fractions f5 for UPF1-UPF2-UPF3b$_{RRM}$, f6 for UPF2-UPF3-EJC and f7 for UPF1-UPF2-UPF3-EJC (**Supplementary Figure 1**).

Amino acid stretches highlighted in bold red are those that have been identified in database search and therefore were not modified by glutaraldehyde. Of note, glutaraldehyde modifies irreversibly lysine-residues. As trypsin cleaves C-terminal to lysine (and arginine residues) the actual cleavage site (or missed cleavages sites within

an identified peptide) defines the non-modified peptide. The complete list of peptides detected includes those that are buried because they are part of core of the proteins as well as those buried by protein-protein interactions. The comparison between the peptides detected for the three samples reveals those corresponding to regions protected due to protein-protein interactions within the UPF-EJC complex. Only these are shown in Figure 5 in the main text.



Supplementary Figure 6. Docking of EJC into the UPF-EJC complex. Two detailed views of the pseudo-atomic model for the UPF-EJC complex to highlight the fitting of the atomic structure of the EJC into the "head" of the cryo-EM map. Color codes are as in Figure 3.

Supplementary Note for

The cryo-EM structure of the UPF-EJC complex shows UPF1 poised towards the RNA 3′ end

Roberto Melero, Gretel Buchwald, Raquel Castaño, Monika Raabe, David Gil, Melisa Lázaro, Henning Urlaub, Elena Conti and Oscar Llorca

Nano-flow liquid chromatography (nanoLC)-MS/MS

Endoproteolytic and nuclease digestion of complexes

Analyses of GraFix treated and non-treated samples were basically carried out as describe in Richter et al, 2010. The peak gradient fractions were diluted to a final volume of 30 μl and thereby adjusted to 4 M urea and 50 mM Tris-HCl pH 7.5. Then, samples were vigorously shaken at room temperature for 5 min. The samples were subsequently diluted with 50 mM Tris-HCl pH 7.5 to yield a urea concentration of 1 M urea in a sample volume of 120 μl (unless otherwise stated, see below). In case of RNA containing complexes (EJC-containing complexes), RNA was digested for 2 h at 52°C by adding 1 μg of RNase A and RNase T1 (Ambion). Samples were chilled briefly on ice, and proteins were digested with 1 μg of trypsin at 37°C overnight. Digestion was stopped by addition of TFA to a concentration of 0.1% (v/v). Samples were stored at –20°C until MS analysis. GraFix treated samples were processed likewise, but were additionally incubated with glycine (final concentration 0.1 M) for 30 min at room temperature prior to denaturation in the presence of urea.

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Off-line nano-liquid chromatography (LC) analysis – Samples were injected into a nano LC system (Dual Gradient, Dionex, Idstein, Germany) in 6 loading cycles with 20 μl per cycle (for a maximum of 120 μl sample volume) unless otherwise stated in the Supplementary Materials and Methods. The nanoLC system was equipped with pre-columns (i.d. 150 μm, length 20 mm, Dr. Maisch C18 5 μm AQ, 120 Å pore size) working in in-line mode with the analytical columns (i.d. 75 μm, length 150 mm, Dr. Maisch C18 5 μm AQ, 120 Å) Peptides were separated on the analytical column by a standard gradient from 10 % solvent B to 60 % solvent B over 3 h (solvent A: 0.1% (v/v) TFA in water; solvent B: 80 % (v/v) ACN, 0.1 % (v/v) TFA in water) at a flow rate of 300 nl/min. The eluted peptides were mixed in a T-piece (MicroTEE, 0.006" void volume, Upchurch Scientific Inc., Oak Harbor, USA) with 10 mg/ml α-cyano-4-hydroxycinnamic acid matrix (HCCA) containing 10 fmol/μl Glu-fibrinogen as internal standard in 70 % (v/v) ACN, 0.1 % (v/v) TFA delivered at a flow rate of 0.9 μl/min. Eluate mixed with matrix was spotted onto a stainless steel MALDI target (Opti-TOF^{TM} LC/MALDI insert; ABSciex) by a Probot Spotter (Dionex, Idstein, Germany) every 15 seconds. Per gradient, approximately 600 peptide-containing fractions were collected.

Sample concentration and amount analyzed:

Non-Grafix-treated samples:

a) UPF1-UPF2-UPF3(RRM) 6.3 μg in 120 μl sample volume (c_{end} urea = 1M) – injected onto column 120 μl
b) UPF1-UPF2-UPF3-EJC 8.2 μg in 120 μl sample volume (c_{end} urea = 1M)- injected onto column 30 μl
c) UPF2-UPF3-EJC 7.9 μg in 120 μl sample volume (c_{end} urea = 1M)- injected onto column 30 μl

Grafix-treated samples

a) UPF1-UPF2-UPF3(RRM), sample f5 (3.8 μg in 120 μl sample volume (c_{end} urea = 1M)) – injected onto column 60 μl .
b) UPF1-UPF2-UPF3-EJC sample f7 (1.8 μg in 120 μl sample volume (c_{end} urea = 1M)), – injected onto column 120 μl.
c) UPF2-UPF3-EJC sample f6 (4.4 μg in 120 μl sample volume (c_{end} urea = 1M)) – injected onto column 60 μl.

MALDI MS and MSMS analysis – Peptide-mixtures eluting from the chromatography were sequenced on a MALDI-ToF/ToF 4800 analyzer (ABSciex) equipped with a Nd:YAG laser (355 nm wavelength and 200 Hz repetition rate). A maximum number of 1000 shots in the positive-ion mode were summed up for each MS spectra and a maximum of 5000 shots were accumulated for each precursor in the MSMS mode; acquisition was terminated depending on the spectral quality being specified within dynamic stop criteria. Upon job-wide interpretation of the MS data, the 15 highest-intensity peptides of every spot were sequenced without repetition of the highest ranking peptide precursors masses within 10 min. The collision energy in MSMS mode was set to 1×10^{-6} torr, with the potential difference between the source II accelerator and the collision cell set to 1 kV.

Protein identification – Peak lists were created using software version TS2 Mascot 1.0.0 (MALDI 4800). Protein identification was performed using a MASCOT v2.2.06 in-house server by searching fragment spectra against the NCBInr 240211 (downloaded 240211) with 13.135.398 protein entries in the database actually searched. The following parameters were used: taxonomy human, enzyme: trypsin, two missed

cleavages allowed, oxidation (M) and carbamylation (K and N-termini) as variable modifications, no fixed modifications; MS mass tolerance was set to 50 ppm and MSMS mass tolerance to 600 mmu. After the evaluation by MASCOT only "bold red" peptides with a peptide score ≥ 20 were considered for further analyses.