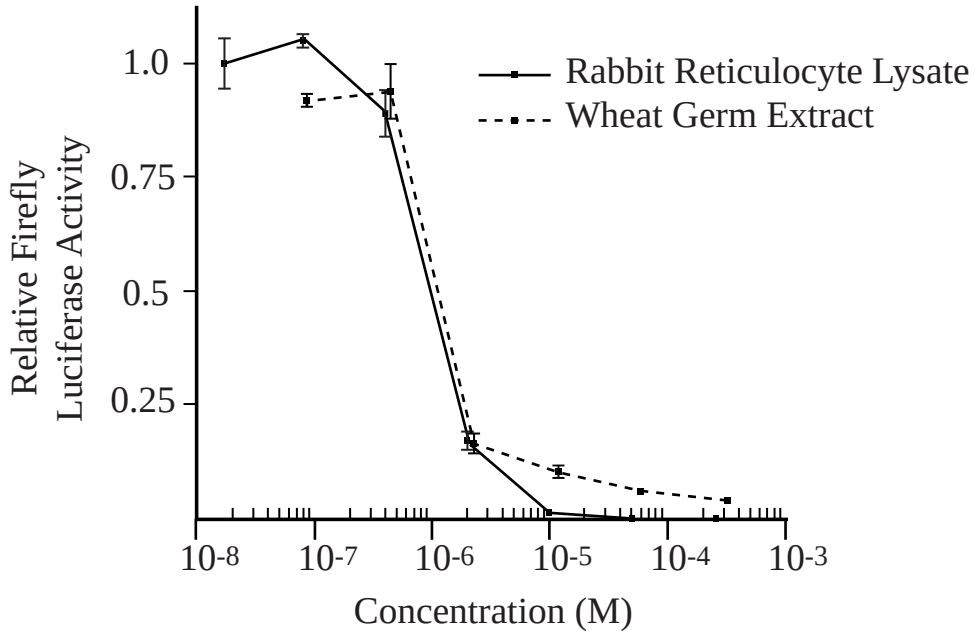
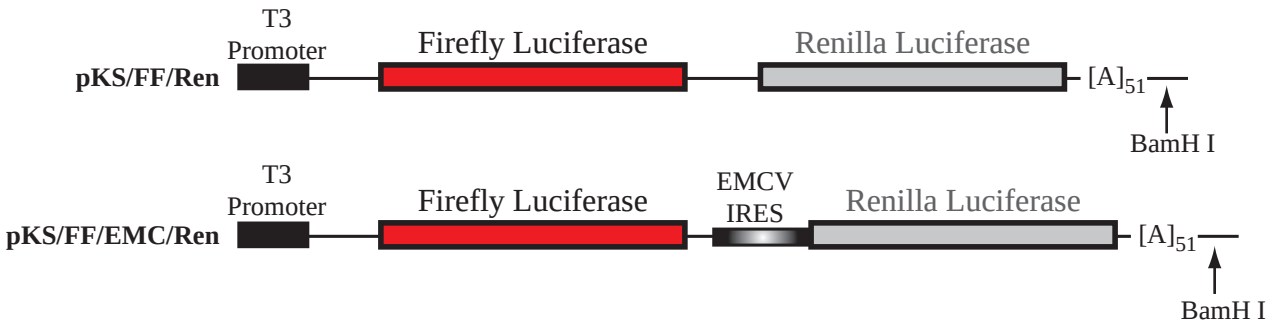
FigS1

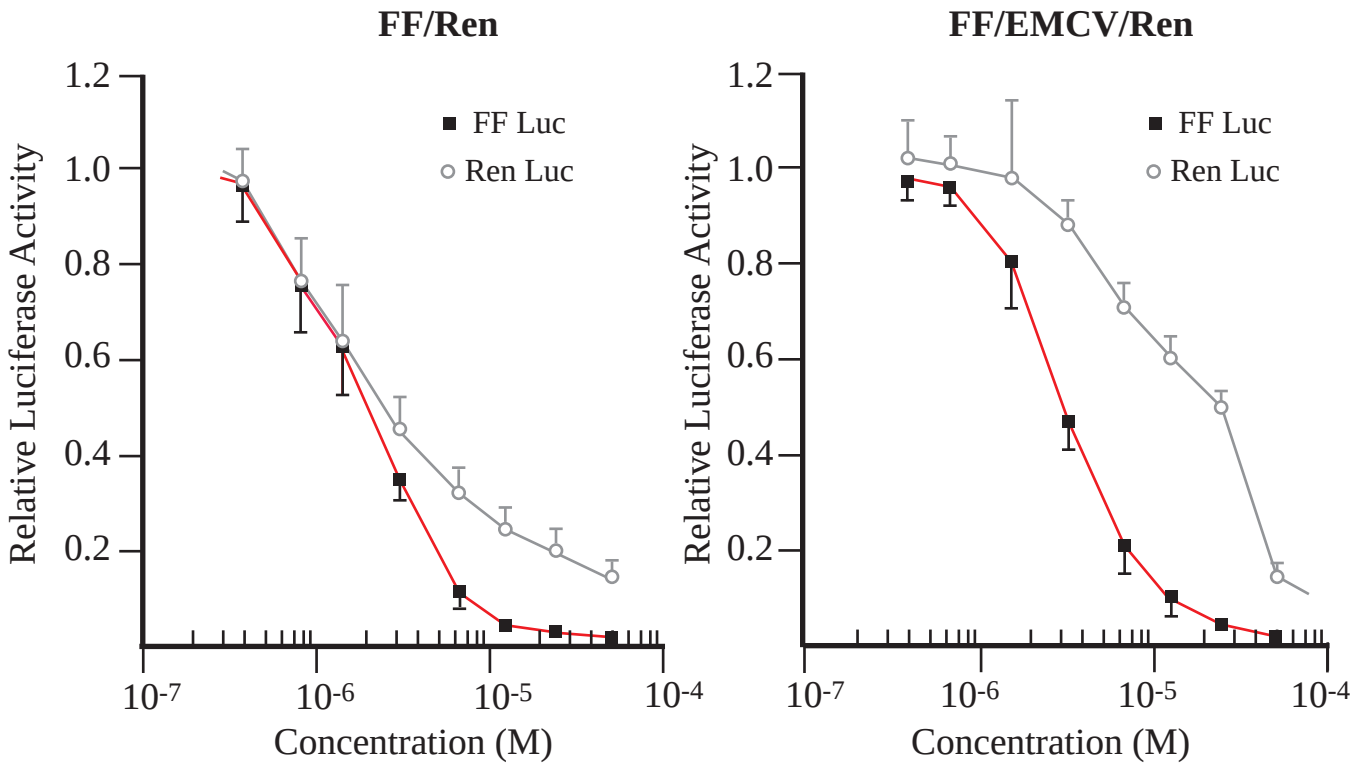

Rabbit Reticulocyte Lysate
Wheat Germ Extract
Relative Firefly Luciferase Activity
1.0
0.75
0.5
0.25
10-8
10-7
10-6
10-5
10-4
10-3
Concentration (M)

FigS2

A

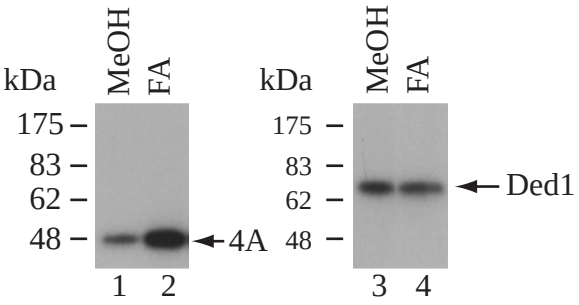

T3
Promoter
pKS/FF/Ren
Firefly Luciferase
Renilla Luciferase
[A]51
BamH I
T3
Promoter
pKS/FF/EMC/Ren
Firefly Luciferase
EMCV
IRES
Renilla Luciferase
[A]51
BamH I

B

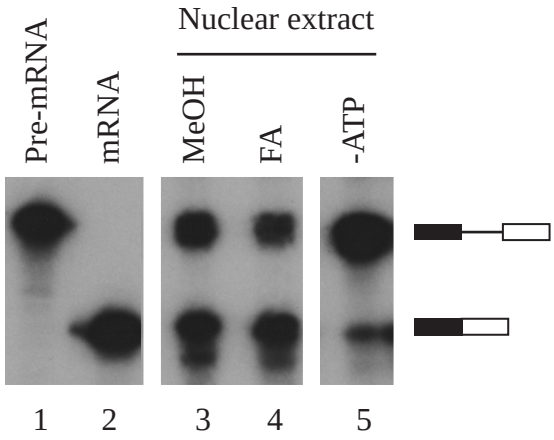

FF/Ren
FF/EMCV/Ren
FF Luc
Ren Luc
Relative Luciferase Activity
1.2
1.0
0.8
0.6
0.4
0.2
10-7
10-6
10-5
10-4
Concentration (M)

FigS3

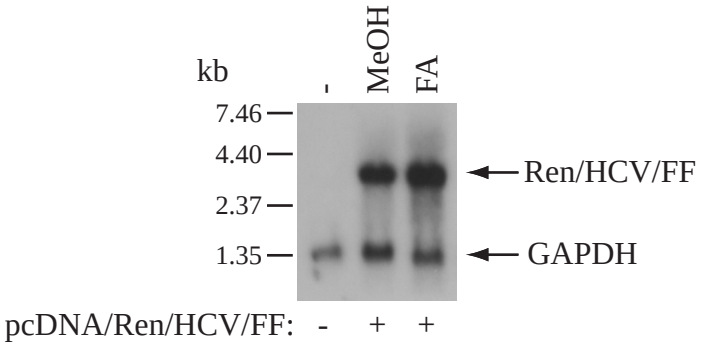
A


kDa
MeOH
FA
175
83
62
48
4A
1
2
kDa
MeOH
FA
175
83
62
48
Ded1
3
4

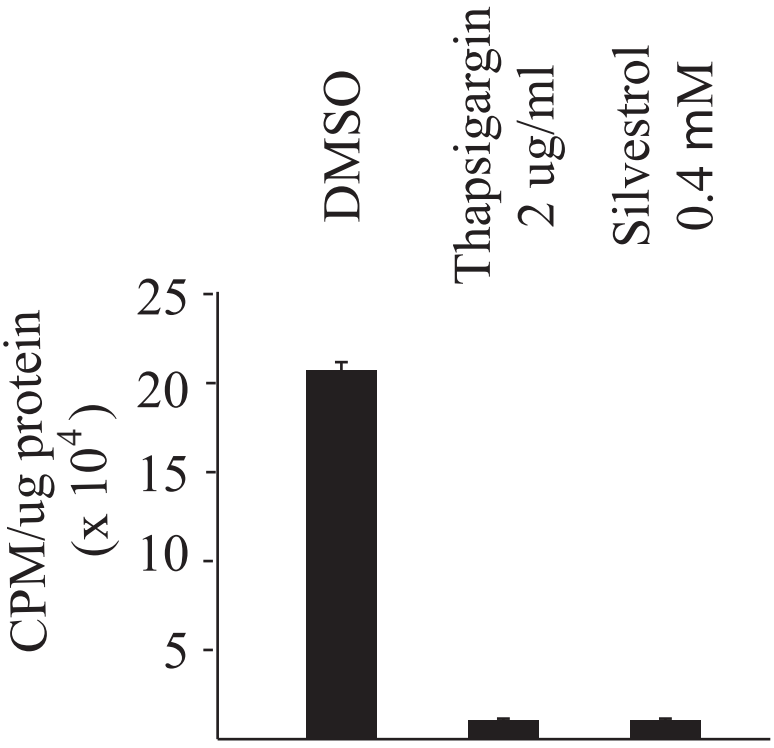
B


Nuclear extract
Pre-mRNA
mRNA
MeOH
FA
-ATP
1
2
3
4
5

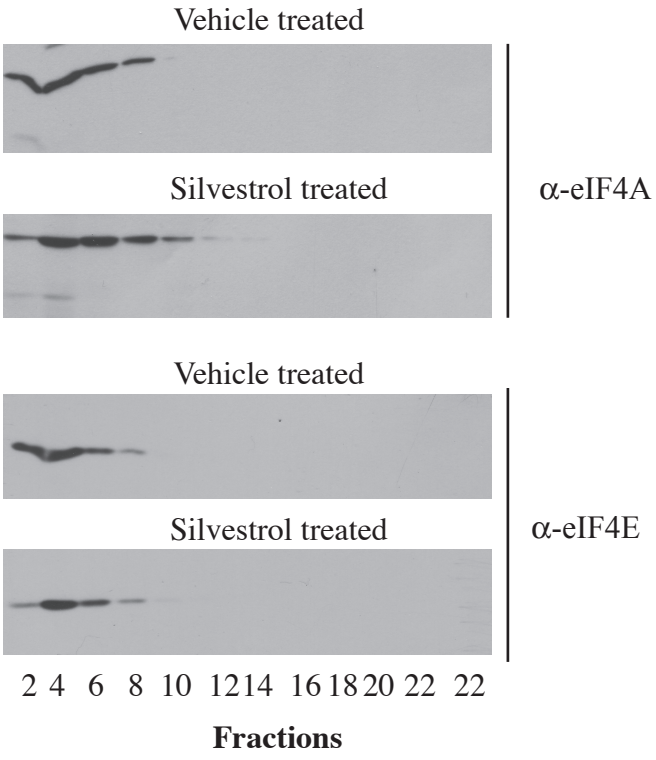
FigS4



FigS5


DMSO
Thapsigargin
2 ug/ml
Silvestrol
0.4 mM
CPM/ug protein
(x 10^4)
25
20
15
10
5

FigS6


Vehicle treated
Silvestrol treated
α-eIF4A
Vehicle treated
Silvestrol treated
α-eIF4E
2 4 6 8 10 12 14 16 18 20 22 22
Fractions

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. FA inhibits cap-dependent translation in rabbit reticulocyte lysate and wheat germ extracts. Titrations of FA were performed in rabbit reticulocyte lysate and wheat germ extracts programmed with FF/HCV/Ren mRNA (10 μg/ml). The FF luciferase activity only was measured and normalized to the activity obtained in the presence of vehicle (MeOH). The results shown are the average of three experiments with the standard error of the mean shown.

Figure S2. FA inhibits EMCV IRES mediated translation. **A.** Schematic diagram of pKS/FF/Ren and pKS/FF/EMCV/Ren plasmids used to generate FF/Ren and FF/EMCV/Ren mRNAs. **B.** Titration of FA in Krebs-2 extracts programmed with bicistronic mRNA reporters. The luciferase activities obtained were normalized to the activity obtained in the absence of compound (which was set at one). Each data point represents the average of 3 translations and the standard error of the mean is presented.

Figure S3. CBFs are not promiscuous DEAD-box helicase modulators. **A.** FA does not stimulate the RNA binding activity of Ded1. [^{32}P]-cap labeled mRNA was cross-linked to recombinant eIF4AI$_f$ (lanes 1 and 2) or Ded1 (lanes 3 and 4) in the presence of vehicle (MeOH) (lanes 1 and 3) or 50 μM FA (lanes 2 and 4). Following nuclease digestion, samples were resolved by SDS-PAGE and the gel was subjected to autoradiography. **B.** FA does not affect *in vitro* splicing reactions programmed with the AdML pre-mRNA. AdML pre-mRNA was incubated with nuclear extracts in the presence (lane 3 and 4) or absence of ATP (lane 5), vehicle (MeOH) (lane 3) or 50 μM FA (lane 4). Splicing of

[^{32}P]-labeled AdML pre-mRNA was performed under standard conditions containing 1 mM ATP in the presence of vehicle (MeOH) or 50 μM FA for 2h at 30°C[1]. Reactions were resolved on a 15 % acrylamide/8M urea gel that was then dried and subjected to autoradiography. The position of migration of the pre-mRNA and spliced product is denoted to the right.

Figure S4. RNA integrity is not altered following transfection of 293 cells with pcDNA/Ren/HCV/FF and treatment with FA. Northern blot analysis of RNA isolated from cells untransfected (-) or transfected (+) with pcDNA/Ren/HCV/FF followed by incubation with 5 μM FA or vehicle (MeOH) for 10 h. The blot was probed with [^{32}P]-labelled Ren/HCV/FF and GAPDH probes and the position of migration of the mRNAs are indicated.

Figure S5. Inhibition of protein synthesis by silvestrol and thapsigargin in Hela cells. HeLa cells were incubated for two hours in the presence of vehicle (DMSO), thapsigargin (2 ug/ml), or silvestrol (400 nM), after which time ^{35}S-methionine was added for 20 min before harvesting. The amount of ^{35}S-methionine in TCA-precipitable material was determined using scintillation counting.

Figure S6. Silvestrol causes a shift in the distribution of eIF4A into heavier sedimenting fractions in *PTEN*$^{+/-}$*Eμ-Myc* lymphomas harvested from silvestrol-treated mice. *PTEN*$^{+/-}$ *Eμ-Myc* tumors were harvested 4 h after treatment of mice with silvestrol (0.5 mg/kg). Cell extracts were prepared and centrifuged through a 10-50% sucrose gradient as in Fig

4A. The presence of eIF4A and eIF4E in the different fractions was determined by Western blot analysis of TCA-precipitation material.

REFERENCES

1. Shibuya, T., Tange, T.O., Sonenberg, N. & Moore, M.J. eIF4AIII binds spliced mRNA in the exon junction complex and is essential for nonsense-mediated decay. *Nat Struct Mol Biol* **11**, 346-51 (2004).