Expanded View Figures

Figure EV1. All HCV IRES-mediated initiation complexes.

A Segmented maps of the indicated 40S ribosomal complexes assembled on the *wt* or ΔdII HCV IRES, showing the 40S subunit (yellow), IRES (red), eIF1A (blue), Met-$tRNA_i^{Met}$ (magenta), and initiation factors eIF2 (green) or eIF5B (cyan). Complexes assembled on the *wt* or ΔdII HCV IRES that share identical 40S subunit conformation and factor composition are enclosed by dashed lines.

B Interaction between the IRES and the 40S subunit at different stages of initiation. Contacts with ribosomal proteins (red) and 18S rRNA (yellow), and the AUG codon (blue) are marked for the indicated complexes. See Appendix Table S4 for additional information.

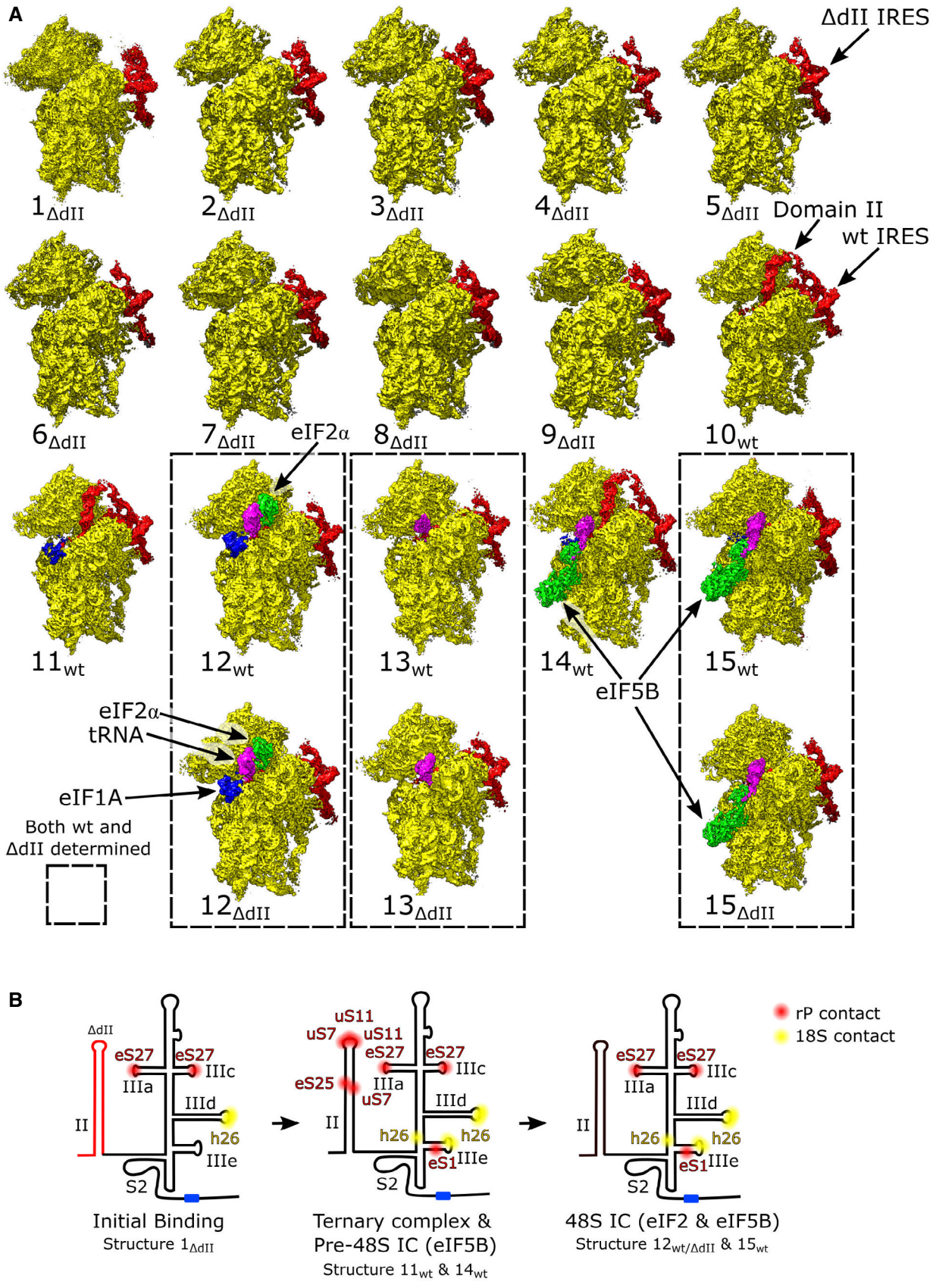

Figure EV1.

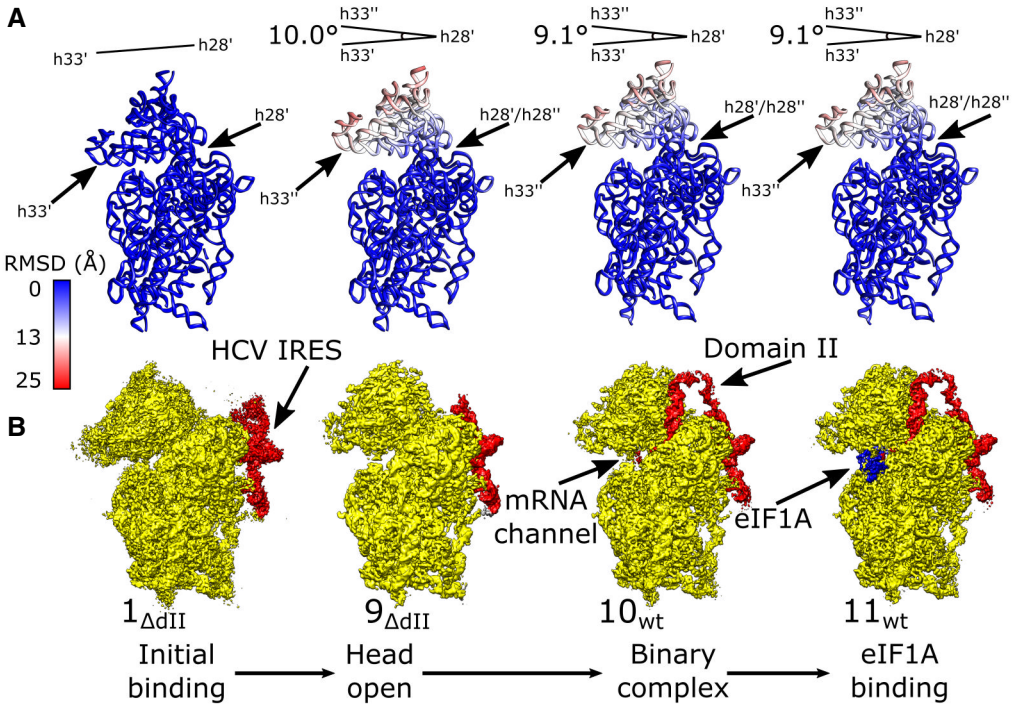

Figure EV2. Conformational changes in the 40S subunit induced by association with the *wt* or ΔdII HCV IRES.

A RMSD (Å) of 18S rRNA for complexes shown directly underneath in (B) relative to the minimally bound state (structure $1_{\Delta dII}$), color-coded as in the inset key. The angle formed between helix 33 (h33′) and helix 28 (h28′) in structure $1_{\Delta dII}$ and helix 33 (h33′′) in other complexes is indicated.

B Segmented maps for the indicated complexes showing 40S subunit (yellow), IRES (red), and eIF1A (blue) organized in a putative sequence showing the minimally bound state (structure $1_{\Delta dII}$), fully opened binary complex containing ΔdII IRES (structure $9_{\Delta dII}$), binary complex containing the *wt* IRES (structure 10_{wt}), and the eIF1A-containing ternary complex (structure 11_{wt}).

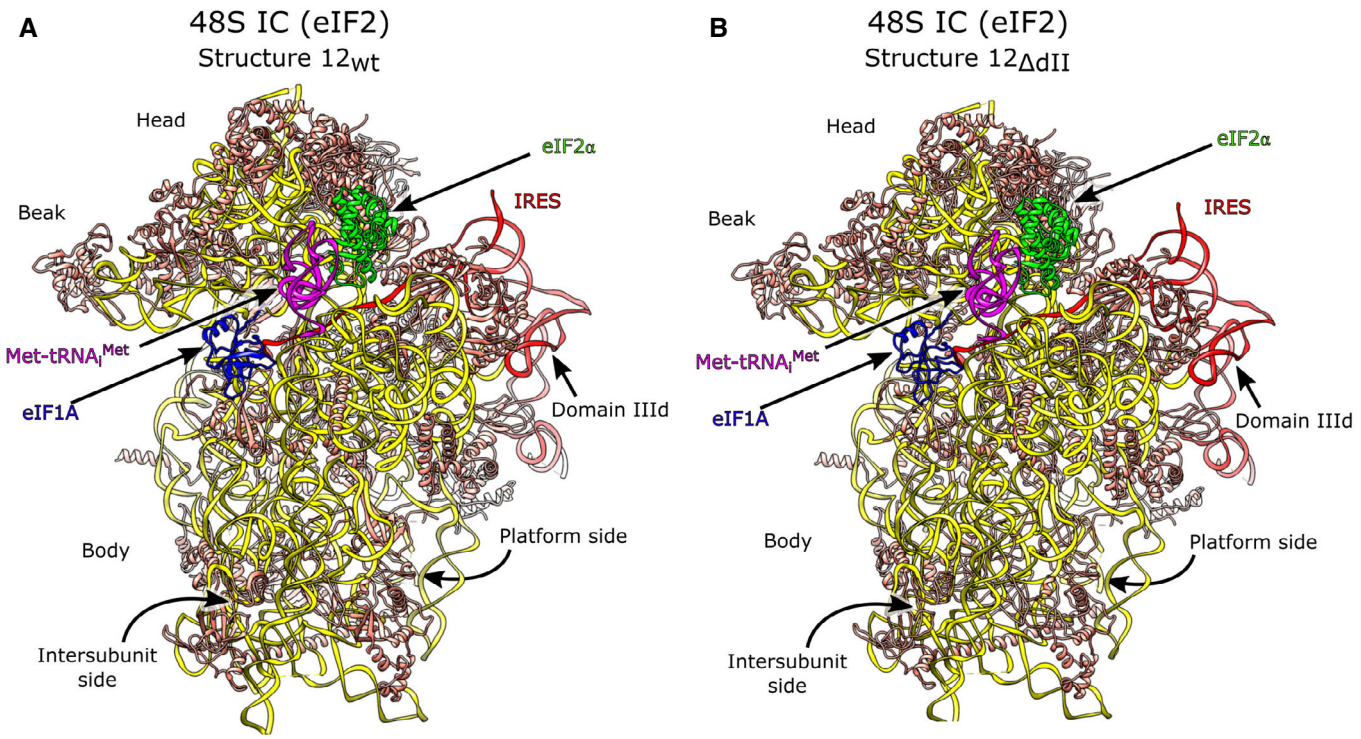

Figure EV3. HCV IRES·eIF2-containing 48S initiation complexes.

A, B Overview of (A) the *wt* IRES eIF2-containing 48S IC (structure 12_{wt}) and (B) the ΔdII IRES eIF2-containing 48S IC (structure $12_{\Delta dII}$).

Figure EV4. The HCV IRES·eIF5B-containing 48S initiation complex.

A, B Overview of (A) the *wt* IRES eIF5B-containing pre-48S IC (structure 14_{wt}) and (B) the *wt* IRES eIF5B-containing 48S IC (structure 15_{wt}).

C Global position of eIF5B bound to the intersubunit face of the 40S ribosomal subunit. Interactions between 18S rRNA (yellow), uS12 (salmon), and eIF1A (blue) are shown.

D Position of eIF5B and tRNA in the 48S and 80S initiation complexes. Domains II, G, and III of eIF5B undergo relatively little movement between the 48S (green) and 80S stages (red), whereas domain IV translates and rotates (see E) causing movement in the tRNA between the two complexes (magenta and orange, respectively). Arrows show displacement for labeled domains or components between the eIF5B-containing 48S IC and the pre-elongation 80S ribosome.

E The position of eIF5B domain IV in the 48S IC (green, left panel) would clash with 28S rRNA H89 and uL16 in the 60S subunit (left panel). To avoid this clash, in the 80S complex, domain IV (red, middle panel) translates by 6.4 Å toward the platform side of the 40S subunit and rotates by 30.9° causing the tRNA to rotate by 12.4° and translate by 6.4 Å toward the head (right panel).

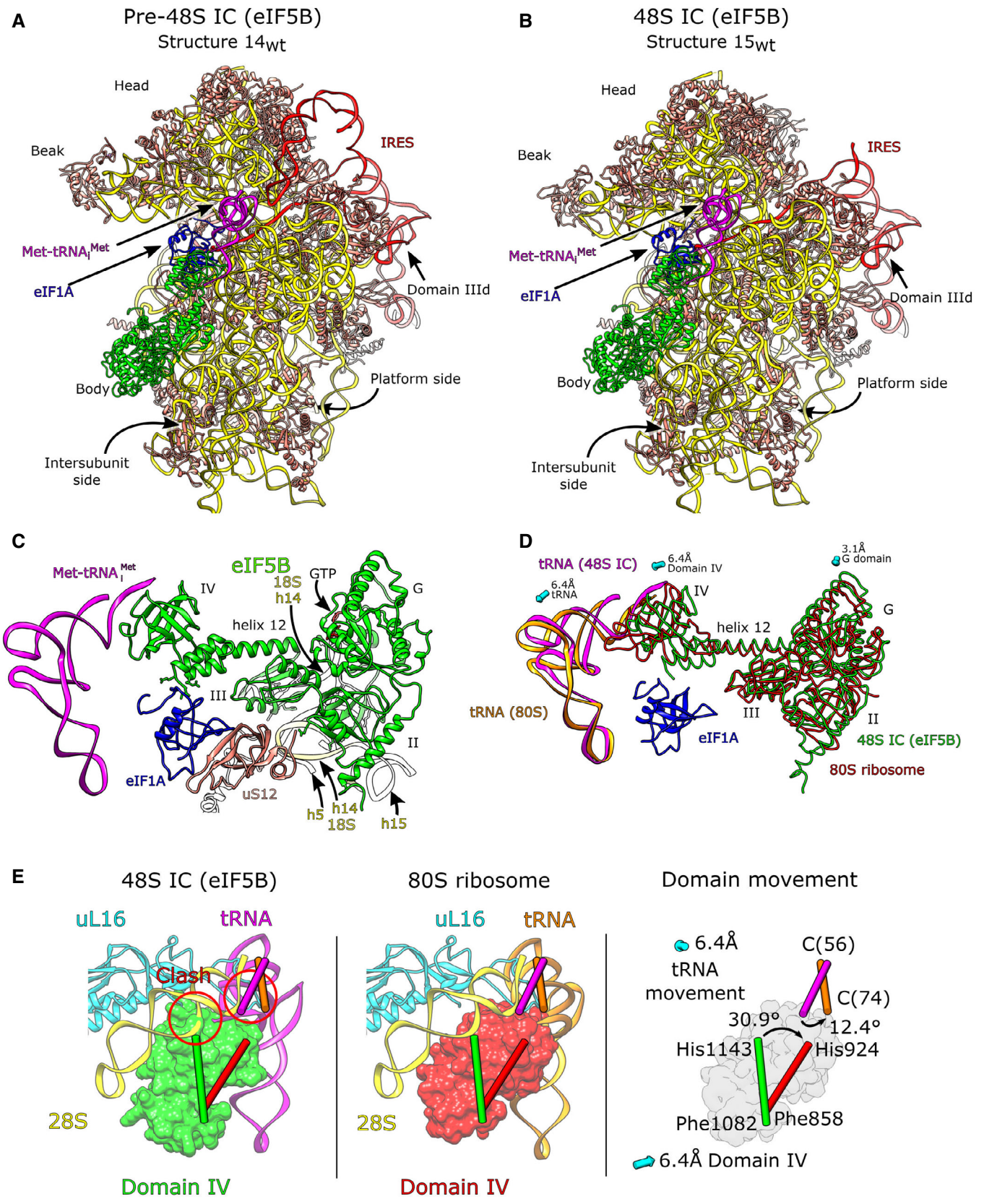
A
Pre-48S IC (eIF5B)
Structure 14wt
Head
Beak
IRES
Met-tRNAiMet
eIF1A
Domain IIId
Body
Platform side
Intersubunit side
B
48S IC (eIF5B)
Structure 15wt
Head
Beak
IRES
Met-tRNAiMet
eIF1A
Domain IIId
Body
Platform side
Intersubunit side
C
Met-tRNAiMet
eIF5B
18S h14
GTP
G
IV
helix 12
III
II
eIF1A
uS12
h5
h14 18S
h15
D
tRNA (48S IC)
6.4Å tRNA
6.4Å Domain IV
3.1Å G domain
IV
G
helix 12
tRNA (80S)
III
II
eIF1A
48S IC (eIF5B)
80S ribosome
E
48S IC (eIF5B)
uL16
tRNA
Clash
28S
Domain IV
80S ribosome
uL16
tRNA
28S
Domain IV
Domain movement
6.4Å tRNA movement
C(56)
C(74)
30.9°
12.4°
His1143
His924
Phe1082
Phe858
6.4Å Domain IV

Figure EV4.