Supplementary Figure 1 GMPPNP is efficiently exchanged from affinity-purified 48S complexes assembled and purified in the absence of recombinant eIF5.

(A) Exchange of GMPPNP for γ32P-GTP in wt and ΔdII HCV 48S complexes detected by UV crosslinking. 48S complexes assembled in the absence or presence of recombinant eIF5 and stalled with GMPPNP were incubated with γ32P-GTP and subjected to UV crosslinking followed by gel electrophoresis on 4-12% NuPAGE gel and PhosphoImager exposure. Size markers are indicated on the left an lanes are numbered below. UV-induced crosslinks to a 52kDa protein corresponding to the molecular mass of eIF2γ (51,8 kDa) are only detected in particles assembled without addition of recombinant eIF5 (lanes 6 and 8).

(B) Analysis of eIF5-mediated GTP hydrolysis in wt and ΔdII HCV 48S complexes. 48S complexes assembled in absence (no eIF5) or presence (eIF5) of recombinant eIF5 and stalled with GMPPNP were incubated with γ32P-GTP or γ32P-GTP and eIF5 as indicated. 32P-Pi was quantified at different time points reflecting the amount of total GTP hydrolysis. Data represents the average of 3 experiments. GTP hydrolysis occurs only with 48S complexes assembled without addition of recombinant eIF5 and requires incubation with GTP and eIF5.

(C, D, E, F) Analysis of eIF5-mediated eIF2 release from wt (C, D) and ΔdII (E, F) HCV 48S complexes. 48S complexes assembled in absence (no eIF5) or presence (eIF5) of recombinant eIF5 and stalled with GMPPNP were incubated with GTP or GTP and eIF5 as indicated. Binding of eIF2 to 48S complexes was assayed over time by immunoblotting using anti-eIF2α antibodies (C, E). Band intensities were quantified using ImageJ software. For every reaction, the initial eIF2 intensity was set to the 100%-bound reference and used to calculate the percentage of eIF2 bound over time (E, F). Release of eIF2 occurs only from 48S complexes assembled without addition of recombinant eIF5 and requires incubation with GTP and eIF5.