Supplementary materials

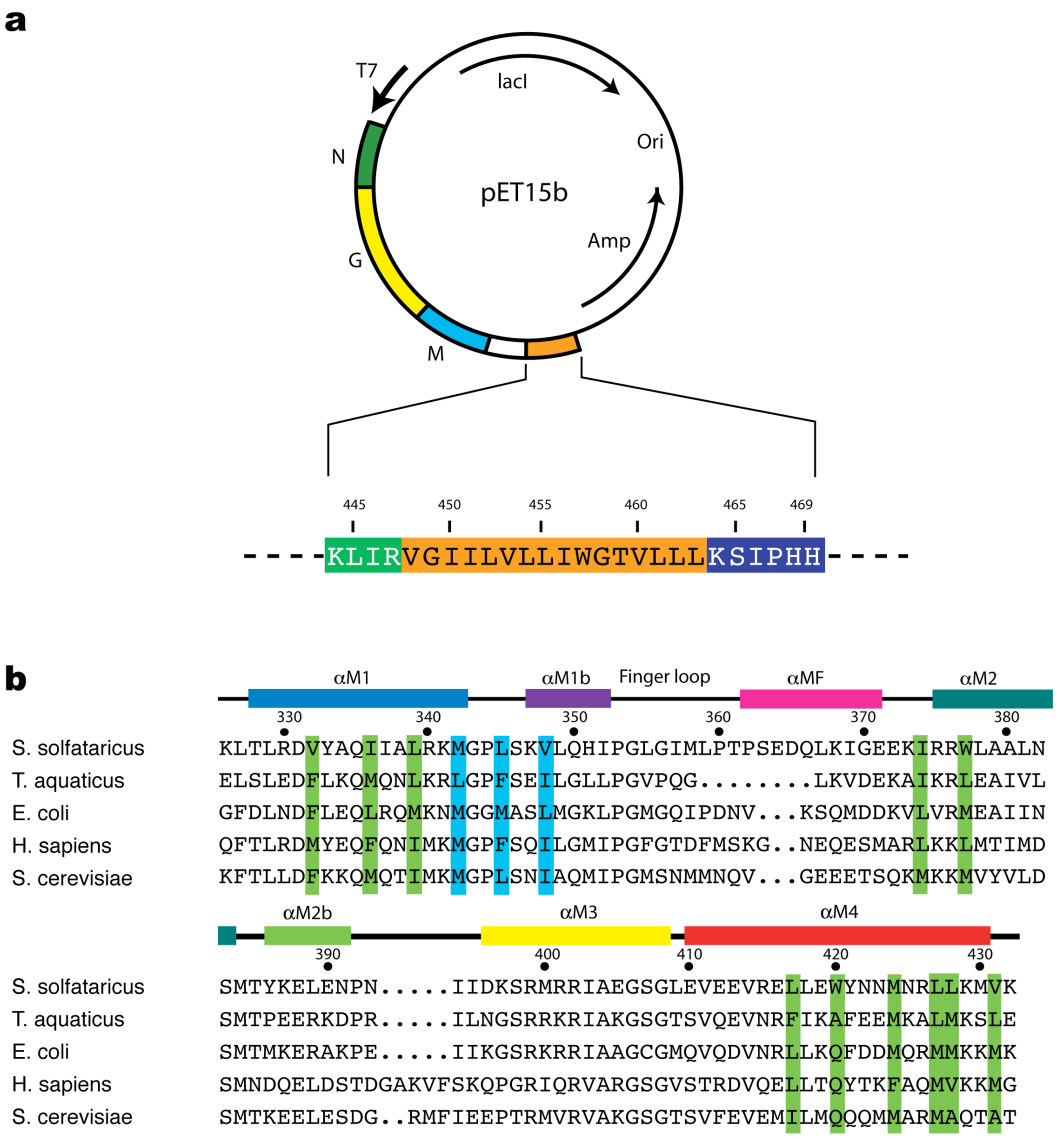

Figure S1 | **Fusion protein of *Sulfolobus solfataricus* SRP54 and a signal peptide**. **a**, Expression vector for the fusion protein. The signal peptide of yeast dipeptidyl aminopeptidase B is fused *via* various linker sequences to SRP54 (see Table S1). The n, h and c-regions of the signal peptide are shown on green, orange and blue background **b**, amino acid sequence alignment of the M domain of SRP54. Green, hydrophobic residues which line the surface of the signal peptide binding groove; cyan, hydrophobic residues which may be involved in coupling the M domain to the NG domain via the linker.

Table S1 | **Amino acid sequences of linker peptides used for this study**

Construct	**Junction on the N-terminal side**[1]	**Linker**	**Junction on the C-terminal side**[1]	**Linker length (residues)**
SRP54*-6mer-5H	(AR)	S G	(GS)	6
SRP54*-11mer-5H	(AR)	S 3 x (GS)	(GS)	11
6H-SRP54*-14mer	(AR)	5 x (GS)	(GS)	14
SRP54*-20mer-5H	(AR)	8 x (GS)	(GS)	20
6H-SRP54*-20mer	(AR)	8 x (GS)	(GS)	20
6H-SRP54*-T28mer	(AR) LVPR / GS (GR)	8 x (GS)	(GS)	28
6H-SRP54*-30merT	(AR)	9 x (GS)	(GT) LVPR / GS (GS)	30
6H-SRP54*-T24merT	(AR) LVPR / GS	4 x (GS)	LVPR / GS (GS)	24
6H-SRP54*-T28merT	(AR) LVPR / GS	6 x (GS)	LVPR / GS (GS)	28
SRP54*-T38 merT-5H	(AR) LVPR / GS (GR)	9 x (GS)	(GT) LVPR / GS (GS)	38
6H-SRP54*-T38merT	(AR) LVPR / GS (GR)	9 x (GS)	(GT) LVPR / GS (GS)	38
10H-SRP54*-T33merP	(ARG) LVPR / GS	6 x (GS)	LEVLFQ / GP (SGGS)	33
6H-SRP54*-34merP	(AR)	9 x (GS)	LEVLFQ / GPVYKD (GS)	34

[1]Amino acid residues in parentheses arise from the restriction sites in the vector. Some linker sequences contain either a thrombin or a TEV protease cleavage site (/) on the N-terminal or C-terminal side or both.

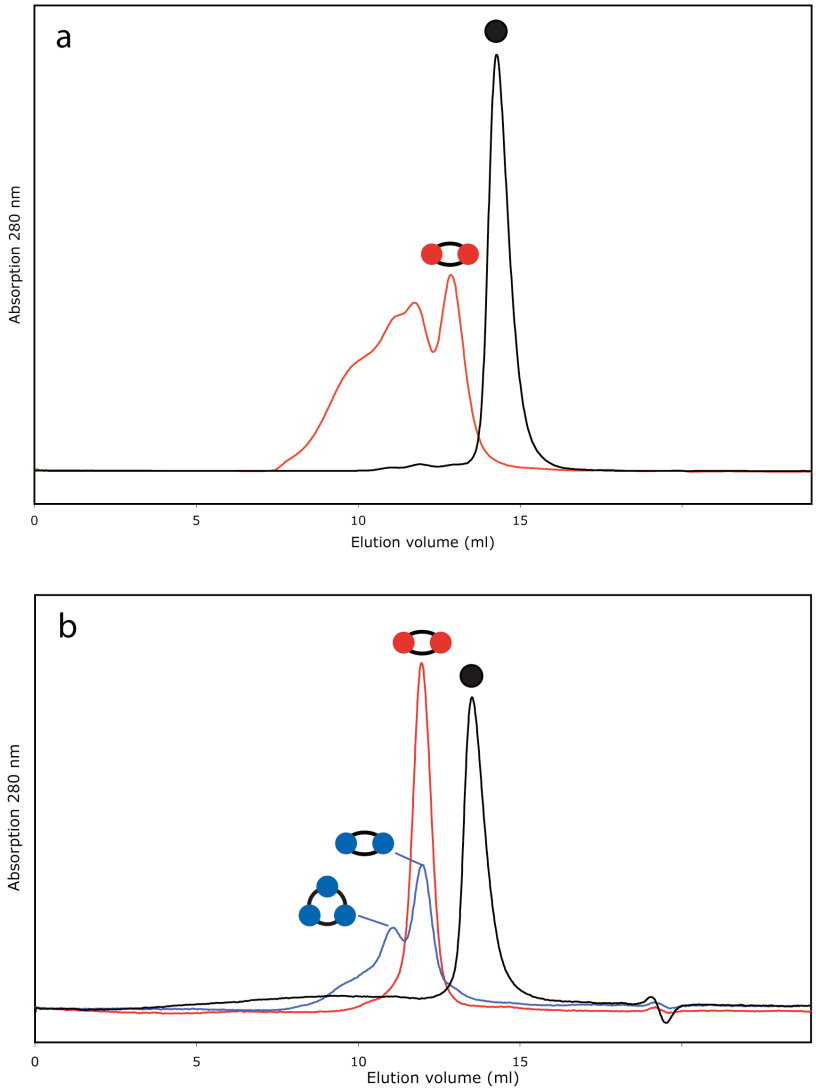

Figure S2 | **Analysis of SRP54* by gel filtration on Superdex S200**. **a**, SRP54*-11mer-5H (red trace) and wild type SRP54 (black trace). **b**, 6H-SRP54*-T28merT pool 1 (red trace) and 2 (blue trace) of the last heparin Sepharose column and wild type SRP54 (black trace). Icons indicate the oligomeric state of the eluted species according to their apparent molecular mass. Superdex S200 column was calibrated with molecular weight standards: cytochrome c (12.3 kDa), BSA (67 kDa), aldolase (158 kDa), ferritin (440 kDa), blue dextran (2,000 kDa).

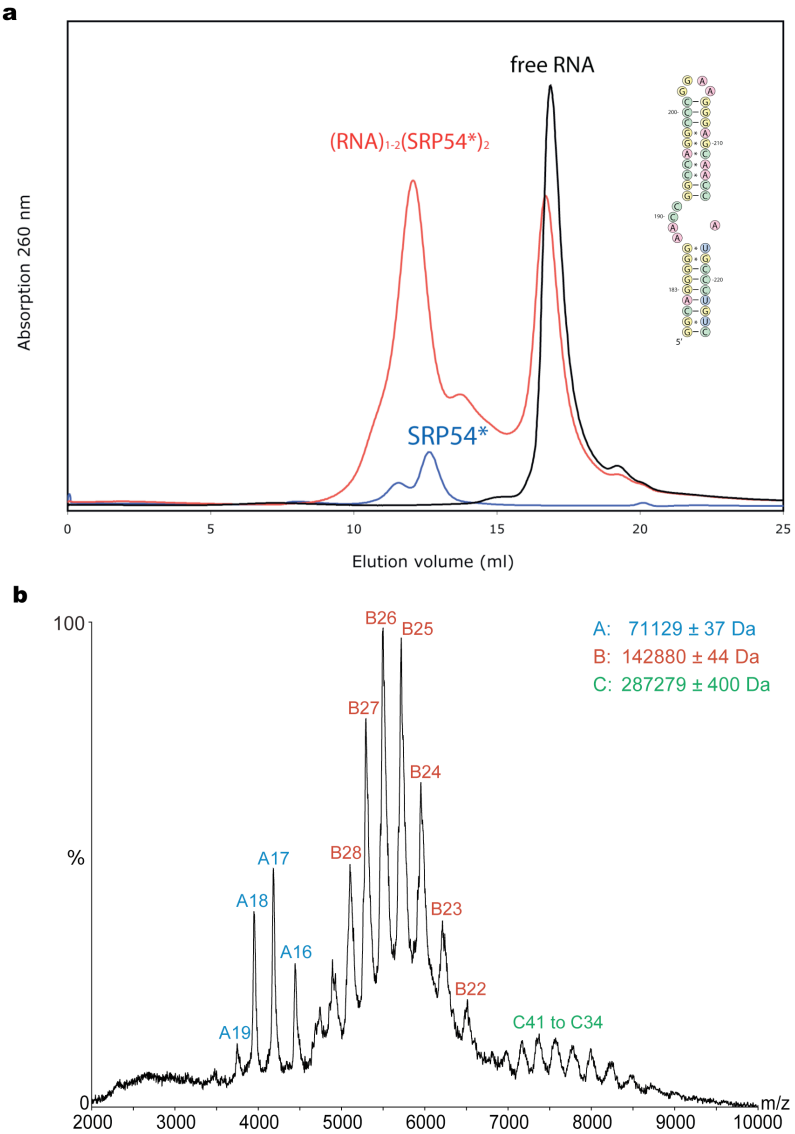

Figure S3 | **Interaction between SRP54* and RNA**. **a**, analysis of SRP54*, RNA and their mixture by gel filtration on Superdex S200. Inset, the secondary structure of helix 8 of *Sulfolobus solfataricus* SRP RNA used for binding assay. **b**, analysis of the SRP54*-RNA complex in 500 mM ammonium acetate with 2 mM Mg^{2+} by nanoflow ESI mass spectrometry. Charge state series labelled with A, B, C correspond to (SRP54*)-(helix 8) (calculated mass: 70,655 Da), $(SRP54^*)_2$-$(helix\ 8)_2$ (calculated mass: 141,310 Da), and $(SRP54^*)_4$-$(helix\ 8)_4$ (calculated mass: 282,620 Da), respectively.

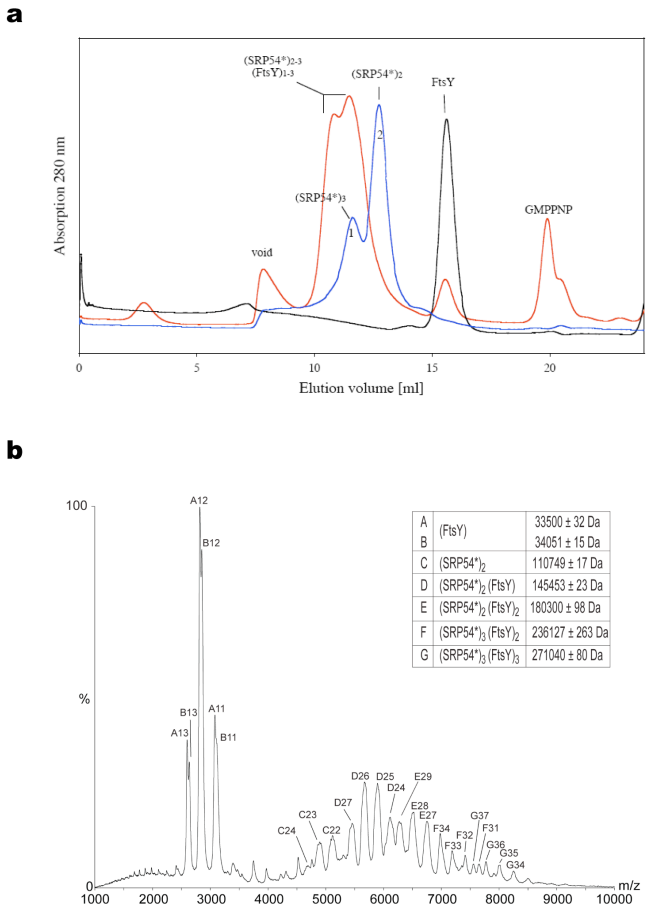

Figure S4 | **Association of SRP54* with the NG domain of FtsY in the presence of GMPPNP.** **a**, SRP54* was mixed with the NG domain of *Sulfolobus solfataricus* FtsY (aa 62-358) and analysed by gel filtration on Superdex S200. Black trace, FtsY; blue trace, a mixture of the dimeric and trimeric forms of SRP54*; red trace, a mixture of SRP54* and FtsY in the presence of GMPPNP. No FtsY binding was observed in the presence of EDTA and GMPPNP, or GTP. **b**, Analysis of the SRP54*-FtsY receptor complex in 500 mM ammonium acetate by nanoflow ESI mass spectrometry. Peak labels indicate complex identity and charge state (see inset table). Calculated masses are FtsY, 33,523 Da; $(SRP54^*)_2$, 110,616 Da; $(SRP54^*)_2$–FtsY: 145,261 Da; $(SRP54^*)_2$–$(FtsY)_2$, 179,906 Da; $(SRP54^*)_3$–$(FtsY)_2$, 235,214 Da; $(SRP54^*)_3$–$(FtsY)_3$, 269,859 Da. Calculated masses of each formed SRP54*-FtsY heterodimer includes the mass of two GMPPNP molecules (519 Da each), two Mg^{2+} ions (24 Da each) and two water molecules (18 Da each).

Table S2 Data collection, phasing and refinement statistics

	Native		SRP54* (N177C)-Hg	
Data collection				
Space group	$P4_12_12$		$P4_12_12$	
Cell dimensions				
a, *b*, *c* (Å)	91.88, 91.88,133.26		92.04, 92.04, 133.68	
α, β, γ (°)	90.00, 90.00, 90.00		90.00, 90.00, 90.00	
		Peak	*Inflection*	*Remote*
Wavelength	0.9340	1.0065	1.0090	0.9185
Resolution (Å)	58.42-3.50 (3.69-3.50)	58.0-4.20 (4.43-4.20)	58.00-4.20 (4.43-4.20)	58.00-4.20 (4.43-4.20)
R_{sym} or R_{merge}	0.081 (0.762)	0.045 (0.251)	0.046 (0.259)	0.086 (0.760)
$I/\sigma I$	29.2 (5.1)	29.4 (10.0)	29.9 (9.0)	16.5 (3.4)
Completeness (%)	99.9 (99.9)	99.6 (100.0)	99.5 (100.0)	99.9 (100.0)
Redundancy	26.7 (27.1)	12.7 (13.5)	12.8 (13.6)	12.9 (13.6)
Refinement				
Resolution (Å)	58.42-3.50 (3.66-3.50)			
No. reflections	7640 (934)			
R_{work}/ R_{free}	30.2/32.3 (32.5/36.0)			
No. atoms				
SRP54	3229			
Signal peptide	156			
Total	3385			
B-factors (Å^2)				
SRP54	150			
Signal peptide	175			
All atoms	152			
R.m.s deviations				
Bond lengths (Å)	0.014			
Bond angles (°)	1.619			

*Highest resolution shell is shown in parenthesis.

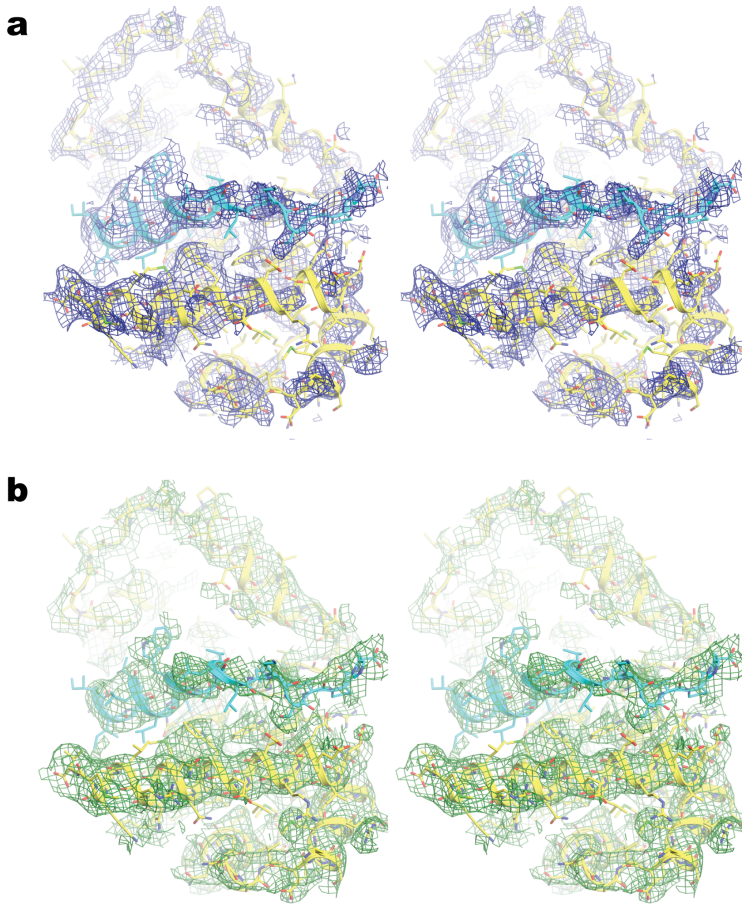

Figure S5 | **Experimental electron density maps. a,** An unbiased map calculated by combining the Hg MAD phases with molecular replacement phases from a homologous NG domain (PDB:1J8M), followed by solvent flattening. **b**, A simulated annealing omit map, omitting both the signal sequence and the finger loop sequence. All the maps are contoured at about 1σ. The atomic model is shown as bonds overlaid by ribbons along the backbone. Atoms are coloured blue (nitrogen), red (oxygen), green (sulphur), yellow (carbon in M domain) and cyan (carbon in signal peptide).

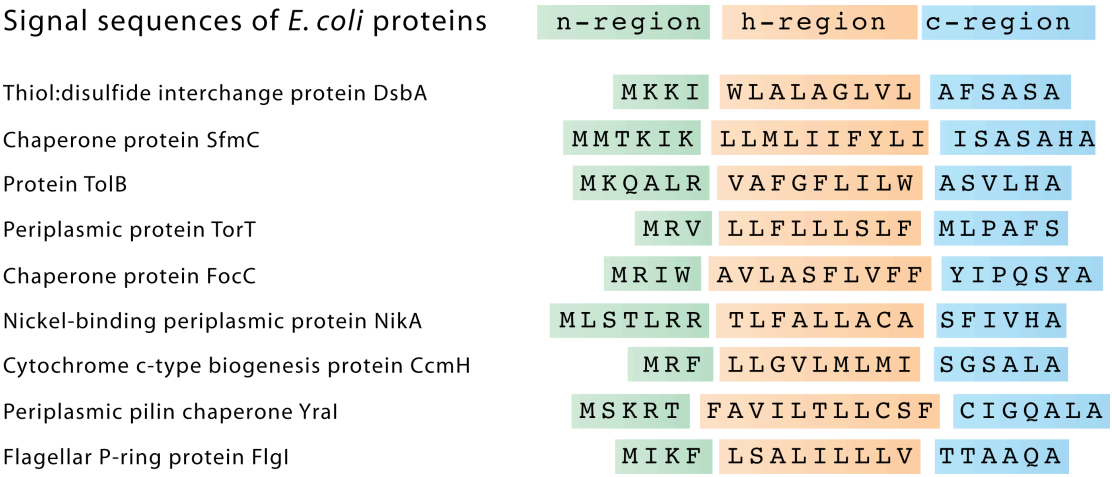

Figure S6 | **List of SRP-dependent signal sequences.** These sequences were experimentally verified to be SRP-dependent by Huber *et al.*[20] The n-, h- and c-regions are defined by SignalP (http://www.cbs.dtu.dk/services/SignalP/)

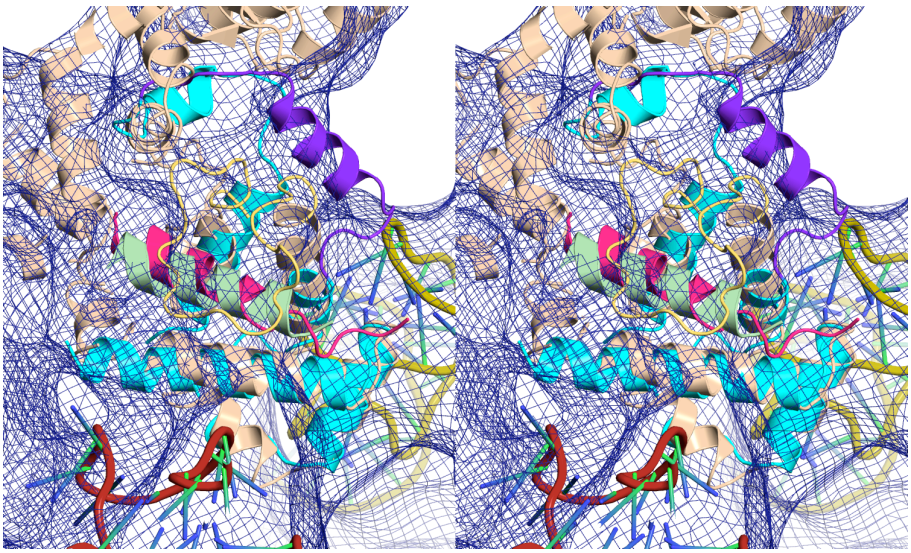

Figure S7 | **Superposition of SRP54* onto a cryoEM model of SRP-RNC complex**. The cryoEM map (pdbe code: emd_1264) and its interpretation (pdb code: 2J37) by Halic *et al.*[15] are shown around the SRP54 M domain. The map has been filtered to 10Å resolution, contoured at about 1 sigma and carved to a 13Å cutoff around the 2J37 model. The crystal structure of SRP54* is aligned to 2J37 by Cα atoms of the M domain with an rms difference of 2.4Å. The signal peptide became superposed with the model helix for the signal sequence. The finger loop and finger helix of the crystal structure is partially contained in the cryoEM envelope. Color code: 2J37, SRP54, tan; signal sequence, pale green; finger loop, yellow. Crystal structure of SRP54* -- SRP54, cyan; signal sequence, magenta; finger loop and finger helix, purple.

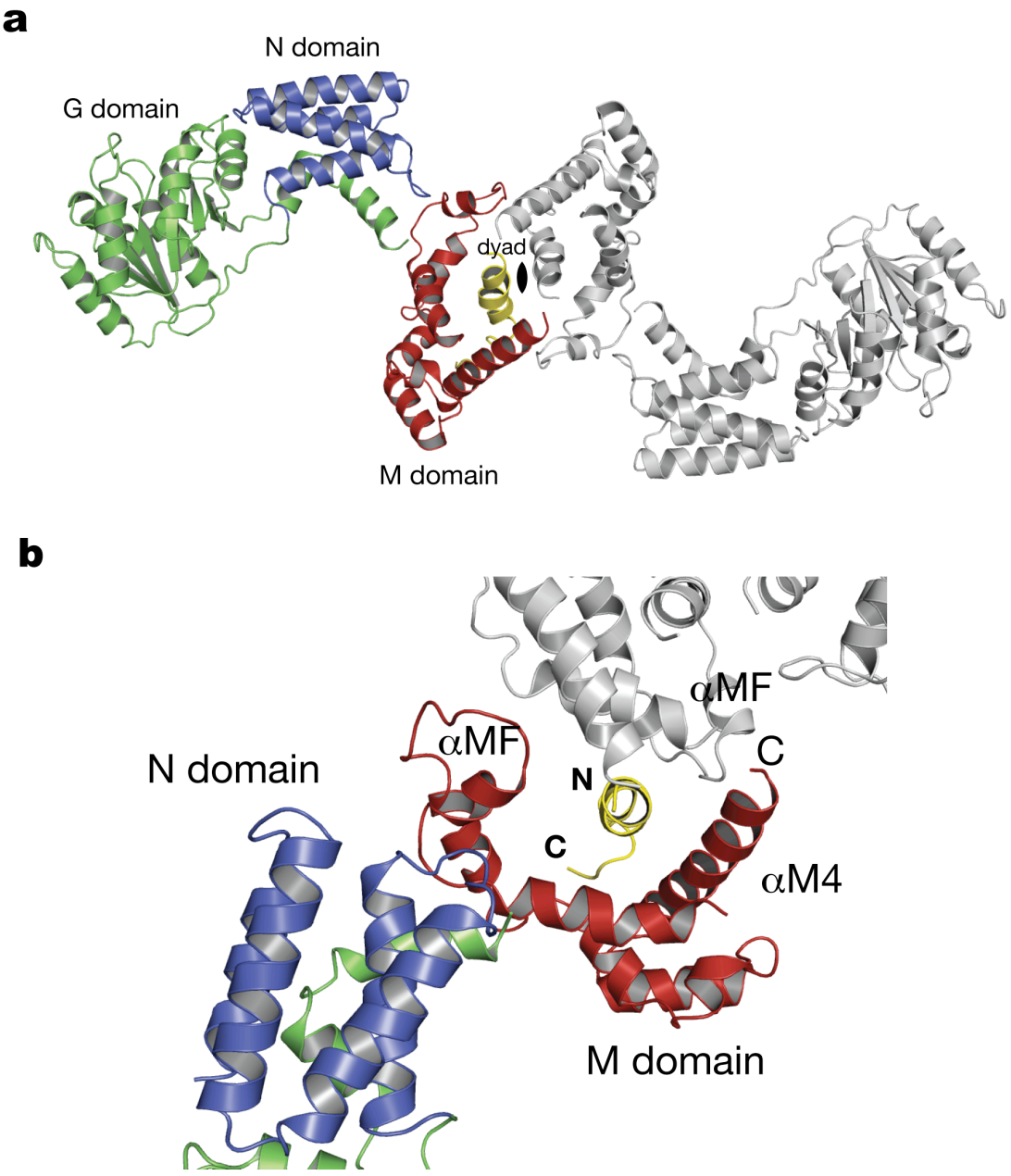

Figure S8 | **Packing of SRP54*. a**, SRP54* dimer related by crystallographic dyad. One monomer is coloured and the other in grey. Signal peptide (yellow) is connected to the grey SRP54 moiety. **b**, SRP54* dimer viewed along the signal peptide to show that the closing movement of αMF towards the signal peptide is restricted in SRP54* by lattice contacts of the αM1b-αMF loop.

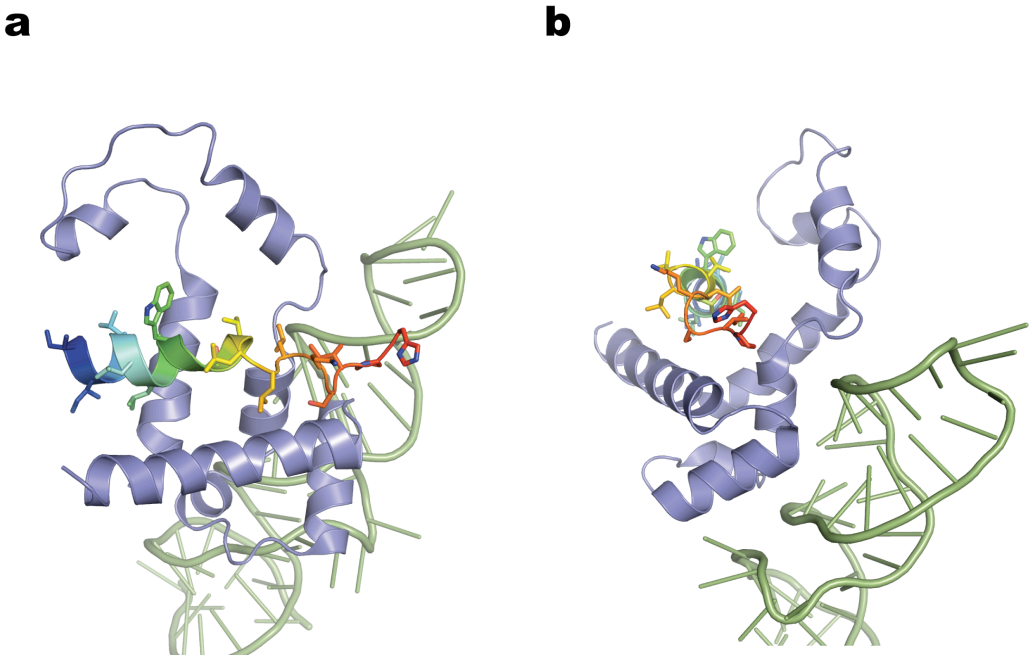

Figure S9 | **A model of the M domain of SRP54* bound to SRP RNA.** The signal peptide is rainbow coloured (N-terminus, blue; C-terminus, red). The M domain of SRP54* was overlaid onto the M domain of the M domain-RNA complex (PDB: 1QZW)[19] by least-square fit of their helices αM2-αM4. **a**, face-on and **b**, end-on views of the signal peptide.