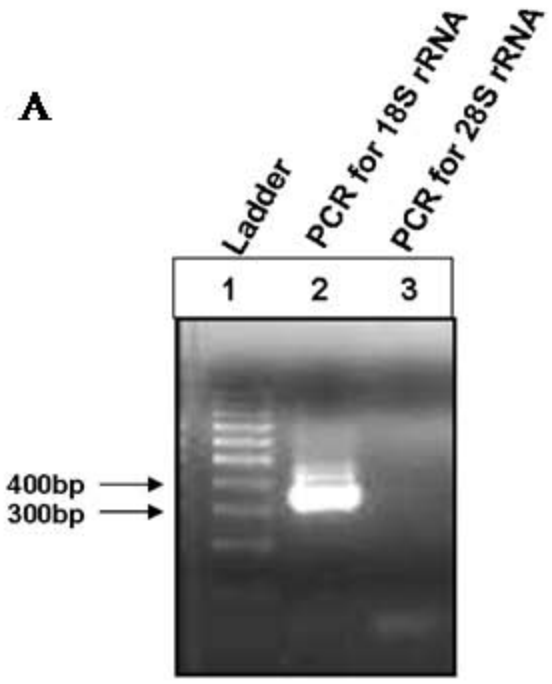
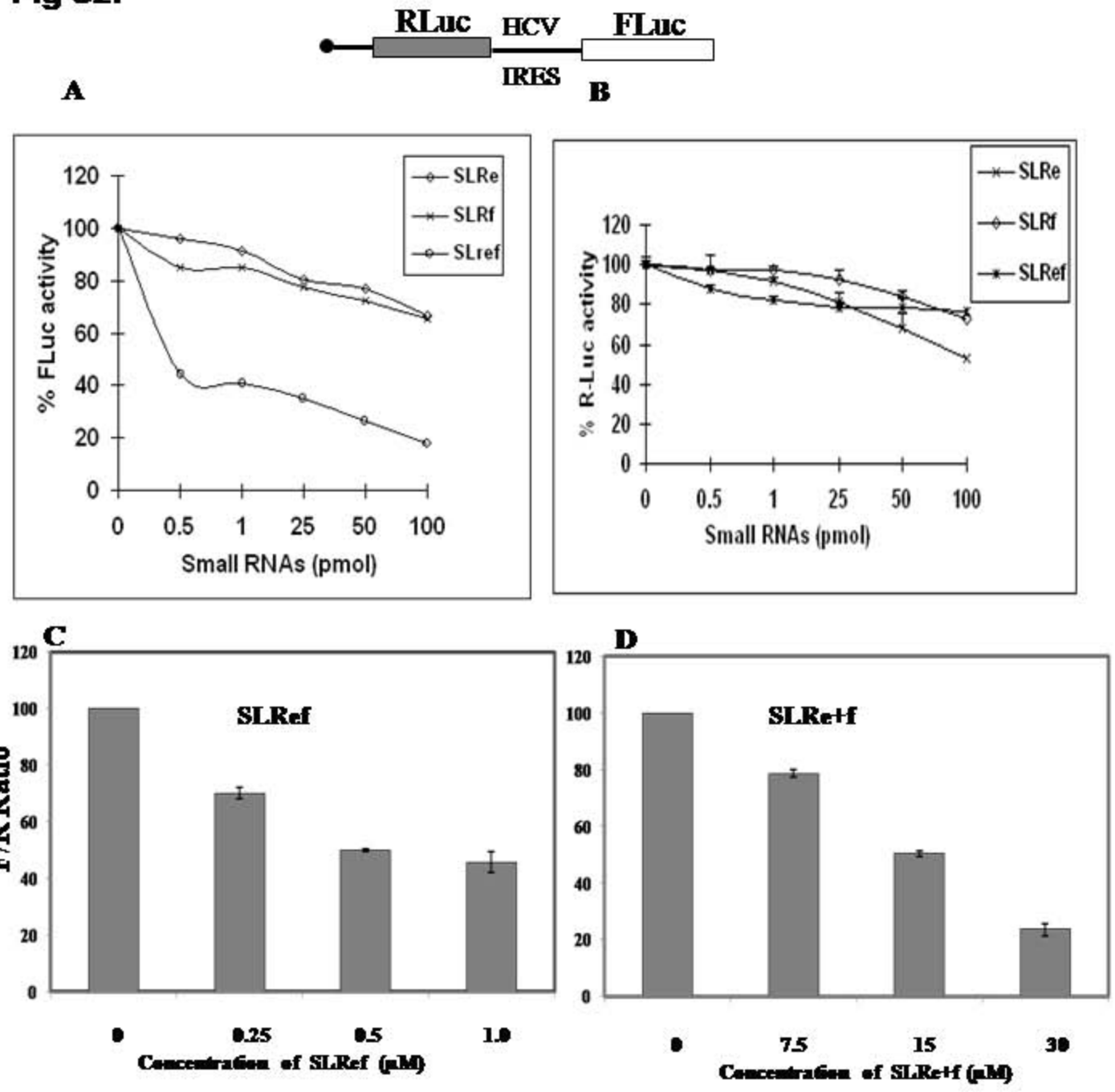
Supplemental Material to: Prasanna Bhat, Sivakumar Gnanasundram, Prashant Mani, Partho Sarothi Ray, Debi P. Sarkar and Saumitra Das. Targeting ribosome assembly on the HCV RNA using a small RNA molecule. RNA Biology 2012; 9(8); DOI: 10.4161/rna.21208;
http://www.landesbioscience.com/journals/rnabiology/article/21208/

FigS1.



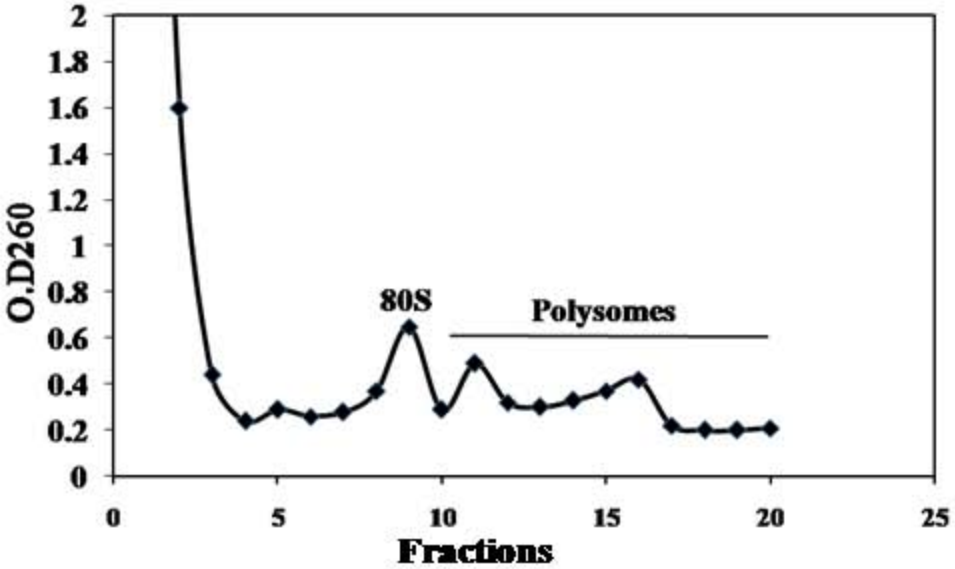
RT PCR to check the purity of 40S sub unit. RNA was isolated from 40S subunits purified from HeLa cells and semi quantitative RT PCR was performed to detect 18S and 28S rRNA.

Fig S2.



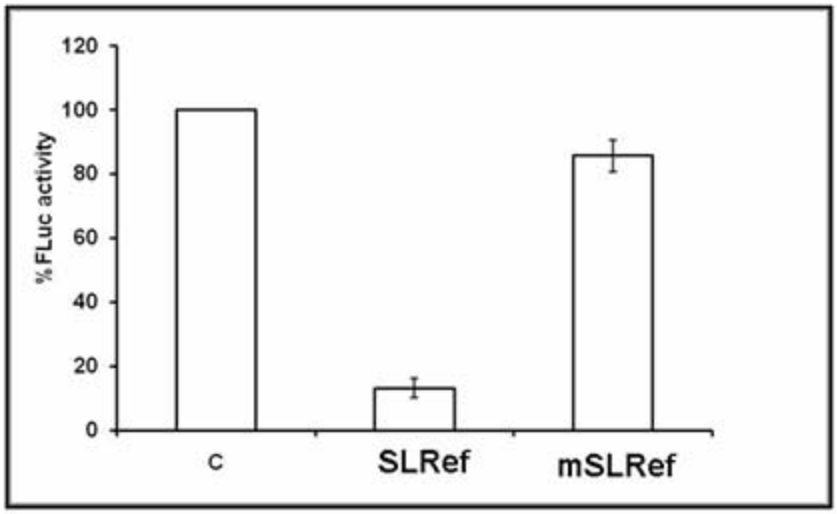
Dose dependent inhibition of HCV IRES mediated translation *in vitro* by SLRef RNA. Different concentrations (0.5 – 100 pmol) of small RNAs were added independently to the *in vitro* translation reactions of HCV bicistronic RNA and luciferase activity was measured. **(A)** Percentage FLuc activity represents the dose dependent effect of various small RNAs of on the HCV IRES mediated translation. **(B).** Percentage RLuc activity represents the effect of various small RNAs on the cap-dependent translation in the same set of experiments.**(C&D)** *In vitro* translation of HCV bicistronic RNA in presence of different concentration of SLRef or SLRe+f RNA's.

Fig S3.



Representative polysome profile of Rep2a cells. RNA was isolated from polysome fractions and free fractions to quantify the HCV RNA level in polysome and free fractions.

Fig S4.



Effect of mSLRef on HCV RNA translation. The SLRef and mSLRef RNA was incubated along with capped HCV bicistronic RNA in RRL reaction, then analyzed for luciferase activity. FLuc activity (representing HCV IRES activity) has been plotted to demonstrate the effect of SLRef and the mutant mSLRef RNA on the HCV IRES function.

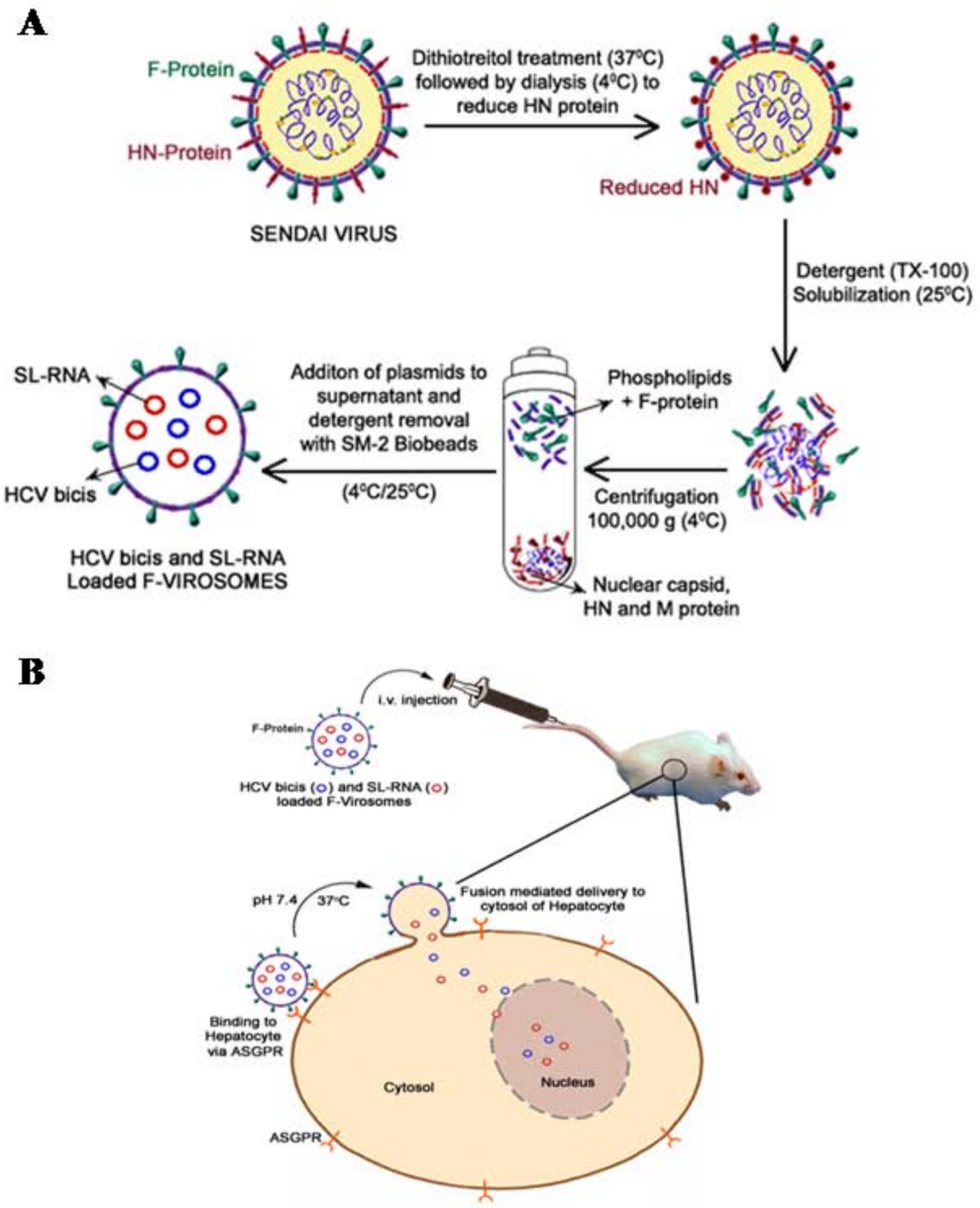
Fig.S5

pSUPERSLRef

pSUPERNSP

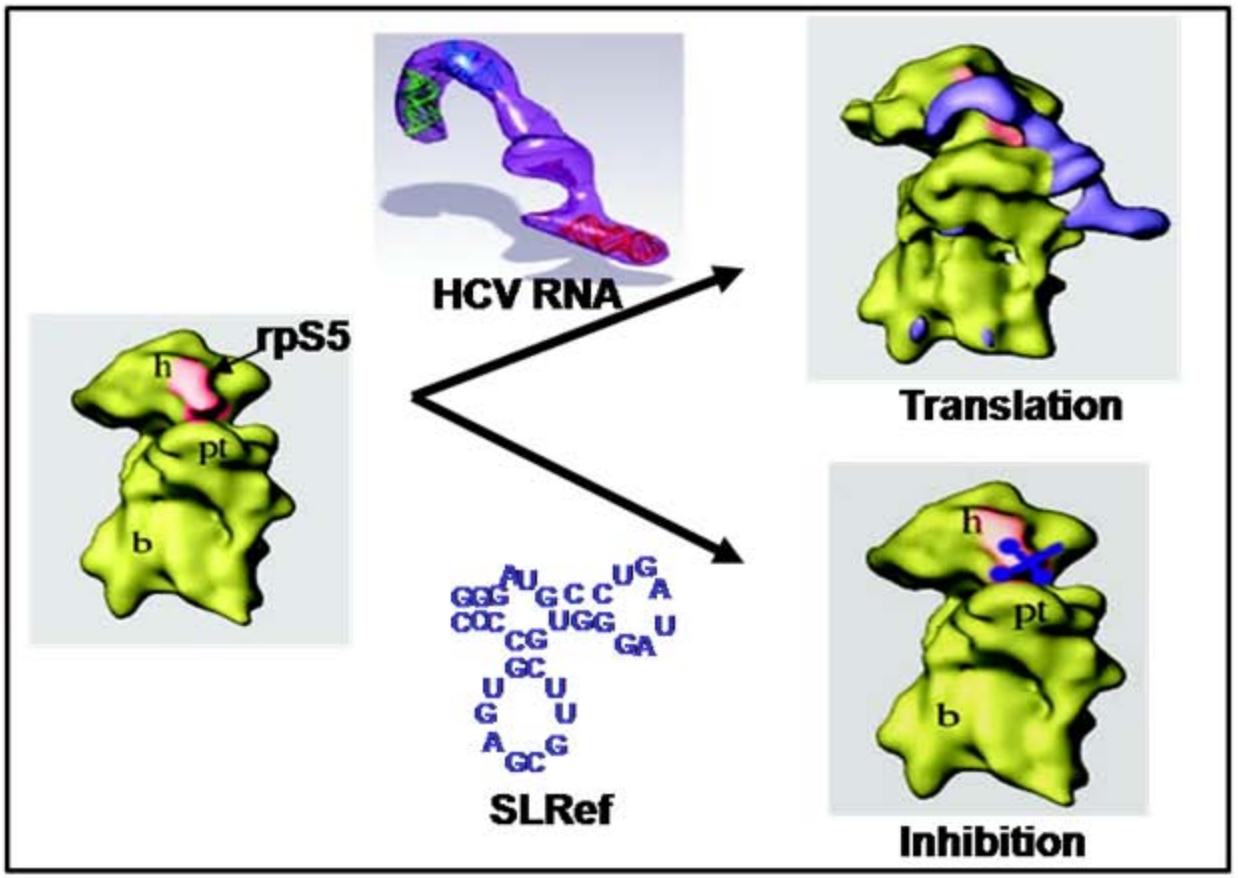
Schematic representation of pSUPER constructs

Fig S6.



Schematic representation of Sendai virosome preparation and fusion mediated delivery into Hepatocytes of mice.

Fig.S7



Schematic representation of the blocking of HCV IRES and ribosome interaction by SLRef RNA. The putative position of ribosomal protein S5 on the 40S is indicated. SLRef binds rpS5 and apparently blocks the HCV IRES-40S interaction site to inhibit translation (Adopted from Spahn et al 2001)

Fig. S8

	R-Luc	F-Luc
HCV alone	2458949±141329	130964.2±17949
SLRef	2232545±154671	19344.17±3647
NSP	2145124±157740	124720±14357

Table depicting luciferase values obtained in Figure 6B.