

Expanded View

Supplemental Figure Legends

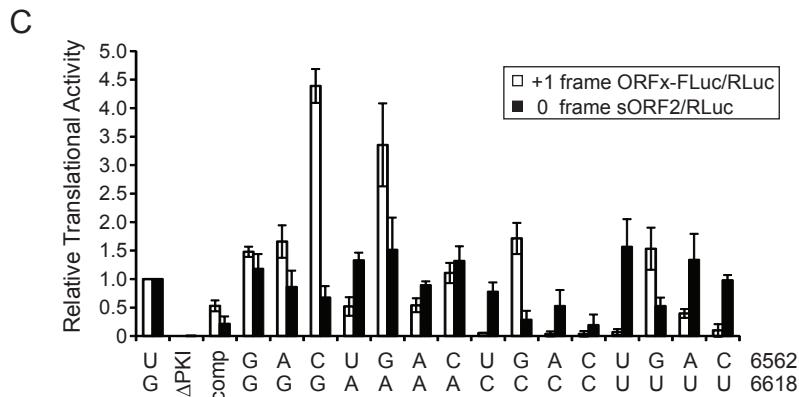
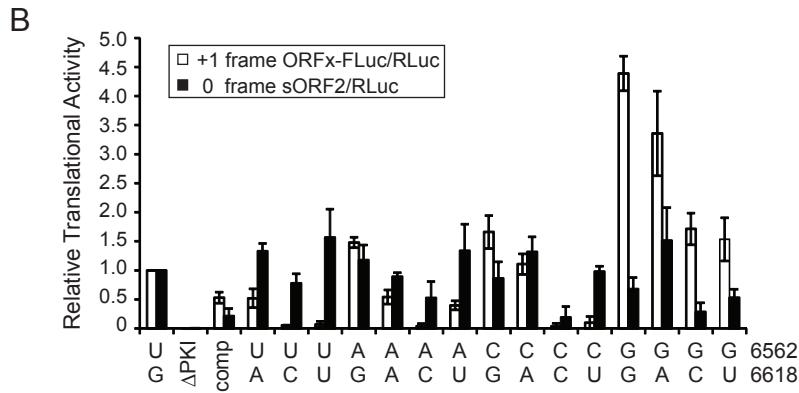
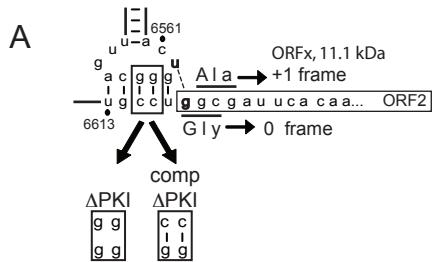
Figure S1 Mutational analysis of U6562 and G6618. (A) PKI domain of the IAPV IGR IRES. The CC6615-6GG mutations that disrupt PKI basepairing and the compensatory mutations that restore basepairing are shown. (B and C) Quantitation of the radiolabeled +1 frame ORFx-Fluc (white bars) and 0 frame sORF2 (black bars) proteins normalized to RLuc. The ratios are normalized to the 0 and +1 frame translation of the bicistronic construct containing the wild-type IAPV IGR IRES given as 1.0. The data are plotted by grouping nucleotide changes to either U6562 (B) or G6618 (C). Averages are shown from at least three independent experiments \pm s.d.

Figure S2 SHAPE analysis of wild-type and mutant IAPV IGR IRESs. Representative sequencing gels, which focus on the PKI domain of the IAPV IGR IRES, are shown. Sequencing from reactions containing dideoxy nucleotides are shown in the left lanes with their respective nucleotides. The sequencing gel was dried and analyzed by phosphorimager analysis.

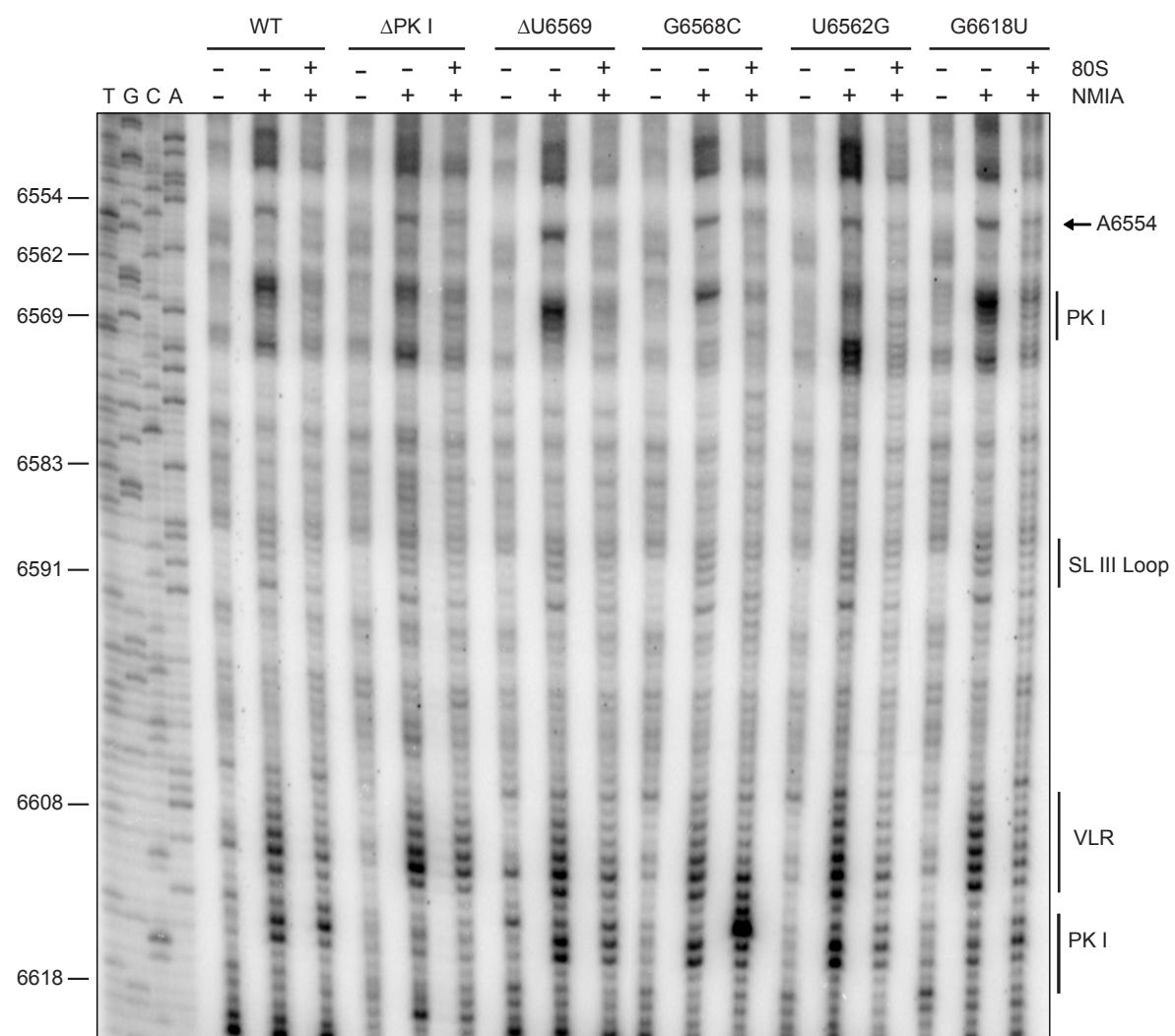
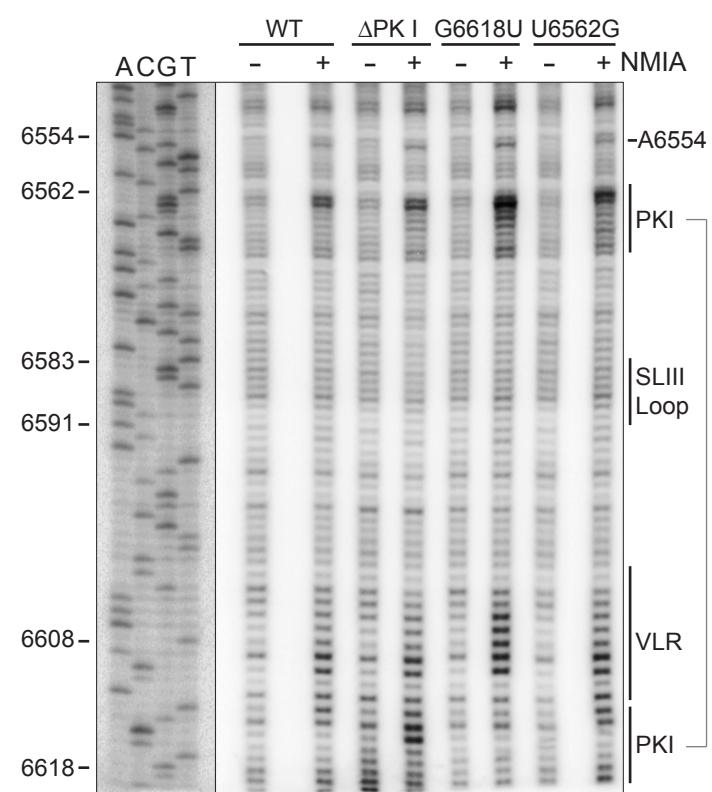
Figure S3 Mutations within a subset Type II IGR IRESs that direct 0 or 1 frame translation. A) Secondary structures of the PKI domain of Type II IGR IRESs, IAPV, KBV, ABPV and SINV-1 IGR IRES. B) A representative gel of IRES-mediated 0 and +1 frame translation. Bicistronic reporter constructs were incubated in Sf21 extracts at 30 °C for 120 min in the presence of [³⁵S]-

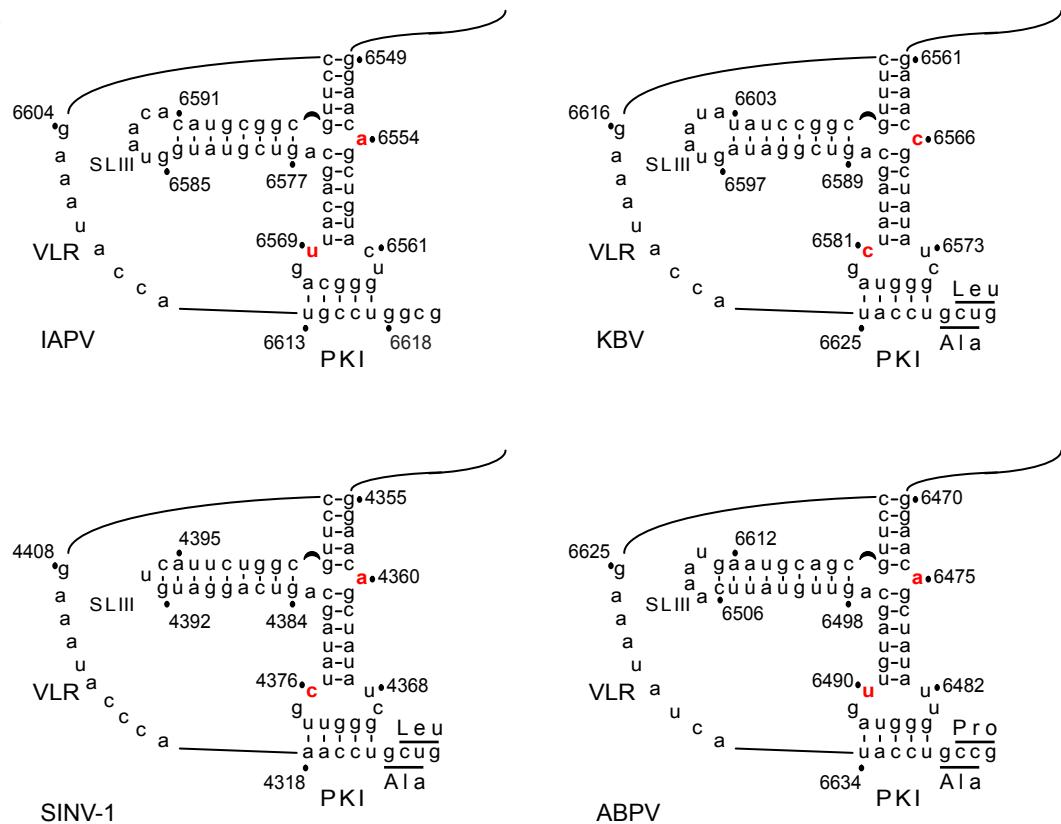
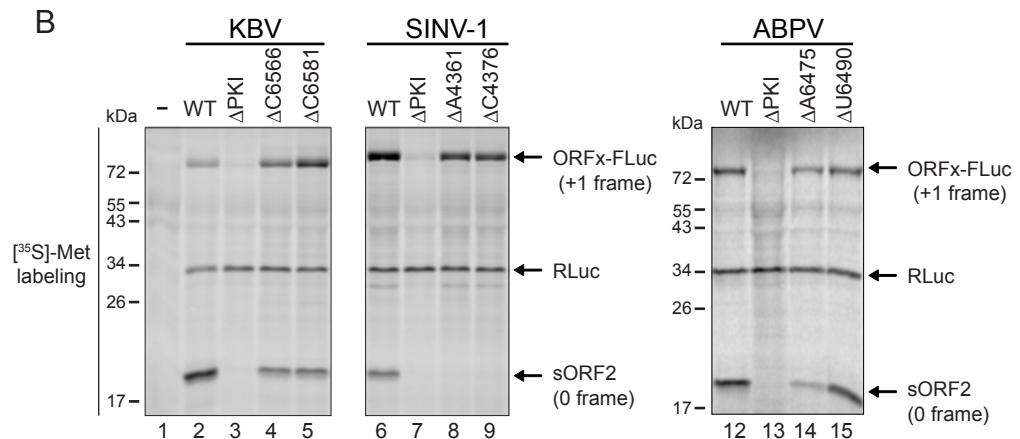
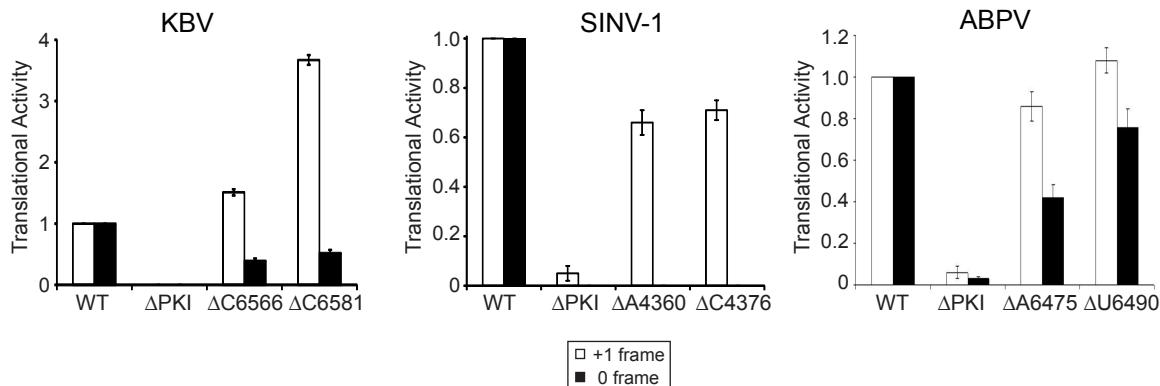
methionine. C) Quantitation of the radiolabeled +1 frame ORFx-Fluc (white bars) and 0 frame sORF2 (black bars) proteins normalized to RLuc. The ratios are normalized to the 0 and +1 frame translation of the wild-type IGR IRES given as 1.0. Averages are shown from at least three independent experiments \pm s.d.

Figure S4 SHAPE analysis of mutant IAPV IGR IRESs. SHAPE data overlayed on the secondary structure of PKI domain of the wild-type and mutant IAPV IGR IRESs. The colour coding indicates the relative SHAPE reactivities of individual nucleotides. Nucleotides are coloured in black, green, orange, and red from unreactive (<0.2) and limited reactivity (0.2-0.5) to moderately (0.5-0.8) and highly reactive (>0.8). SHAPE results are from at least three independent experiments \pm s.d.



Ren_Fig. S2



A**B****C**

Ren_Fig. S4

