APPENDIX

Brown et al., Figure S1

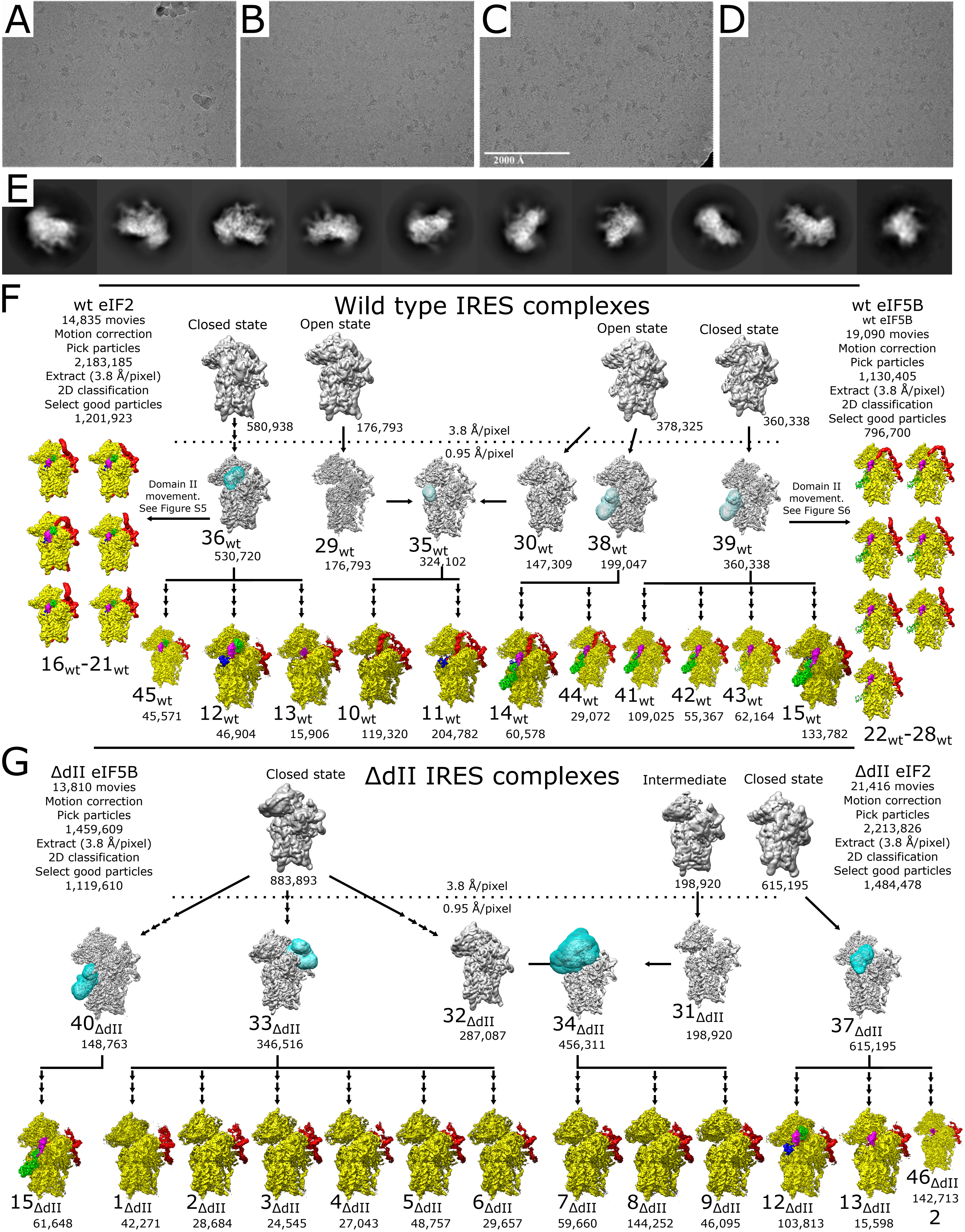

Figure S1. Classification of HCV IRES-containing ribosomal complexes.

(A-D) Example micrographs for (A) the *wt* IRES/eIF2-containing, (B) the *wt* IRES/eIF5B-containing, (C) the ΔdII IRES/eIF2-containing, and (D) the ΔdII IRES/eIF5B-containing samples. Further details are in Table S2. Scale bar is 2000 Å.

(E) Example 2D classification results for the *wt* IRES/eIF2-containing sample.

(F-G) Classification scheme for (F) *wt* and (G) ΔdII IRES-containing samples. For full details regarding classification see Methods. Final maps are colored showing the 40S subunit (yellow), IRES (red), eIF1A (blue), Met-tRNA$_i^{Met}$ (magenta), and initiation factors eIF2 or eIF5B (green). Full details for complexes are available in Table S3 (structures $1_{\Delta dII}$-$15_{wt/\Delta dII}$), Table S5 (16_{wt}-28_{wt}), and Table S7 (structures 29_{wt}-$46_{\Delta dII}$).

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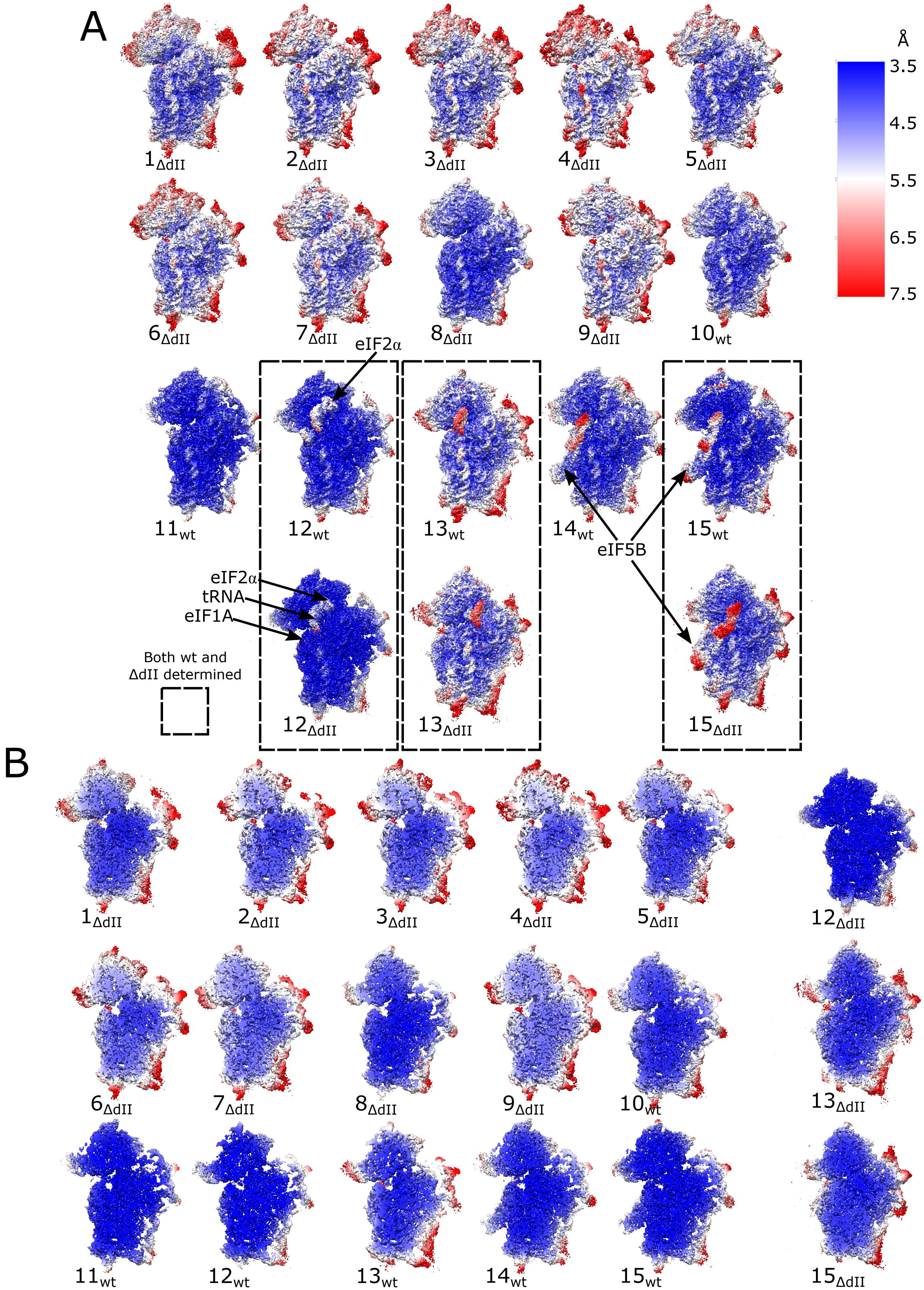

Figure S2. Local resolution of complexes.

(A) Local resolution of the indicated HCV IRES complexes.

(B) Central slice of each complex.

Local resolution values and filtered maps can be found under EMDB accession codes for each structure (see Table S3).

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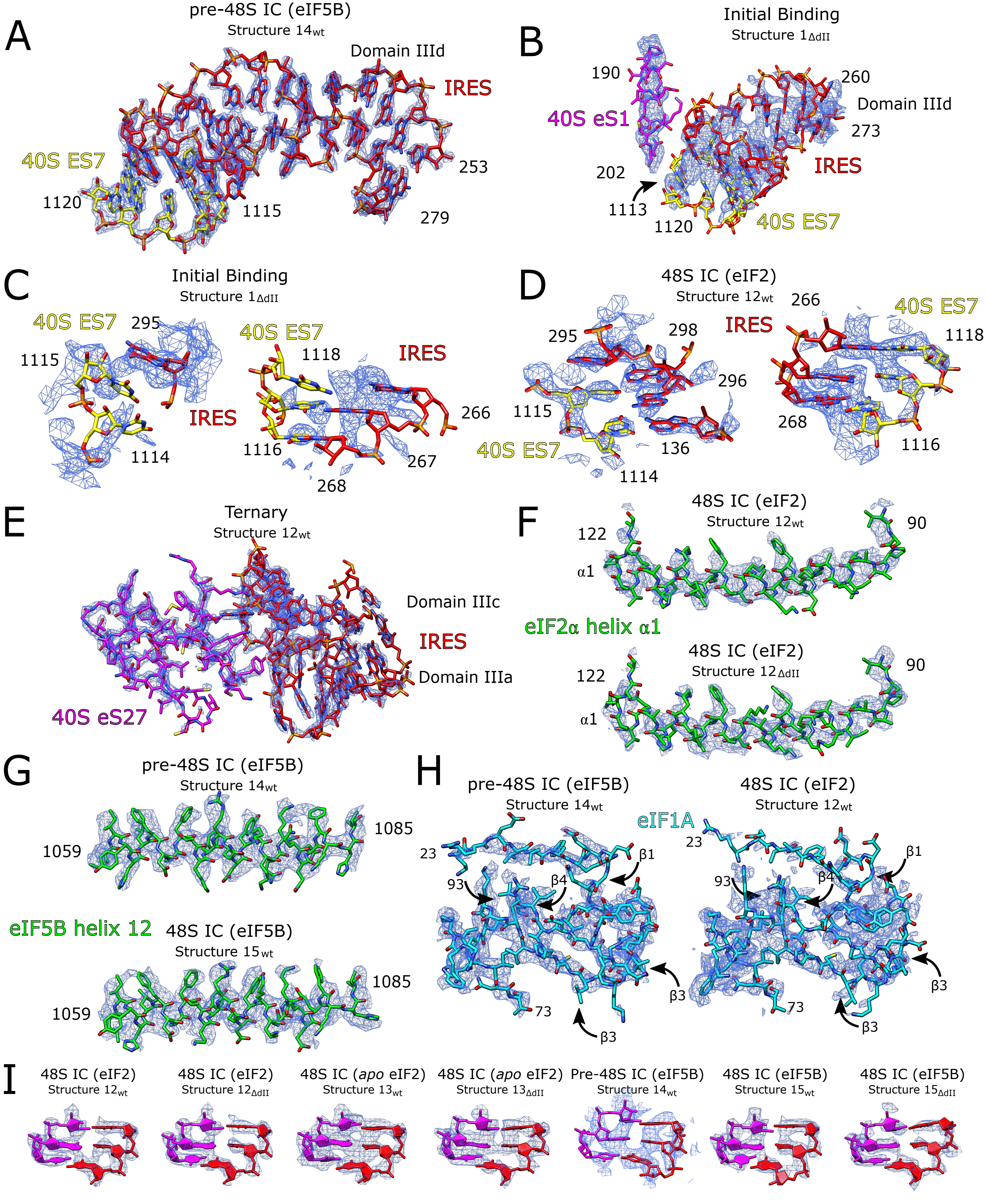

Figure S3. Example densities.

(A) Domain IIId (red) and ES7 (yellow) for the pre-48S IC (structure 14_{wt}).

(B) Domain IIId (red) and ES7 (yellow) for the minimally bound complex (structure $1_{\Delta dII}$).

(C) ES7 and IRES domain IIId for the minimally bound complex (structure $1_{\Delta dII}$).

(D) ES7 and IRES domain IIId for the 48S IC (structure 12_{wt}).

(E) Contact between domain IIIa/IIIc (red) and riboprotein eS27 (magenta) in the ternary complex (structure 12_{wt}).

(F) eIF2 α-helix 1 in the *wt* IRES 48S IC (structure 12_{wt}), top, and the ΔdII IRES 48S IC (structure $12_{\Delta dII}$), bottom.

(G) eIF5B α-helix 12 in the eIF5B-containing pre-48S IC (structure 14_{wt}), top, and the 48S IC (structure 15_{wt}), bottom.

(H) eIF1A example densities from the eIF5B-containing pre-48S IC (structure 14_{wt}), left, and the eIF2-containing 48S IC (structure 12_{wt}), right.

(I) Initiation codon (red) and tRNA anticodon (magenta) for all complexes where mRNA is present in the P site. Diagram shows identity of nucleotides and hydrogen bonds for tRNA and initiation codon bases in each accompanying figure.

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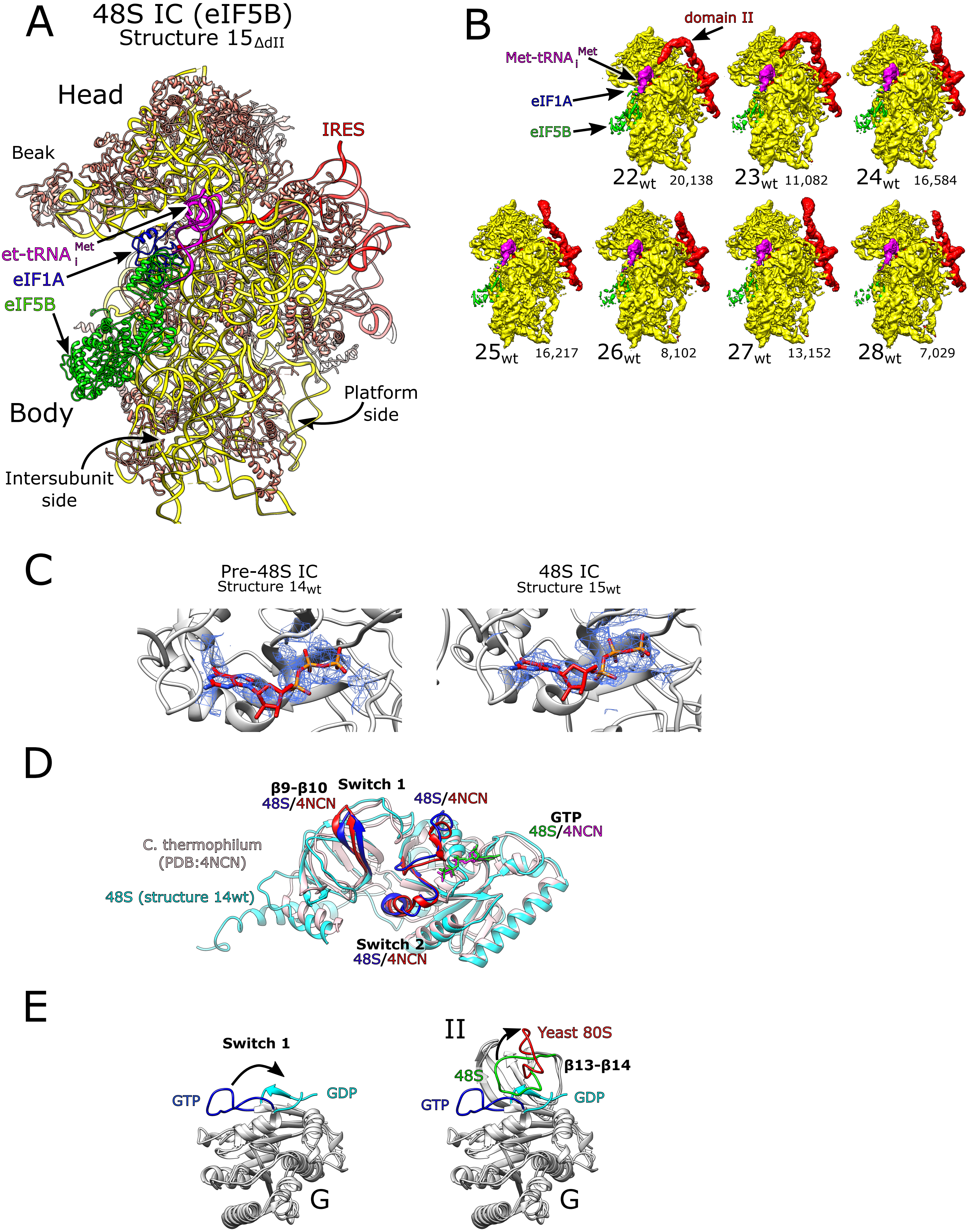

Figure S4. eIF5B related complexes.

(A) Overview of the ΔdII IRES eIF5B-containing 48S initiation complex (structure $15_{\Delta dII}$).

(B) Maps showing that domain II occupies multiple positions in the *wt* IRES eIF5B-containing 48S IC.

(C) Coulomb potential for the GTP nucleotide in eIF5B G domain for pre-48S IC (structure 14_{wt}), left, and 48S IC (structure 15_{wt}), right.

(D) Comparison of the conformations of switch 1, switch 2 and the β9-β10 loop of GTP-bound *O. cuniculus* eIF5B in the pre-48S IC (structure 14_{wt}) (cyan) with corresponding elements of GTP-bound eIF5B from *C. thermophilum* (PDB: 4NCN) (light pink). Switch 1, switch 2 and the β9-β10 loop are labelled and colored for *O. cuniculus* (blue) and *C. thermophilum* (red). The GTP nucleotides bound to eIF5B from *O. cuniculus* and *C. thermophilum* are green and magenta, respectively.

(E) Repositioning of the β13- β14 loop in domain II of eIF5B in 80S compared to 40S ribosomal complexes. (*Left*) The GTP-bound G domain of *O. cuniculus* eIF5B (structure 14_{wt}) aligned with the GDP-bound *C. thermophilum* eIF5B (PDB: 4NCL) with switch 1 colored blue or cyan, respectively. (*Right*) Domain II of GTP-bound *O. cuniculus* eIF5B in pre-48S ICs (structure 14_{wt}) aligned with domain II of GTP-bound yeast eIF5B in 80S ribosomes (PDB: 6WOO) with the β13-β14 loop colored green or red, respectively. Notably, the position of the β13- β14 loop in 40S complexes (green) would interfere with transition of switch 1 from GTP-bound (blue) to GDP-bound (cyan) conformations.

Table S1. **Sample composition.**

IRES	Wild type	Wild type	ΔdII	ΔdII
Ternary Complex	eIF2	eIF5B	eIF2	eIF5B
40S small subunit	3.5 pmol	3.5 pmol	3.5 pmol	3.5 pmol
Met-tRNA$_i^{Met}$	3.5 pmol	3.5 pmol	3.5 pmol	3.5 pmol
eIF2	6 pmol	-	6 pmol	-
eIF5B	-	10 pmol	-	10 pmol
eIF3	4.5 pmol	4.5 pmol	4.5 pmol	4.5 pmol
eIF1A	10 pmol	10 pmol	10 pmol	10 pmol
wt HCV IRES	7 pmol	7 pmol	-	-
ΔdII HCV IRES	-	-	7 pmol	7 pmol

Table S2. Data collection statistics.

IRES	Wild type	Wild type	ΔdII	ΔdII
Ternary Complex	eIF2	eIF5B	eIF2	eIF5B
Electron microscope	Polara F30	Polara F30	Polara F30	Polara F30
Magnification	52,000	52,000	52,000	52,000
Detector	K3 Summit	K3 Summit	K3 Summit	K3 Summit
Pixel size (Å)	0.95	0.95	0.95	0.95
Voltage (kV)	300	300	300	300
Electron exposure (e-/Å2)	70.9	70.9	70.9	70.9
Defocus range (µm)	1-2.5	1-2.5	1-2.5	1-2.5
Symmetry imposed	C1	C1	C1	C1
Total collection sessions	2	4	3	2
Total micrographs	14,815	27,263	22,735	13,809
No. collected at 30° tilt	14,815	20,509	17,695	13,809
Initial no. picked particles	2,183,185	1,133,335	2,213,826	1,459,609
Initial particles per micrograph	147	42	97	106
Particles after 2D classification	1,201,923	736,700	1,484,658	1,119,610
After 2D per micrograph	81	36	84	81

Table S3. Refinement and validation statistics.

Structure	1ΔdII	2ΔdII	3ΔdII	4ΔdII	5ΔdII	6ΔdII	7ΔdII	8ΔdII	9ΔdII	10wt	11wt	12wt	12ΔdII	13wt	13ΔdII	14wt	15wt	15ΔdII
IRES	ΔdII	ΔdII	ΔdII	ΔdII	ΔdII	ΔdII	ΔdII	ΔdII	ΔdII	wt	wt	wt	ΔdII	wt	ΔdII	wt	wt	ΔdII
name										Binary	Ternary	48S IC (eIF2)	48S IC (eIF2)	48S IC (w/o eIF2)	48S IC (w/o eIF2)	pre-48S IC (eIF5B)	48S IC (eIF5B)	48S IC (eIF5B)
EMD	25527	25528	25529	25530	25531	25532	25533	25534	25535	25536	25537	25538	25539	25540	25541	25542	25543	25544
PDB	7SYG	7SYH	7SYI	7SYJ	7SYK	7SYL	7SYM	7SYN	7SYO	7SYP	7SYQ	7SYR	7SYS	7SYT	7SYU	7SYV	7SYW	7SYX
Reconstruction																		
Particles	42,271	28,684	24,545	27,043	48,757	29,657	59,660	144,252	46,095	119,320	204,782	46,904	103,813	15,598	15,906	60,578	133,782	49,524
Map resolution (Å)	4.3	4.6	4.5	4.8	4.2	4.5	4.8	4.0	4.6	3.8	3.8	3.6	3.5	4.4	4.6	3.8	3.7	3.7
FSC threshold	0.143	1.143	2.143	3.143	4.143	5.143	6.143	7.143	8.143	9.143	10.143	11.143	12.143	13.143	14.143	15.143	16.143	17.143
Map resolution range	3.65-22.06	3.74-27.01	3.74-23.97	3.88-35.75	3.64-19.89	3.77-22.88	3.89-31.38	3.47-21.68	4.10-22.32	3.44-24.85	3.31-21.92	3.14-19.71	3.00-26.78	3.64-30.36	3.56-32.65	3.42-19.24	3.27-50.00	3.26-17.65
Refinement																		
Model resolution (Å)	4.4	4.8	4.7	4.8	4.5	4.8	4.6	4.0	4.8	4.1	3.9	3.8	3.4	4.7	4.3	3.9	3.8	4.2
FSC threshold	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5
Map sharpening factor	-103.01	-113.25	-93.88	-102.84	-104.30	-101.41	-142.94	-128.11	-138.92	-111.66	-114.32	-81.81	-83.72	-101.78	-76.23	-74.85	-89.27	-57.82
Model composition:																		
Chains	37	38	38	38	38	38	38	38	38	40	39	40	40	38	38	41	40	41
Protein residues	4891	4891	4891	4891	4891	4891	4891	4891	4891	4896	4997	5177	5177	4896	4896	5644	5622	5622
Nucleotides	1859	1859	1859	1859	1859	1859	1859	1859	1863	1943	1944	1960	1960	1960	1960	2019	1960	1960
non-hydrogen atoms	78773	78761	78761	78761	78761	78748	78761	78761	78846	80500	81427	83005	82981	80962	80962	88186	86758	86744
Ligands (Zn, Mg, GTP)	2, 0, 0	2, 0, 0	2, 0, 0	2, 0, 0	2, 0, 0	2, 0, 0	2, 0, 0	2, 0, 0	2, 0, 0	2, 0, 0	2, 0, 0	1, 0, 0	1, 0, 0	1, 0, 0	1, 0, 0	1, 2, 1	1, 1, 1	1, 1, 1
B factors (Å):																		
Protein	101.6	107.24	109.73	118.76	100.54	77.22	101.23	64.92	125.01	73.5	82.13	51.25	52.65	76.99	97.14	78.23	64.67	37.31
RNA	122.84	125.22	119.39	138.94	115.81	94.45	115.38	87.93	120.7	95.98	101.52	75.46	71.77	108.65	120.61	94.3	84.42	58.41
RMS deviations:																		
Bond lengths (Å)	0.003	0.003	0.004	0.003	0.001	0.004	0.003	0.002	0.003	0.004	0.003	0.003	0.003	0.004	0.003	0.003	0.003	0.003
Bond angles (°)	0.744	0.786	0.869	0.789	0.83	0.865	0.833	0.495	0.854	0.817	0.81	0.775	0.748	0.855	0.797	0.776	0.78	0.778
Validation:																		
Molprobity score	1.61	1.66	1.74	1.70	1.68	1.77	1.65	1.84	1.65	1.59	1.56	1.50	1.45	1.59	1.56	1.59	1.52	1.53

Clashscore	4.83	5.13	5.70	5.37	5.27	6.16	5.28	9.66	5.43	4.63	4.35	3.73	3.53	4.83	4.35	4.43	3.89	4.26
Poor rotamers (%)	0	0.02	0	0.02	0.02	0.02	0	0	0.05	0.07	0.02	0.07	0.11	0.07	0.07	0.02	0.02	0
Ramachandran plot:																		
Disallowed (%)	0.02	0.02	0.04	0	0.02	0.02	0	0	0	0.02	0.02	0	0	0	0	0	0	0
Favoured (%)	94.84	94.23	93.32	93.90	94.05	93.38	94.73	95.25	94.90	94.84	95.03	95.16	95.59	95.05	95.09	94.61	95.06	95.39
Allowed (%)	95.58	95.02	94.19	94.69	94.88	94.25	95.56	95.75	95.75	95.66	95.84	95.94	96.34	95.91	95.89	95.39	95.84	96.17
RNA validation:																		
Length outliers (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Angle outliers (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sugar pucker outliers (%)	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Average suit	0.494	0.495	0.472	0.482	0.499	0.462	0.483	0.519	0.475	0.510	0.519	0.546	0.549	0.519	0.519	0.520	0.534	0.535

Table S4. Contacts between the HCV IRES and 40S subunit.

IRES Domain	**No.**		**40S**	**No.**	**Res.**	**1ΔdII**	**2ΔdII**	**3ΔdII**	**4ΔdII**	**5ΔdII**	**6ΔdII**	**7ΔdII**	**8ΔdII**	**9ΔdII**	**10wt**	**11wt**	**12wt**	**12ΔdII**	**13wt**	**13ΔdII**	**14wt**	**15wt**	**15ΔdII**
II	68	G	eS25	78	Lys	no	no	no	no	no	no	no	no	no	yes	yes	no	no	no	no	yes	no	no
II	81	A	uS7	130, 133	Arg, Thr	no	no	no	no	no	no	no	no	no	yes	yes	no	no	no	no	yes	no	no
II	83-86	C	uS11	66	Arg	no	no	no	no	no	no	no	no	no	yes	yes	no	no	no	no	yes	no	no
II	101	U	eS25	76	Arg	no	no	no	no	no	no	no	no	no	yes	yes	no	no	no	no	yes	no	no
hIII1	136	A	18S	1115	U	no	no	no	no	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
IIIa	163/164	G/U	eS27	41	Tyr	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
IIIc	233	G	eS27	43,80	Ile,Arg	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
IIId	266	G	18S	1118	C	no	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
IIId	267	G	18S	1117	C	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
IIId	268	G	18S	1116	C	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
IIIe	295	G	18S	1114	U	no	no	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
IIIe	296	A	18S	1114	U	no	no	no	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
IIIe	300	G	eS1	147	Asn	no	no	no	no	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes

Table S5. Maps showing HCV IRES domain II movement.

Structure	16_{wt}	17_{wt}	18_{wt}	19_{wt}	20_{wt}	21_{wt}	22_{wt}	23_{wt}	24_{wt}	25_{wt}	26_{wt}	27_{wt}	28_{wt}
Sample:	eIF2	eIF2	eIF2	eIF2	eIF2	eIF2	eIF5B	eIF5B	eIF5B	eIF5B	eIF5B	eIF5B	eIF5B
EMDB	25545	25546	25547	25548	25549	25550	25551	25552	25553	25554	25555	25556	25557
Reconstruction													
Particles	41,393	39,148	49,034	66,090	24,190	36,155	20,138	11,082	16,584	16,217	8,102	13,152	7,029
Map resolution (Å)	3.8	3.8	3.7	3.6	3.9	3.8	5.0	5.4	5.2	4.9	6.0	5.3	6.2
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
Map sharpening factor	-79.90	-90.02	-71.72	-86.21	-93.54	-82.41	-169.5	-144.1	-156.7	-120.5	-135.7	-108.5	-132.1
Map resolution range	3.2-13.1	3.3-13.7	3.2-20.2	3.1-14.3	3.4-16.4	3.3-14.5	4.3-20.6	4.6-21.2	4.4-23.8	4.0-42.6	4.4-49.0	4.1-23.9	45.-26.3

Table S6. Contacts between eIF5B and the 40S subunit. Contacts between eIF5B in the pre-48S IC (structure 14_{wt}), 48S IC (structure $15_{wt/\Delta dII}$), or yeast eIF5B-containing 80S (PDB: 6WOO).

Domain	**no.**	**res.**	**chain**	**Helix**	**no.**	**res.**	**14wt**	**15wt**	**15ΔdII**	**PDB: 6WOO**
II	957	GLU	uS12	n/a	142	ARG	yes	no	yes	yes
II	965	ARG	18S	15	478	G	yes	yes	yes	no
II	965	ARG	18S	15	488	OP1	yes	yes	yes	yes
II	980	ASN	18S	5	50	A	yes	yes	yes	no
II	984	LYS	18S	5	488	U	yes	yes	no	yes
II	999	LYS	18S	5	487	U	yes	yes	yes	yes
III	1077	HIS	18S	5	480	OP1	yes	yes	yes	yes
III	1078	LYS	18S	5	482	OP1	no	no	no	no
III	1103	ARG	18S	14	460	A	yes	yes	yes	no
III	1103	ARG	18S	14	461	U	yes	yes	yes	no
III	1106	ARG	18S	14	459	U	yes	yes	yes	no

Table S7 Additional maps produced during data processing.

Structure	EMDB (PP job)	Particles	Map resolution (Å)	FSC threshold	Map sharpening factor	Note
29_{wt}	25588	176,793	3.8	0.143	-132.09	Pre-consensus (binary, eIF2)
30_{wt}	25589	147,309	4.1	0.143	-113.23	Pre-consensus (binary, eIF5B)
$31_{\Delta dII}$	25590	198,920	3.5	0.143	-85.69	Pre-consensus (head movement, eIF2)
$32_{\Delta dII}$	25591	287,087	4.5	0.143	-218.18	Pre-consensus (head movement, eIF5B)
$33_{\Delta dII}$	25592	346,516	3.6	0.143	-112.77	Consensus (IRES binding)
$34_{\Delta dII}$	25593	456,311	3.5	0.143	-104.49	Consensus (head movement)
35_{wt}	25594	342,102	3.3	0.143	-99.38	Consensus (binary)
36_{wt}	25595	530,720	3.1	0.143	-114.46	Focused (eIF2)
$37_{\Delta dII}$	25596	615,195	3.3	0.143	-108.92	Focused (eIF2)
38_{wt}	25597	199,047	3.6	0.143	-84.45	Focused (open, eIF5B)
39_{wt}	25598	360,338	3.4	0.143	-107.37	Focused (closed, eIF5b)
$40_{\Delta dII}$	25599	148,763	3.4	0.143	-65.49	Focused (closed, eIF5B)
41_{wt}	25600	109,025	3.7	0.143	-97.61	Closed, eIF5B
42_{wt}	25601	55,367	3.8	0.143	-89.55	Closed, eIF5B
43_{wt}	25602	62,164	3.9	0.143	-117.14	Closed, eIF5B
44_{wt}	25603	29,072	4.2	0.143	-70.09	Open, eIF5B
45_{wt}	25604	45,571	3.6	0.143	-82.06	Closed, eIF2
$46_{\Delta dII}$	25620	142,713	3.6	0.143	-104.37	Closed, eIF2 ΔdII