Experimental Procedures

Protein expression, preparation, and purification- Full length CRHSP-24 from *Homo sapiens* was cloned into a pGEX-6p-1 vector (GE Healthcare) and expressed in *Escherichia coli* BL21 (DE3) in rich (LB) medium as a fusion protein with an N-terminal GST tag. Expression of GST-CRHSP-24 in *E. coli* BL21 was induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside for 4 h. Bacteria were lysed by sonication in phosphate buffered saline (PBS, 10 mM sodium phosphate, pH7.3, 150 mM NaCl), and the lysates were cleared by ultracentrifugation. GST fusion proteins were purified by affinity chromatography on glutathione-Sepharose resin (GE Healthcare), and the tag was removed by cleavage with PreScission protease (GE Healthcare), leaving a five-residue Gly-Pro-Leu-Gly-Ser N-terminal extension. The cleaved protein was additionally purified using size-exclusion chromatography (Superdex-75, GE Healthcare).

Surface Plasmon resonance (SPR)-based Biosensor Analysis- The interaction of CRHSP-24 with the synthetic thymine-rich nucleotides was analyzed by SPR (1) using a BIACORE 3000 optical biosensor (Biacore AB, Uppsala, Sweden), using research grade streptevidin-coated (SA) chips. 5'-biotinylated ssDNA sequences were synthesized and captured onto a SA chip flow cell previously prepared by three successive pulses of 1 M NaCl in 50 mM NaOH, according to the manufacturer's recommendations. At least one flow-cell containing no biotinylated ssDNA was employed as a reference channel. Protein dilutions were performed in running buffer containing 10 mM HEPES at pH 7.4, 150 mM NaCl, 0.05% (v/v) Tween 20. The concentration of CRHSP-24 was quantified by optical spectroscopy (280nm) and Biosensor experiments were repeated a minimum of three times and corrected for binding to a separated control flow cell (activated and blocked). Regeneration of the chip between experiments was performed in the same way with 0.1 M NaOH. CRHSP-24 was injected at variable concentrations at 20 °C using a flow rate of 30 µl/min, and binding to the biotinylated ssDNA immobilized on the chip was monitored in real time. Response curves were prepared by subtracting the signal generated from the control flow cell. Thermodynamic dissociation constants (K_D) were determined using the software BIA evaluation 3.0.

Crystallization- Conditions were identified by the hanging drop vapor diffusion method with Crystal Screen reagent kits I and II (Hampton Research). Crystals suitable for diffraction were obtained after 9 days from the condition 0.1 M sodium acetate trihydrate pH 4.9, 2.0 M sodium formate. For data collection, crystals were picked up in a nylon loop and flash-cooled in a N_2 cold stream. The cryoprotectant solution contained the reservoir solution with addition of 20% (v/v) glycerol.

Structure Solution and Refinement- Diffraction data from a single crystal of Se-CRHSP-24 were collected on an ADSC-Q270t CCD detector (Marresearch GmbH Norderstedt Germany) at beamline BL17A of the High Energy Accelerator Research Organization (KEK, Tsukuba, Japan) (Table 1). Data processing and scaling were performed with HKL2000 (2). The structure was determined by the single-wavelength anomalous dispersion (SAD) method. Crystals of Se-CRHSP-24 belonged to the space group $P6_1$ with unit cell parameters a=b=84.6 Å, c=186.5 Å, α=β=90°, γ=120°, and contained four molecules per asymmetric unit. Four selenium sites were located and refined at 3.3 Å resolution using the SHELX program suite (3) and the electron density map was traced using PHENIX (4). After automatic model building with PHENIX, about 60% of the backbone and 30% of all side chains were modeled into the experimental map. The remaining residues were traced manually with

COOT (5) and PHENIX.REFINE (4) was used for refinement.

Data from the native CRHSP-24 crystal were collected at wavelength 0.97945 Å at the Advanced Photon Source (APS, Argonne, USA). The crystal also belonged to the space group $P6_1$ and contained four molecules per asymmetric unit, but with the unit cell parameters a=b=79.9 Å, c=182.0 Å, α=β=90°, γ=120°. The final structure of CRHSP-24 was phased by molecular replacement using PHASER (6) with the model of Se-CRHSP-24 as a starting model. Model building and fitting were carried out using COOT, and refinement and addition of water molecules were performed using PHENIX.REFINE. Data collection, processing, and refinement statistics are given in Table 1. Model quality was checked with PROCHECK (7).

cDNA Construction and Cell Culture- To generate GST-tagged full-length CRHSP-24, PCR-amplified CRHSP-24 cDNA was digested with BamHI and XholI and then cloned into the pGEX-6p-1 vector (GE Healthcare). The eukaryotic constructs of CRHSP-24 were cloned into the pEGFP-C1 vector (Clontech) with BglII and SalI digestion, because BamHI and BglII, XholI and SalI are isocaudamer. GST-tagged non-disulfide bonds, phosphormimetic, and non-nucleotide binding mutants of CRHSP-24 were created by the Easy Mutagenesis System kit (Transgen). All constructs were sequenced in full.

HeLa cells from the American Type Culture Collection (Manassas, VA) were maintained as subconfluent monolayers in DMEM (Invitrogen) with 10% fetal bovine serum (Hyclone, Logan, UT) and 100 units/ml penicillin plus 100 μg/ml streptomycin (Invitrogen) at 37 °C with 8% CO_2. Cells were treated with arsenite for 50 mins to induce the formation of stress granules.

Antibodies- Goat monoclonal antibodies to CRHSP-24 and G3BP were purchased from Santa Cruz Biotechnology and diluted at 1:100 and 1:500 for immunofluorescence. Mouse monoclonal antibody to GFP was purchased from Abcam and diluted at 1:2000 for Western blotting. A mouse monoclonal antibody to FLAG were purchased from Abcam and diluted at 1:2000 for Western blotting.

Immunoprecipitation- Co-immunoprecipitation was performed as described previously (e.g. ref. (8)). Briefly, HeLa cells growing on Petri dishes were transfected with appropriate plasmids for 24 h and were exposed to arsenite for 50 mins. Cells were lysed in lysis buffer (50 mM Hepes, pH 7.4, 150 mM NaCl, 2 mM EGTA, 0.1% Triton X-100, 1 mM $MgCl_2$, 1 mM PMSF, and protease inhibitors). After centrifugation, the supernatant was incubated with 25 μl of 50% slurry of anti-FLAG M2 affinity resin (Sigma) for 4 h at 4°C. After washing 3 times in cold lysis buffer, the beads were boiled in 50 μl of SDS sample buffer without/with DL-Dithiothreitol. For immunoblotting, the mouse monoclonal anti-FLAG M2 antibody (Sigma) was diluted to 2 μg/ml.

Transfection and Immunofluorescence- Cells were transfected with GFP-tagged plasmids in a 24-well plate by Lipofectamine 2000 (Invitrogen), according to the manufacture's manuals.

For immunofluorescence, HeLa cells were seeded onto sterile, acid-treated 12-mm coverslips in 24-well plates (Corning Glass Works, Corning, New York). The HeLa cells were then transfected with 1 μl of Lipofectamine 2000 premixed with 1 μg of various plasmids as described above. In general, 24-36 h after transfection and 50 mins after arsenite treatment, HeLa cells were rinsed for 1 min with PHEM buffer (100 mM PIPES, 20 mM HEPES, pH 6.9, 5 mM EGTA, 2 mM $MgCl_2$, and 4 M glycerol) and permeabilized for 1 min with PHEM plus 0.1% Triton X-100. Extracted cells were then fixed in freshly prepared 4% paraformaldehyde in PHEM and rinsed three times in PBS. Cells on the coverslips were blocked with 0.05% Tween-20 in PBS (TPBS) with 1% bovine serum

albumin (Sigma). These cells were incubated with various primary antibodies in a humidified chamber for 1 h and then washed three times in TPBS. Primary antibodies were visualized using fluorescein isothiocyanate-conjugated mouse anti-goat IgG or rhodamine-conjugated mouse anti-goat IgG (Jackson Immunoresearch). DNA was stained with 4', 6-diamidino-2-phenylindole (Sigma).

Antibodies against CRHSP-24 and G3BP were used at dilutions of 1:100 and 1:500, respectively. Images were acquired using an Axiovert 200 inverted Microscope (Carl Zeiss) with Axiovision 3.0 software.

siRNA- For siRNA studies, the 21-mer of siRNA duplexes against CRHSP-24 was purchased from Santa Cruz Biotechnology. The efficiency of siRNA-mediated protein suppression was judged by Western blotting analysis and the transfection efficiency was judged based on the uptake of the FITC-conjugated oligonucleotides. After trial experiments using a series of concentration and time course assay, treatment of 100 nM for 48 h was finally selected as the most efficient conditions for repressing the target protein.

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SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. CRHSP-24 siRNA suppressed the CRHSP-24 protein expression. Aliquots of HeLa cells were transfected with CRHSP-24 siRNA and scramble control as described under "Materials and Methods". Cells were then harvested for SDS-PAGE and subsequent Western blotting analyses of the efficiency on suppression of CRHSP-24 protein (*upper panel*) and specificity of this siRNA-mediated CRHSP-24 depletion (*lower panel*).

Fig. S2. Structural surface of chain B of CRHSP-24. Three thymines (colored in magenta) are docked in the pocket of chain B of CRHSP-24. Cys^{69} and Cys^{71} are marked in yellow. Leu^{43} is indicated in blue, other than in the chain A of CRHSP-24, it does not prevent CRHSP-24 from binding with these thymines.

Fig. S3. Characterization of phosphomimetic mutant S41D upon GSSG treatment. In the gel filtration profiles, cyan line shows the S41D mutant, yellow line represents S41D in the presence of 0.5 mM GSSG for two hours at room temperature, indicating that mutant S41D can not block the formation of polymerization.

Fig. S4. Characterization of CRHSP-24 upon GSH treatment. The peak of native CRHSP-24 is shown by a blue solid line. The red line shows the CRHSP-24 in the presence of 0.5 mM GSH to mimic the reducing environment of the cytoplasm. They have the similar peak patterns.

Fig. S5. CRHSP-24 localization to SGs after knocking down calcineurin. *A*. Endogenous CRHSP-24 (green) localized to G3BP-marking stress granules (red) in response to arsenite treatment. *B*. Dephosphorylation of CRHSP-24 was inhibited by cyclosporin A, an inhibitor of calcineurin, and CRHSP-24 (green) persisted in stress granules (red). These results suggest that dephosphorylation of CRHSP-24 by calcineurin has no effect on the localization to SGs. DAPI staining (blue) indicates the location of nuclei. Scale bar represents 5μm.

Figure S1

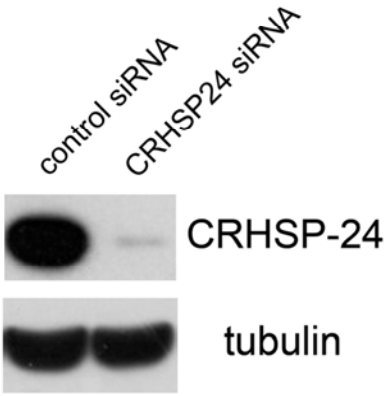


Figure S2

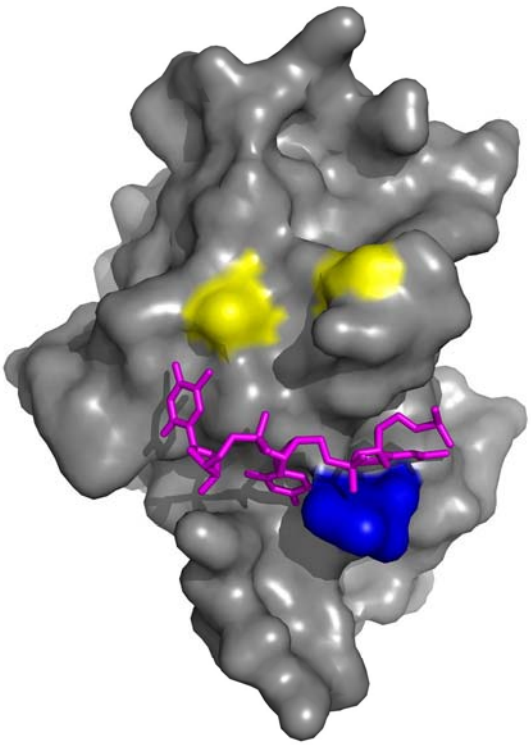


Figure S3

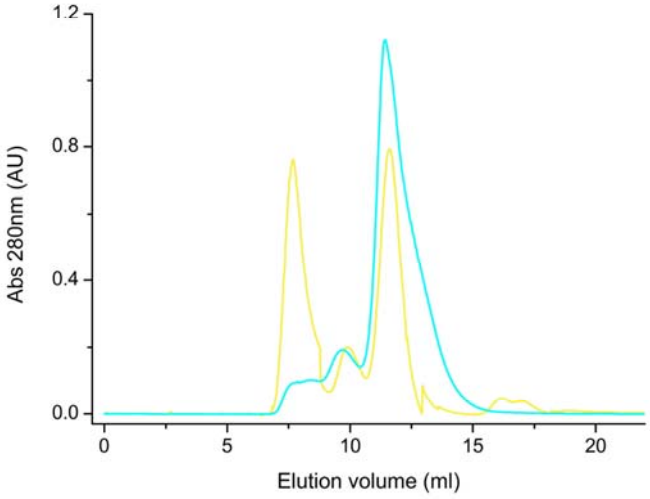


Figure S4

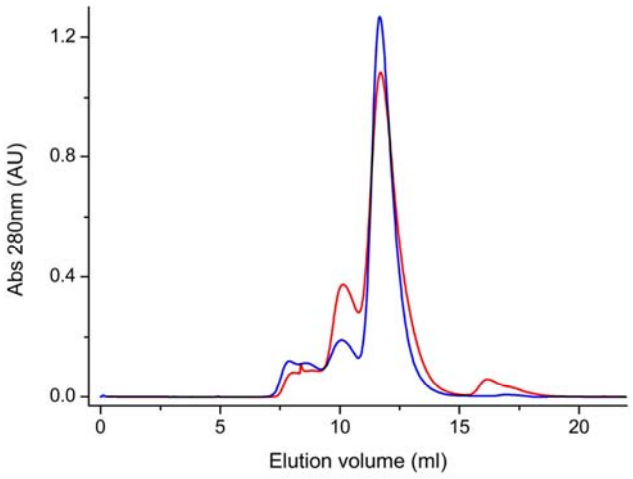
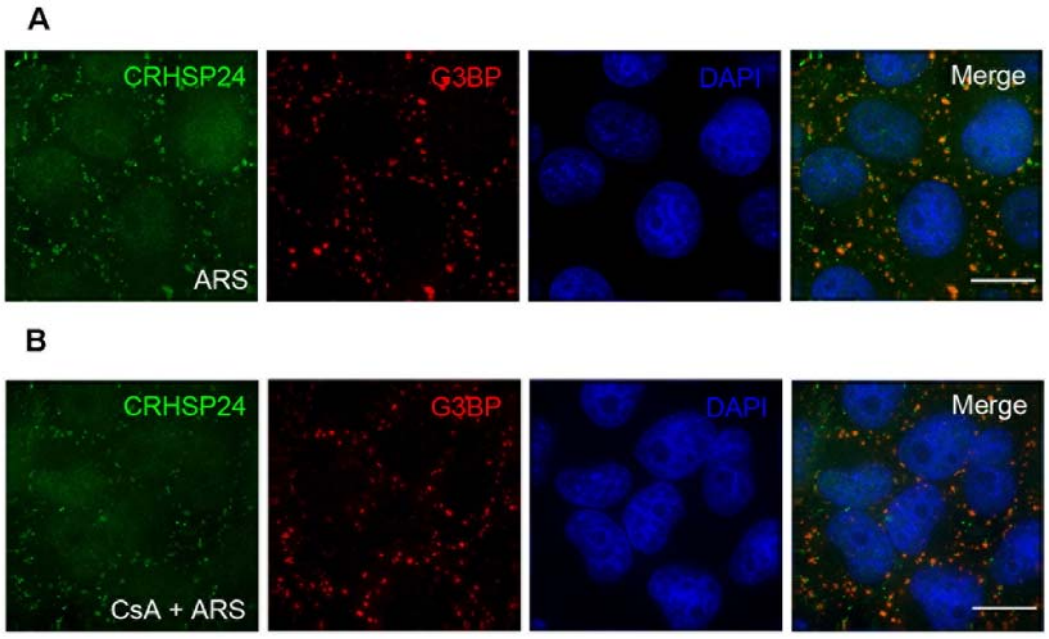


Figure S5


A
CRHSP24
G3BP
DAPI
Merge
ARS
B
CRHSP24
G3BP
DAPI
Merge
CsA + ARS