

## **Supplementary Data**

The supplementary data includes:

**Supplementary material and methods**

**Supplementary references**

**Supplementary figures S1-S7**

## Supplementary Material and Methods

### Real-time quantitative RT-PCR

Total RNA was extracted using Tri reagent (Molecular Research Center, Inc). After DNase treatment (DNase I Amplification Grade, Invitrogen), reverse transcription was performed using the Roche Transcriptor High Fidelity cDNA Synthesis Kit with random primers according to the manufacturer's instructions. Real-time PCR was performed using the Power SYBR Green PCR Master Mix (Applied Biosystems). Primers for amplification of the SRP RNA are described in Lakkaraju et al., 2007 (1); primers 5'GAAGGTGAAGGTCGGAGTC3' and 5'GAAGATGGTGATGGGATTTC were used to amplify GAPDH mRNA.

### Purification of recombinant proteins, RNAs and 40S ribosomal subunits

Human recombinant SRP9 and SRP14 proteins (h9 and h14) were overexpressed in BL21pLysS for 4 hours at 37°C. Bacterial pellets were lysed by sonification in 50 mM NaCl, 50 mM Tris-HCl pH 7.5, 10% glycerol, 10 mM MgCl<sub>2</sub>, 10 mM DTT and protease inhibitors. Lysate containing h9 was combined with the one containing h14 and incubated 30 min to allow formation of the heterodimer. h9/14 was purified by heparin-sepharose affinity chromatography, the eluted protein was dialysed overnight against 50 mM Hepes pH 7.5, 300 mM KOAc, 1 mM EDTA, 0.01% Nikkol, 10 mM DTT and further purified by ion-exchange chromatography on cation exchanger (MonoS 5/50 GL, GE Healthcare). Fractions that contained h9/14 were pooled, dialysed against 20 mM Hepes pH 7.5, 500 mM KOAc, 0.01% Nikkol, 10 mM DTT, 10% glycerol and stored at -80°C in aliquots.

scAlu and scB1 RNAs were synthesized with T7 polymerase from plasmids pPscAlu/ $\alpha$ -feto (2) and pscB1 (2). U6 RNA was synthesized with SP6 polymerase from plasmid pSP6-U6 (3). After DNase digestion, the transcripts were purified on G50 column, run on 8% denaturing acrylamide gel and visualized by UV shadowing. They were then eluted in a buffer containing 0.25% SDS, 1 mM EDTA, 0.25 M sodium acetate and 20 mM Tris-HCl pH 7.5, extracted with phenol, precipitated and resuspended in water. After purification on G50 column, they were extracted with phenol and resuspended in water. Synthesis of the randomly biotinylated scAlu and scB1 RNAs was obtained with the same procedure except that biotinylated-11-UTP was added in the transcription mix in equimolar amount with UTP. RNA integrity was confirmed on denaturing acrylamide gel and RNA concentrations

were determined by OD<sub>260</sub>.

40S ribosomal subunits were purified from rabbit reticulocyte lysate (Green Hectares, Oregon) as described in (4).

### **Glycerol gradients**

Arsenite-treated and control HeLa or NIH 3T3 cells were lysed in 50 mM Tris-HCl, pH 7.5; 250 mM sucrose; 25 mM potassium chloride; 5 mM magnesium chloride; 1 mM dithiothreitol; 0.01% Nikkol ; 0.25% Triton X100 ; AEBSF (Uptima, London, UK); peptide inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA); and RnaseOUT (Invitrogen). The cell extracts were centrifuged for 10 min at 800g and the postnuclear supernatants (500 µg of protein) were loaded on 12-30% glycerol gradients equilibrated with lysis buffer. Centrifugation was performed for 16h at 40'000 rpm in a with Centrikon TST 60.4 rotor, 12 or 14 fractions were collected, precipitated with 10% TCA and analyzed by Western blot using anti-SRP14 and anti-SRP19 antibodies.

### **RNA binding assay**

Hela Kyoto cells were transfected with *TransIT*®-2020 (Mirus) and after 48h lysed in a buffer containing 20 mM Hepes-KOH; 150 mM KOAc; 2.5 mM MgOAc; 0.01% Nikkol; 1 mM DTT; AEBSF (Uptima, London, UK); peptide inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA); and RnaseOUT (Invitrogen). An aliquot of the cell extract corresponding to 60 µg of protein was incubated for 1 h at 4°C with 4 pmoles of randomly biotinylated *scAlu* RNA synthesized *in vitro* from the *Alu* element of the  $\alpha$ -fetoprotein gene using T7 RNA polymerase (2). Biotinylated *scAlu* RNA and *scAlu* RNP were removed from the extract using magnetic streptavidin beads (Dynabeads M-280, Dynal). After three washes, the amounts of SRP9/14 in the immobilized and supernatant fractions were determined by Western blots using anti-SRP14 antibodies.

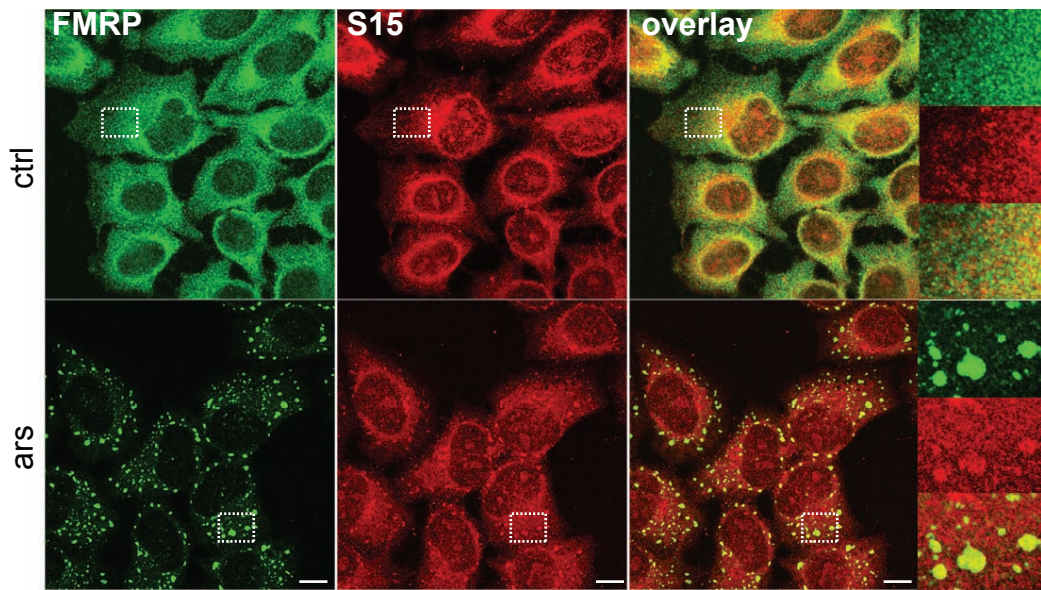
### **Statistical methods**

Error bars in the figures represent the standard deviation (SD).  $SD = \sqrt{\frac{\sum(x-\bar{x})^2}{(n-1)}}$ , where  $x$  is the individual data point and  $n$  is the number of samples. Student's  $t$  test was used for statistical analysis. Unpaired one-tailed  $t$ -tests were used for the quantification following knockdown of SRP9 and SRP14

and overexpression of 14-9VN proteins during stress recovery, while unpaired two-tailed t-test was used for the quantification of SG formation upon *Alu* overexpression.

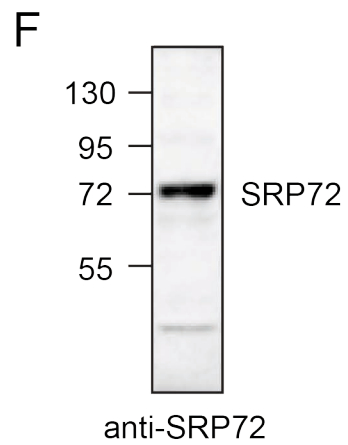
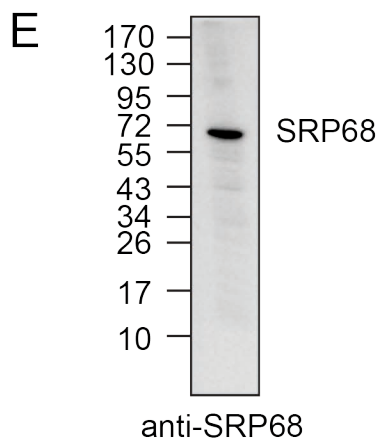
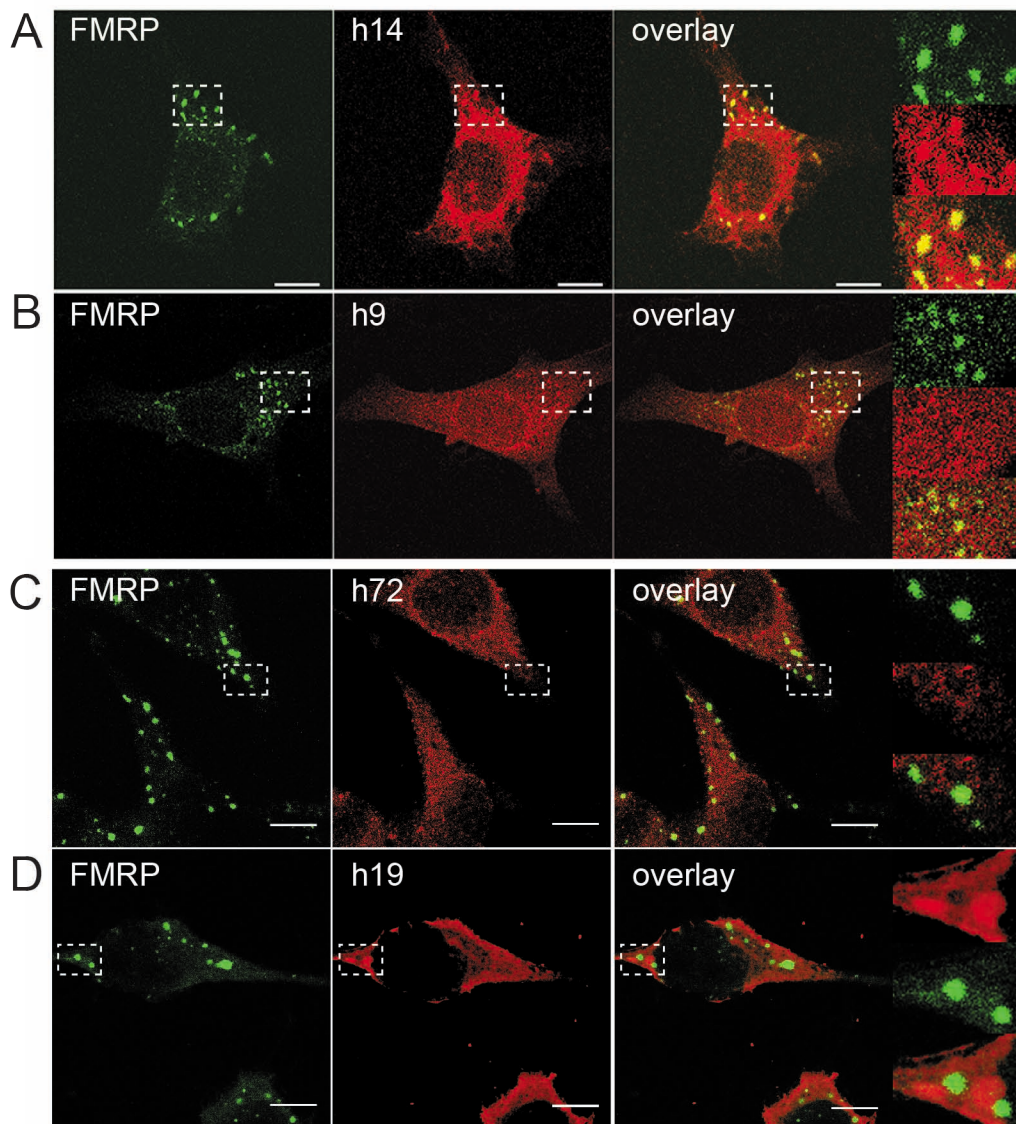
## Supplementary References

1. Lakkaraju, A.K., Luyet, P.P., Parone, P., Falguieres, T. and Strub, K. (2007) Inefficient targeting to the endoplasmic reticulum by the signal recognition particle elicits selective defects in post-ER membrane trafficking. *Exp. Cell Res.*, **313**, 834-847.
2. Bovia, F., Wolff, N., Ryser, S. and Strub, K. (1997) The SRP9/14 subunit of the human signal recognition particle binds to a variety of Alu-like RNAs and with higher affinity than its mouse homolog. *Nucleic Acids Res.*, **25**, 318-326.
3. Hausner, T.P., Giglio, L.M. and Weiner, A.M. (1990) Evidence for base-pairing between mammalian U2 and U6 small nuclear ribonucleoprotein particles. *Genes Dev.*, **4**, 2146-2156.
4. Pestova, T.V., Hellen, C.U. and Shatsky, I.N. (1996) Canonical eukaryotic initiation factors determine initiation of translation by internal ribosomal entry. *Mol. Cell. Biol.*, **16**, 6859-6869.



**Fig. S1.** SG localization of 40S subunits in arsenite-treated cells. Double immunofluorescence staining of arsenite-treated (ars) and control (ctrl) HeLa Kyoto cells with anti-S15 and anti-FMRP antibodies. Images were captured using a 63x lens on the LSM-710 Laser scanning microscope. Surface areas denoted by rectangles are shown at higher magnification. Scale bars: 10  $\mu$ m.

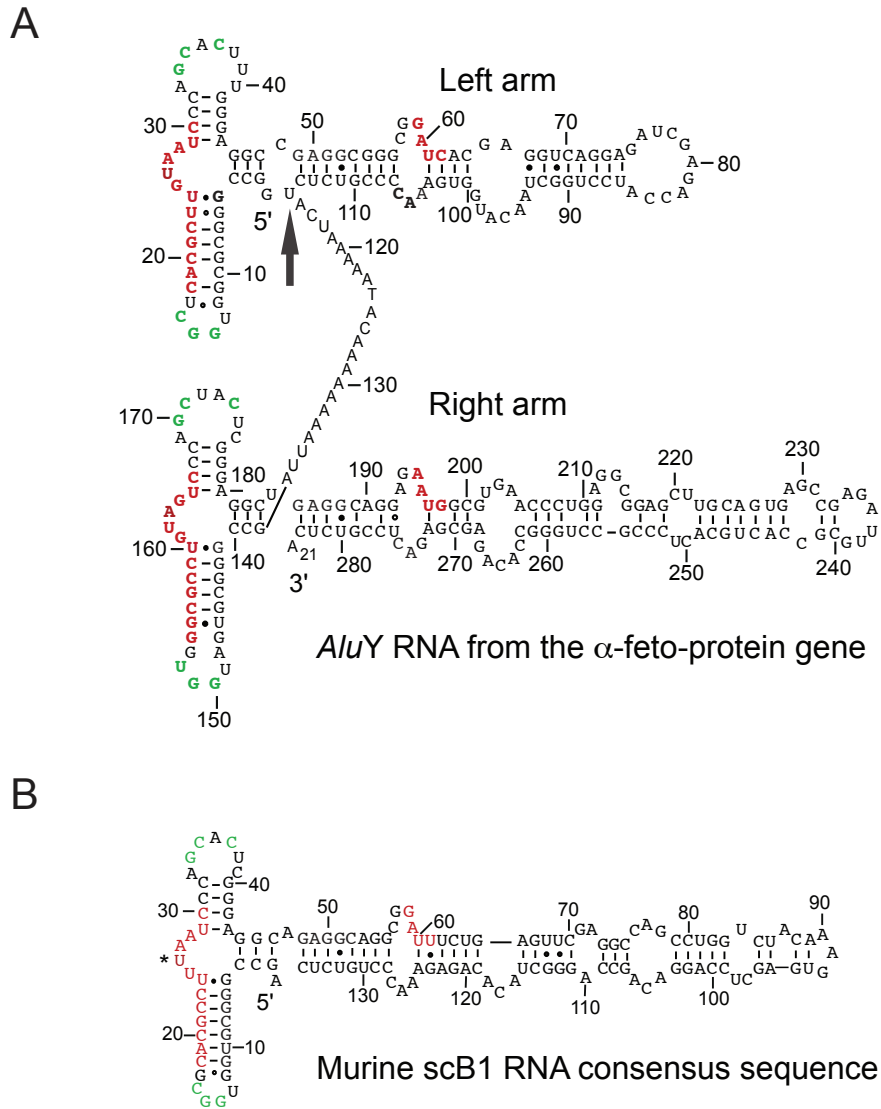
Berger et al., Fig. S2



**Fig. S2.** After hippuristanol treatment of HeLa cells, SGs contain h9 and h14 but not h72 and h19.

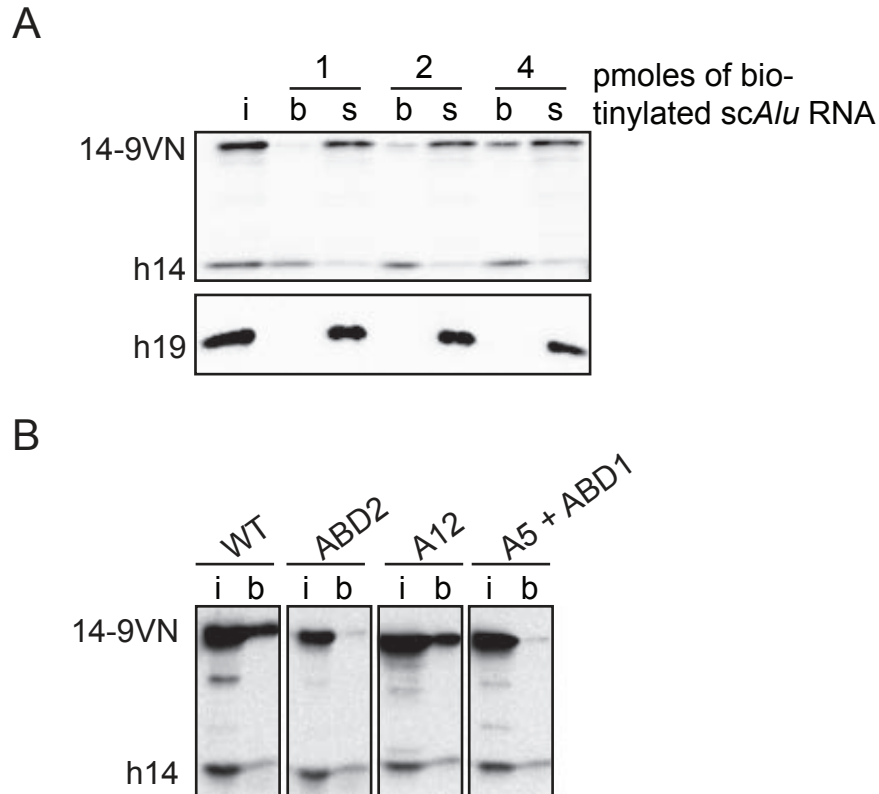
Double immunofluorescence staining of HeLa cells treated with 1  $\mu$ M hippuristanol for 30 min with antibodies against h14 (**A**), h9 (**B**), h72 (**C**) and h19 (**D**) in combination with anti-FMRP antibody . Images were captured at the SP2 laser scanning confocal microscope (63x/1.4 numerical aperture, Plan-Apo). Surface areas denoted by rectangles are shown at higher magnification. Scale bars: 10  $\mu$ m. (**E**) and (**F**): Western blots of HeLa total cell lysates using anti-human SRP68 (**E**) and SRP72 antibodies (**F**).





**Fig. S3.** Secondary structure models for the dimeric *AluY* RNA and scB1 RNA.

(**A**) Dimeric *Alu* RNA. It can be processed into a smaller left arm *Alu* RNA, named *scAlu* RNA (black arrow). (**B**) scB1 RNA. The structure models were drawn based on the structure of the *Alu* portion of 7SL RNA. Arrow: 3' end of the smaller, processed *scAlu* RNA. Red and green nucleotides represent the binding site of SRP9/14 and the tertiary base-pairs in the 7SL-*Alu* RNA, respectively. The high conservation of these nucleotides indicates that the tertiary structure and SRP9/14 binding to *Alu* and scB1 RNAs are associated with an important function.

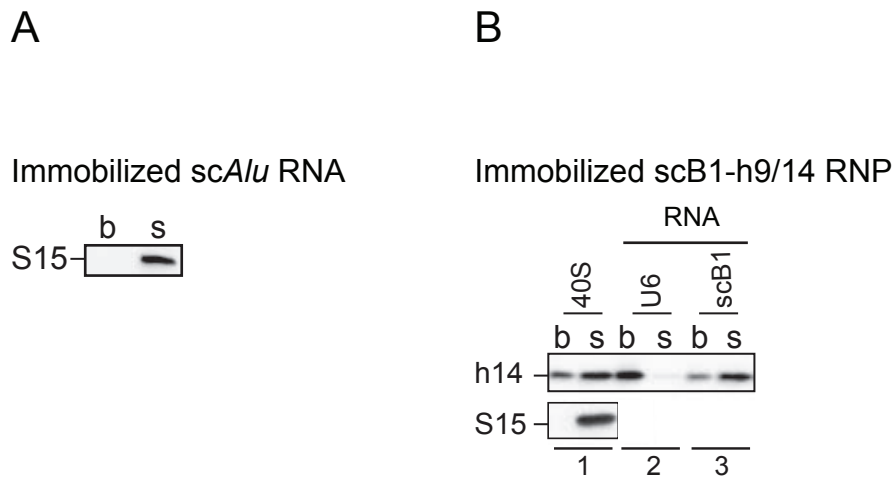


**Fig. S4.** RNA binding assays.

(**A**) The postnuclear supernatant of HeLa Kyoto cells expressing 14-9VN was incubated with increasing amounts of synthetic biotinylated *scAlu* RNA immobilized on streptavidin beads. 14-9VN and endogenous h14 were revealed in the input (i), bead (b) and supernatant (s) fractions using an anti-h14 antibody. The addition of 4 pmoles of biotinylated *scAlu* RNA allows the binding of both h9/14 and 14-9VN proteins. The endogenous h19 was revealed using anti-h19 antibody. Its absence in the bead fractions confirms the specificity of the RNA binding assay.

(**B**) Western blot of input (i) and *Alu* RNA-bound (b) fractions of the indicated fusion protein variants expressed in HeLa cells and assayed as described in Fig. 4F. Quantification is shown in Fig. 4H.

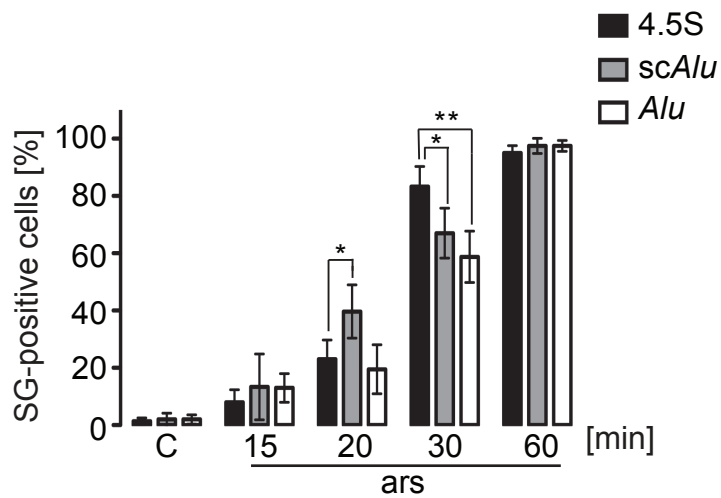
Berger et al., Fig. S5



**Fig. S5.** Protein transfer assay with *scAlu* RNA alone and scB1 RNP.

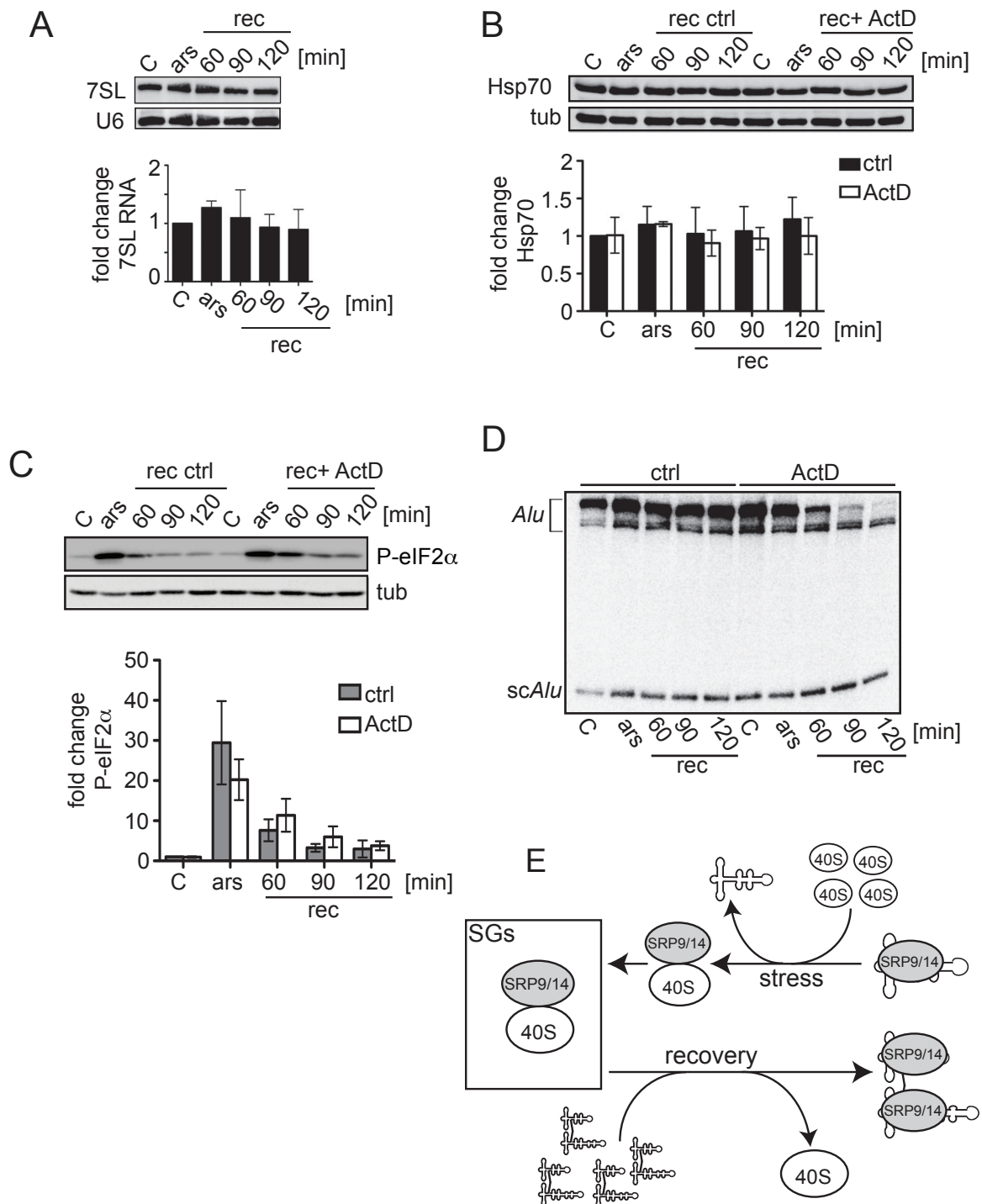
(**A**) *scAlu* RNA was immobilized on beads and incubated with 40S subunits purified from rabbit reticulocyte lysate. Analysis of bound (b) and supernatant (s) fractions by Western blot using anti-S15 antibodies shows that 40S does not bind to *scAlu* RNA.

(**B**) The immobilized scB1 RNP was directly formed on the streptavidin beads and the excess of h9/14 removed by washes. After addition of 40S, U6 or scB1 RNAs, the presence of h14 and S15 in the supernatant (s) and bound (b) fractions was assessed by Western blot. Sample 1: 40S, sample 2: U6 RNA, sample 3: non-biotinylated scB1 RNA.



**Fig. S6.** Time course of SG formation in the presence of *Alu* RNAs.

Quantification of SG formation. HeLa cells expressing any of the RNAs *scAlu* (grey), *Alu* (white) or 4.5S (black) together with the VN protein as a transfection control were incubated with 100 μM sodium arsenite for the indicated time periods and processed for double immunofluorescence staining with anti-flag antibodies to detect transfected cells and with anti-eIF3 antibodies to mark SGs. Error bars are shown as SD,  $n \geq 3$ .



**Fig. S7.** Controls for the experiments shown in Fig. 7 and a model for the role of SRP9/14 in SGs.

(A) Northern blot of 7SL and U6 RNAs for the experimental samples shown in Fig. 7B. For quantification, 7SL RNA was standardized to U6 RNA in each sample and normalized to C, which was set to 1. (B) and (C) Western blots of Hsp70 and phosphorylated eIF2 $\alpha$  (P-eIF2 $\alpha$ ) of the samples shown in Fig. 7D. For quantification, the protein levels of each sample were standardized to tubulin (tub) and normalized to C, which was set to 1. (D) Northern blot of *Alu* RNA in the experimental samples shown in Fig. 7E. ars: 0.5 mM sodium arsenite for 30 min; rec: recovery; C: control cells; ActD: 8  $\mu$ M actinomycin D. (E) Model. See discussion for details.