LEGENDS TO SUPPLEMENTARY FIGURES

Supplemental Figure1. Purification and identification of a factor from RRL RSW which binds Met-tRNA$_i^{Met}$ to the complex 40S x HCV IRES in the absence of GTP. (A) SDS-PAGE of fractions from MonoQ fractionation of the material eluted from PC between 250 and 350 mM KCl (see the scheme of purification in Fig. 2A). Lane M – protein molecular weight markers; F/t – flow-through fraction (material not adsorbed at the starting concentration of KCl, 25 mM). Maximum activity was detected in fraction 17 (see Fig. S1C); (B) Western blot of indicated MonoQ fractions with antibodies against factor eIF2A; (C) Determination of Met-tRNA$_i^{Met}$ binding activity in the indicated MonoQ fractions by toe-printing. "S" denotes the RSW fraction before fractionation on MonoQ (see the text and Fig. 1A); (D) Further purification of eIF2A-containing fraction (F/t) from the MonoQ column (Fig. S1A) on MonoS. Designations are as in (A); (E) Western blots of fractions presented in Fig. S1D with eIF2A antibodies.

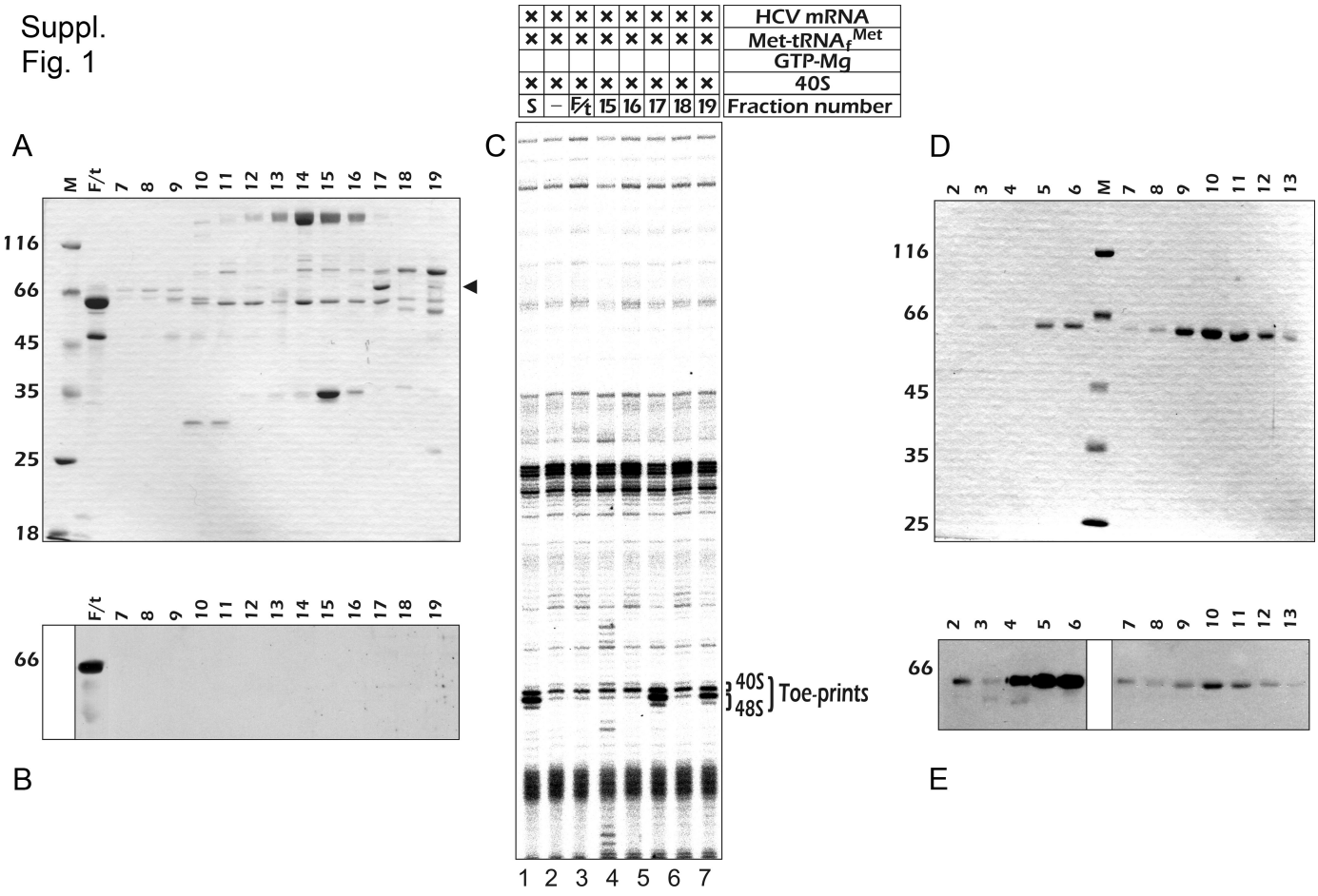
Supplemental Figure 2. eIF2A does not possess any activity in Met-tRNA$_i^{Met}$ binding to the 40S x HCV IRES complex. (A) SDS-PAGE of the two forms of eIF2A sequentially eluted from MonoS and purified to homogenity. eIF2A-1 and eIF2A-2 reveal similar mass spectra. (B) Determination of Met-tRNA$_i^{Met}$ binding activity in the indicated MonoQ fractions by toe-printing.

Supplemental Figure 3. Reconstitution of 48S complexes on the beta-globin mRNA using either the eIF2A containing RSW fraction or eIF2 (control). For details, see the legend to Fig. 1.

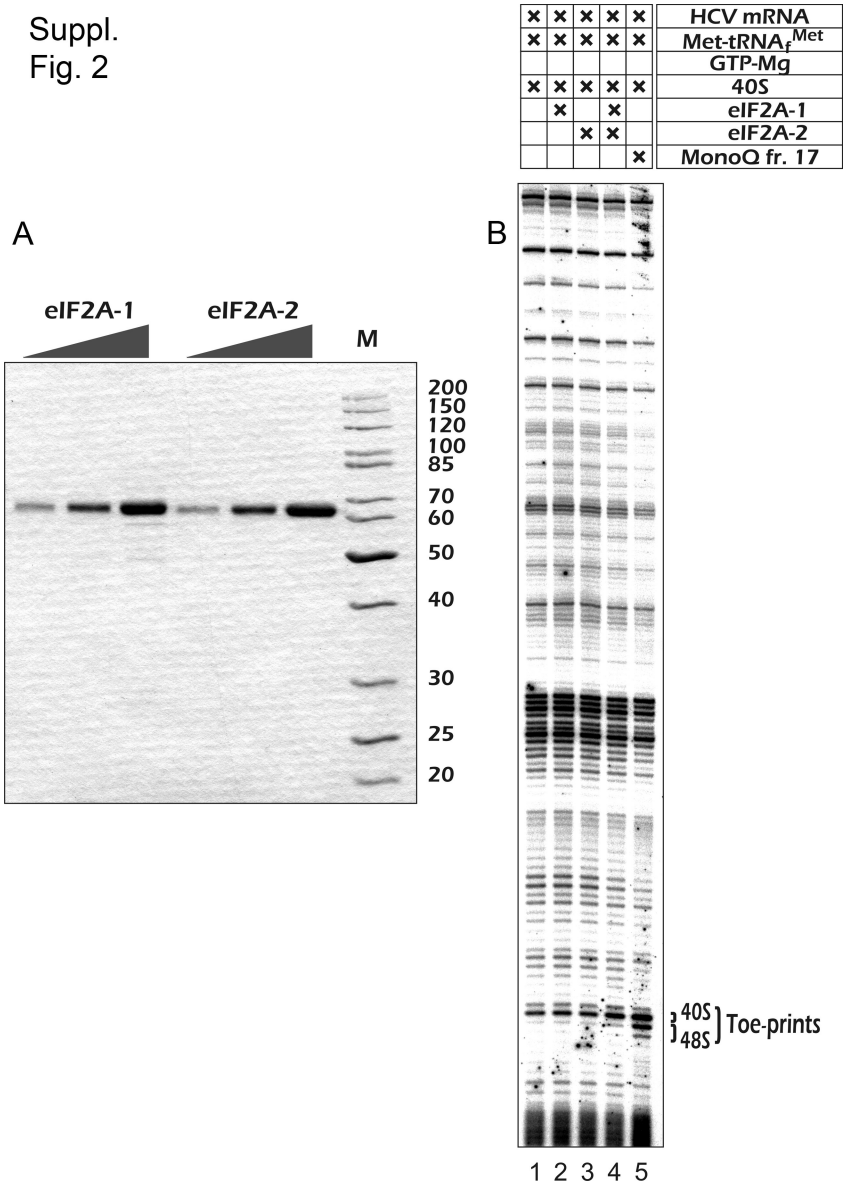
Supplemental Figure 4. Transiently expressed eIF2D colocalizes with eIF3 in the cytoplasm of HeLa cells. Cells were fixed 24 hours after transfection with pcDNA3.1-eIF2D and stained with anti-eIF3a (top panel) and anti-eIF2D (middle panel) antibodies. At the bottom panel, a merge view is shown with eIF2D colored in green, endogenous eIF3a in red and DAPI stained nuclei in blue. Bar: 10 μm. Note that only one cell in the frame seems to be transfected and expresses high level of eIF2D, while the others show only a background staining for a low amount of the endogenous protein.

Supplemental Figure 5. Overexpressed eIF2D partially co-migrates with ribosomes during centrifugation in sucrose gradient. (A) Polysomal profile of cell lysates containing overexpressed eIF2D. (B) Western blot analysis of gradient fractions using antibody to eIF2D or 40S ribosomal protein RPSA. Ps, polysome fraction.

Suppl.
Fig. 1


A
M
F/t
7
8
9
10
11
12
13
14
15
16
17
18
19
116
66
45
35
25
18
B
66
C
HCV mRNA
Met-tRNA$_f^{Met}$
GTP-Mg
40S
S
–
F/t
15
16
17
18
19
Fraction number
40S
48S
Toe-prints
1 2 3 4 5 6 7
D
2
3
4
5
6
M
7
8
9
10
11
12
13
116
66
45
35
25
E
66

Suppl.
Fig. 2


HCV mRNA
Met-tRNA$_f^{Met}$
GTP-Mg
40S
eIF2A-1
eIF2A-2
MonoQ fr. 17
A
B
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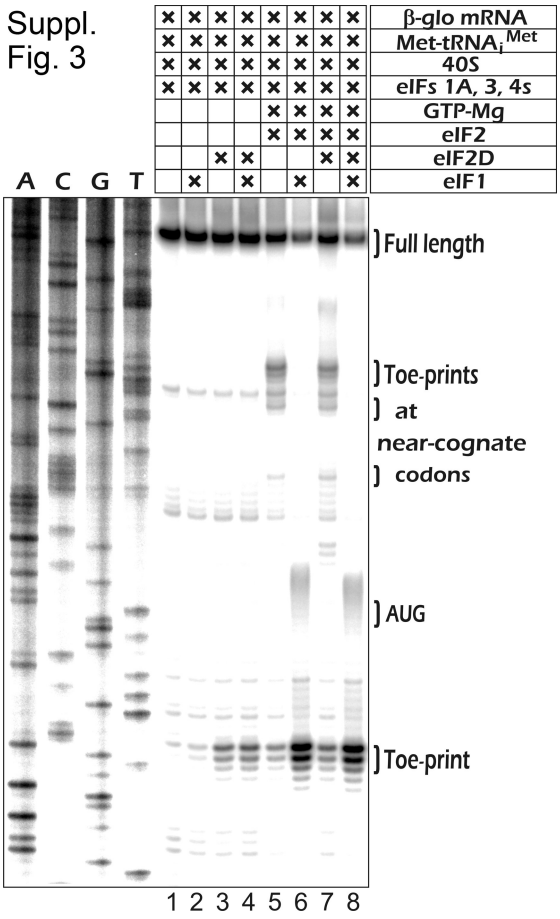
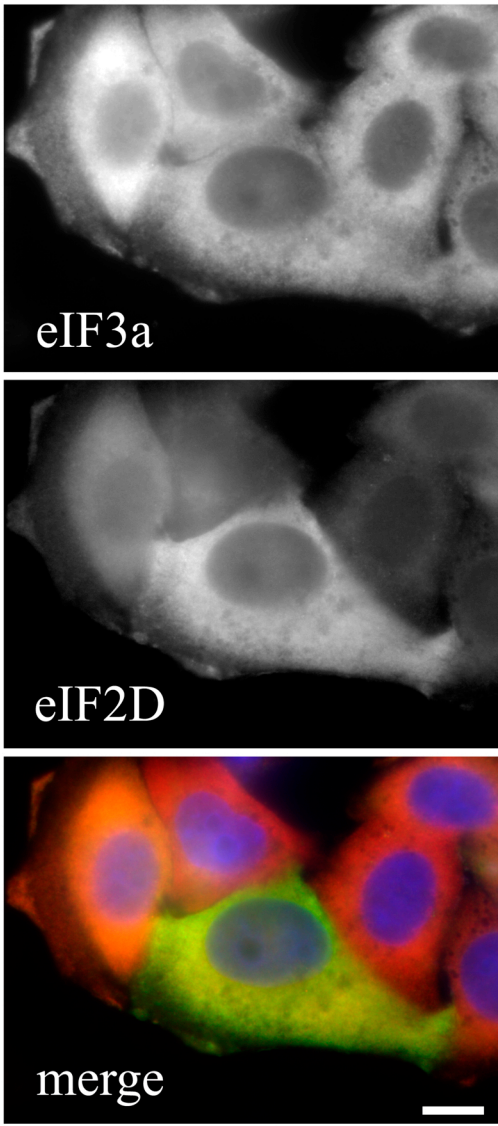
eIF2A-2
M
200
150
120
100
85
70
60
50
40
30
25
20
40S
48S
Toe-prints
1 2 3 4 5

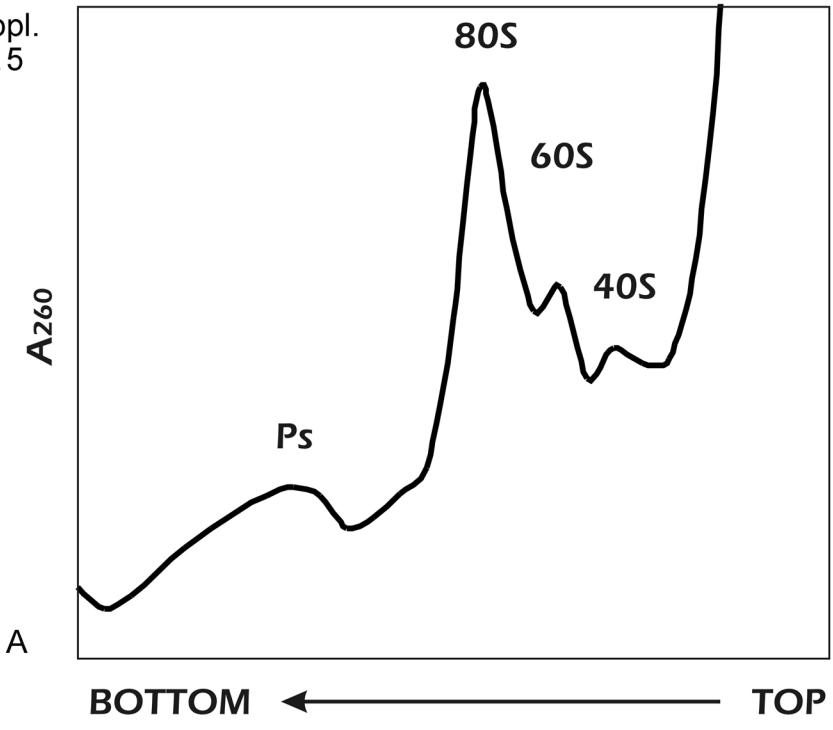
Suppl.
Fig. 3

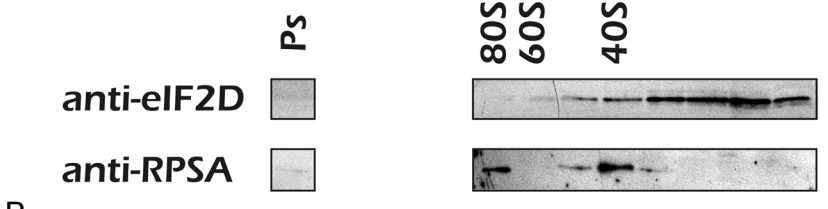

β-glo mRNA
Met-tRNA$_i^{Met}$
40S
eIFs 1A, 3, 4s
GTP-Mg
eIF2
eIF2D
eIF1
A C G T
Full length
Toe-prints
at
near-cognate
codons
AUG
Toe-print
1 2 3 4 5 6 7 8

Suppl.
Fig.4



Suppl. Fig. 5


80S
60S
40S
Ps
A260
A
BOTTOM
TOP


Ps
80S
60S
40S
anti-eIF2D
anti-RPSA
B