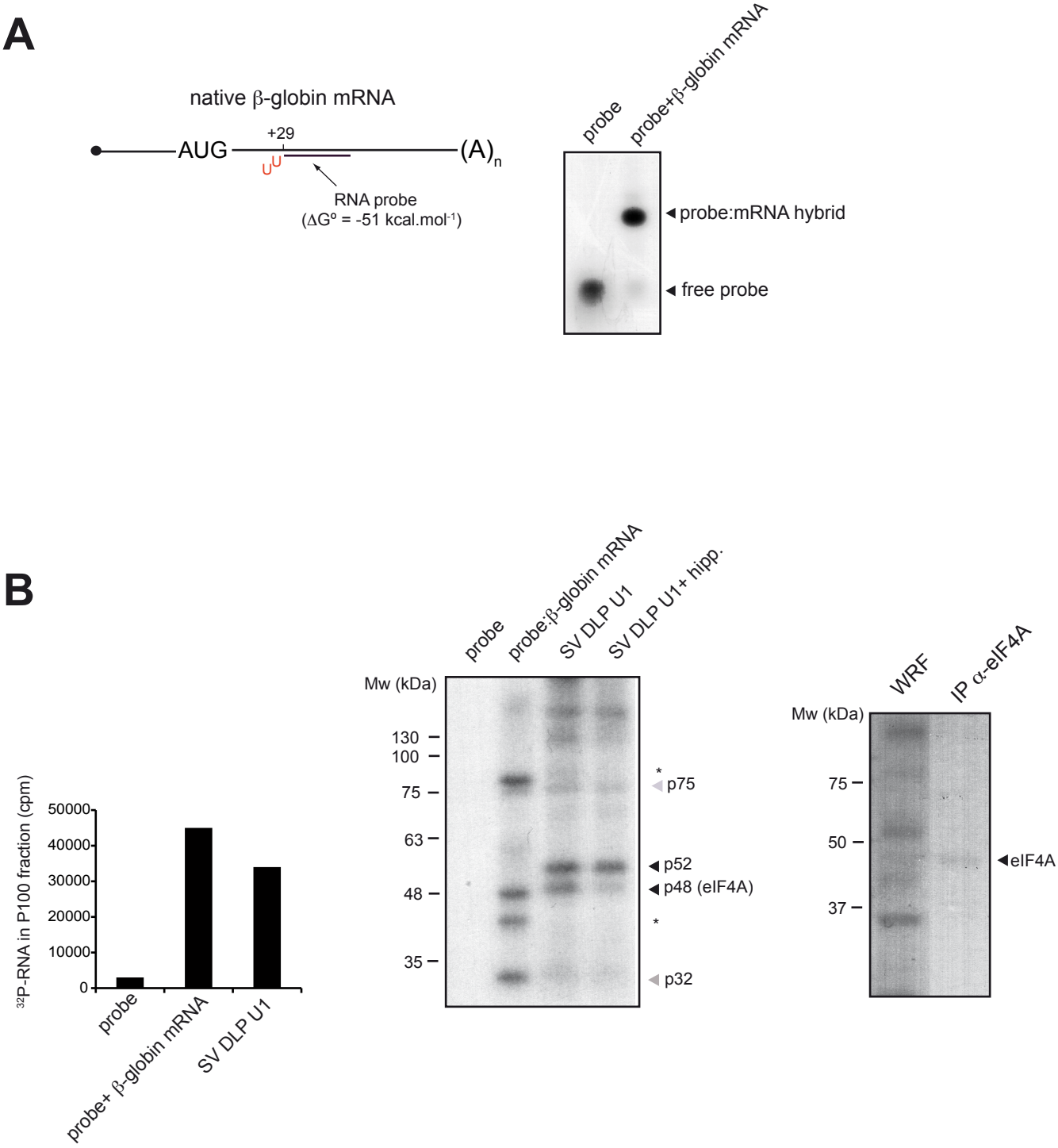
Supplementary Figures

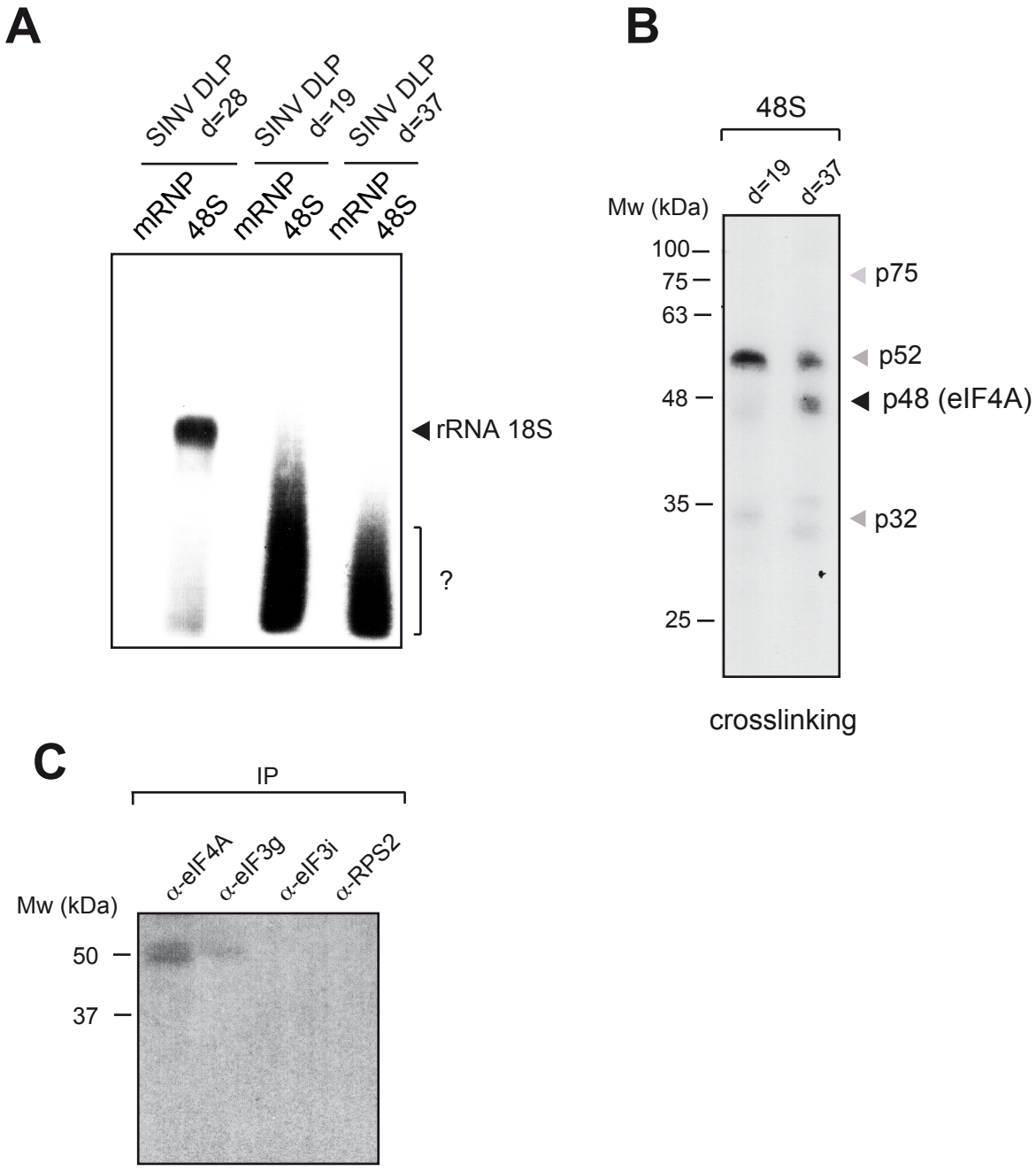
Supplementary Figure 1. eIF4A within the 48S complex can be crosslinked to positions 27-28 of β-globin mRNA. **(A)** Schematic diagram showing the strategy, placing 4-thio-U on nt 27-28 of native β-globin by creating a dsRNA stretch with position and stability equivalent to the SV DLP. The RNA oligo bearing 2 residues (red) at the 3´-end was labeled with [α–^{32}P]GTP and annealed to rabbit β-globin mRNA, purified through oligo(dT) beads and analyzed in a native 1.5% agarose gel (right panel). **(B)** Equivalent amounts of RNA (10^5 cpm) were used to assemble 48S complex in RRL followed by UV crosslinking. The radioactivity found in the ribosome pellet (P100 fraction) of each sample is shown (left panel), along with proteins crosslinked to the indicated RNAs (central panel). One of the samples assembled with SV DLP U1 RNA was treated with 25 μM of hippuristanol (+hipp). Protein bands (p80 and p42) found exclusively in samples with probe:β-globin mRNA are marked with *. A denaturing immunoprecipitation using anti-eIF4A antibodies was performed as described inmaterials and methods (right panel).

Supplementary Figure 2. Crosslinking assays of DLP d=19 and d=37 mRNAs within the 48S complex. **(A)** Analysis of RNA-RNA crosslinking of the indicated mRNAs with 18S rRNA in the 48S complex and postribosomal fractions (mRNP). After crosslinking and ultracentrifugation, the P100 and mRNP fractions were digested with proteinase K, phenolized, ethanol precipitated, and analyzed by denaturing agarose electrophoresis and blotting to nitrocellulose as described recently. The band corresponding to 18S rRNA was identified after staining the blots with methylene blue. In some experiments as shown here, SV DLP d =19 and d= 37 mRNAs generated crosslinking products of unknown origin with lower molecular weights. **(B)** A comparasion of crosslinking patterns generated by d=19 and d=37 mRNAs. The gels were intentionally run longer to better separate eIF4A from the upper band. Note that d=19 mRNA gave no detectable eIF4A crosslinking. **(C)** Evidence that eIF3g also crosslinks to SV-DLP mRNA in 48S complex. Denaturing immunoprecipitation was carried out as described in Materials and Methods with the indicated antibodies.

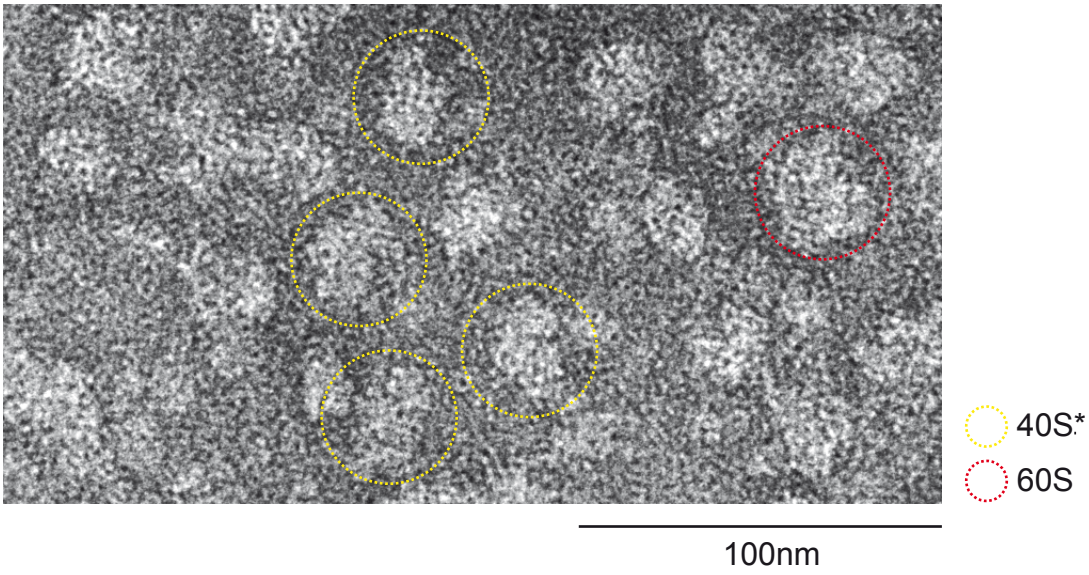
Supplementary Figure 3. (A) A general view of the 48S preparation analyzed in Fig. 6 before nanogold labelling. Some 40S-containing particles are encircled in yellow,

whereas the orange circle shows a 60S subunit. The sample was negatively stained before EM examination. **(B)** Comparation of the number of nanogold particles detected in and out of 40S containing particles in control sample (-) and in a sample that was preincubated with α-eIF4A antibody. Following the 17 nm rule, a nanogold particle located ≤17 nm from the border of 40S particle was scored as in. Neither nanogold particles were detected inside the 40S particle in the absence of eIF4A antibody, nor labelling of 60S particles was detected in any of samples analyzed. **(C)** Annealing of 5´biotinylated RNA oligo to SV-ΔDLP capsid mRNA restored its translation in RRL. The stability of RNA:RNA hybrid compared to DLP stem is shown. *In vitro* translation was programmed with the indicated mRNAs as described in materials and methods and Figure 4B.

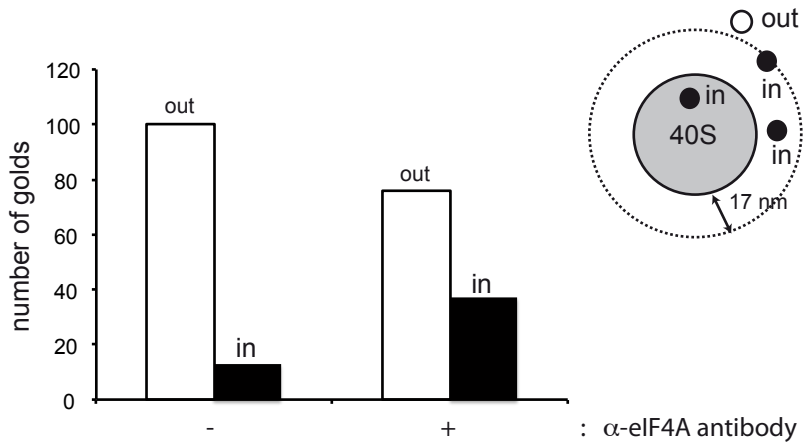

A
native β-globin mRNA
AUG
+29
(A)n
UU
RNA probe
(ΔG° = -51 kcal.mol-1)
probe
probe+β-globin mRNA
probe:mRNA hybrid
free probe
B
32P-RNA in P100 fraction (cpm)
50000
40000
30000
20000
10000
0
probe
probe+ β-globin mRNA
SV DLP U1
probe
probe:β-globin mRNA
SV DLP U1
SV DLP U1+ hipp.
Mw (kDa)
130
100
75
63
48
35
p75
p52
p48 (eIF4A)
p32
WRF
IP α-eIF4A
Mw (kDa)
75
50
37
eIF4A


A
SINV DLP d=28
SINV DLP d=19
SINV DLP d=37
mRNP
48S
mRNP
48S
mRNP
48S
rRNA 18S
?
B
48S
d=19
d=37
Mw (kDa)
100
75
63
48
35
25
p75
p52
p48 (eIF4A)
p32
crosslinking
C
IP
α-eIF4A
α-eIF3g
α-eIF3i
α-RPS2
Mw (kDa)
50
37

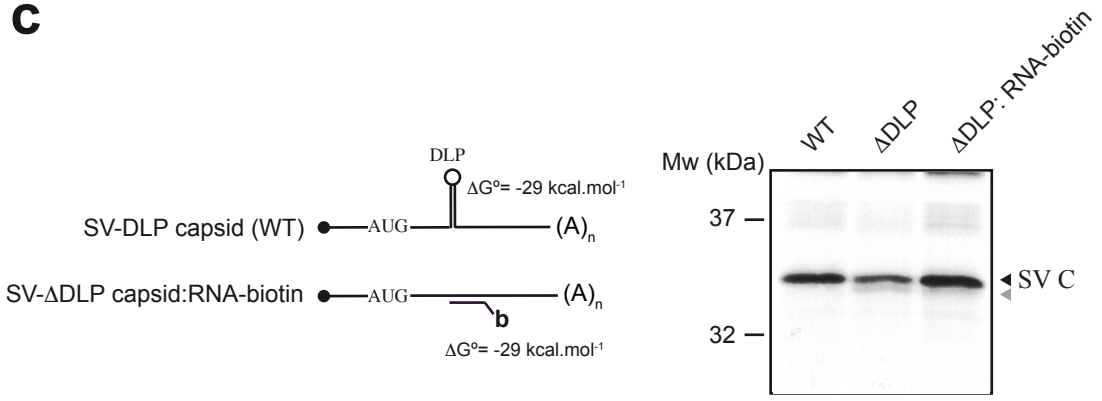
A


40S*
60S
100nm

B


number of golds
120
100
80
60
40
20
0
out
in
out
in
-
+
: α-eIF4A antibody
out
in
in
40S
in
17 nm

C


DLP
ΔG°= -29 kcal.mol-1
SV-DLP capsid (WT)
AUG
(A)n
SV-ΔDLP capsid:RNA-biotin
AUG
(A)n
b
ΔG°= -29 kcal.mol-1
Mw (kDa)
WT
ΔDLP
ΔDLP: RNA-biotin
37
32
SV C