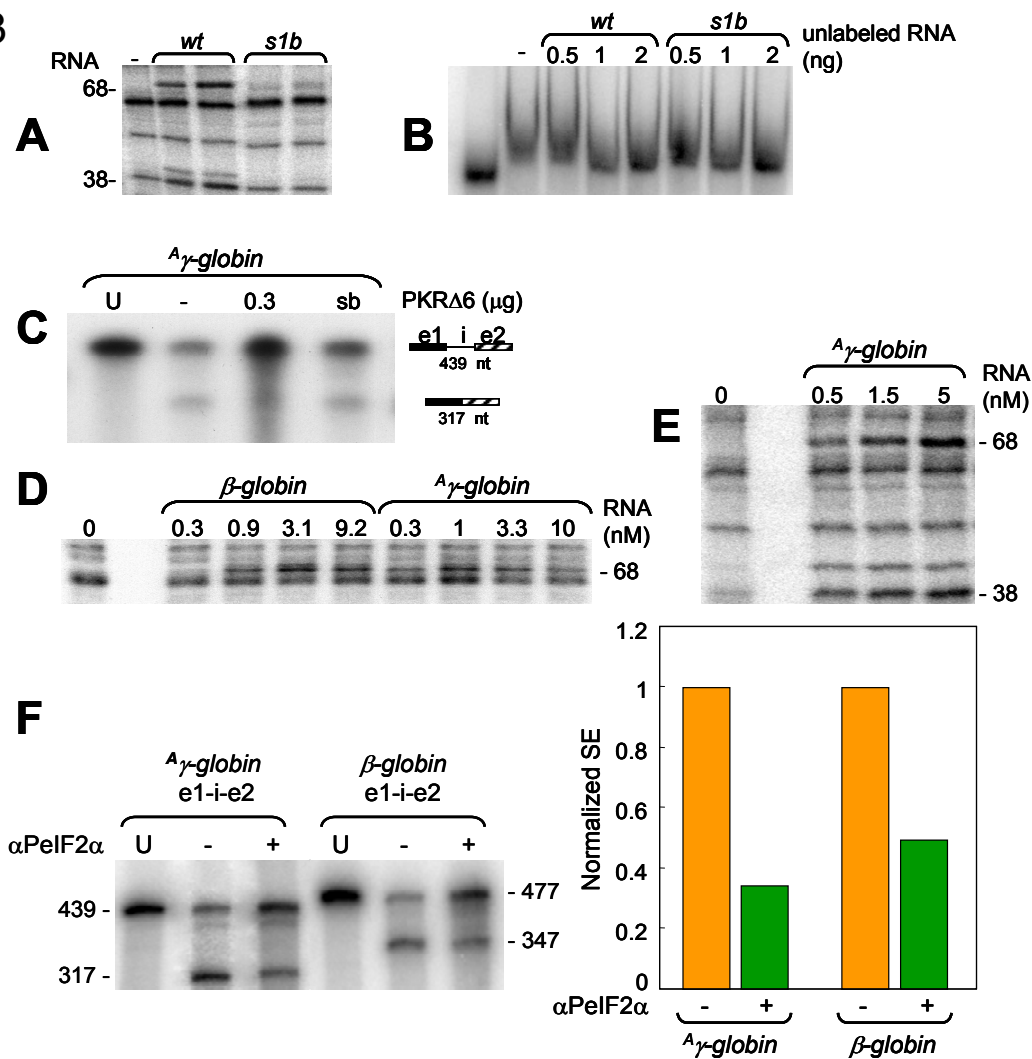


Figure S3



**Figure S3. Properties of *s1b* mutant  $\beta$ -globin RNA and PKR- and phospho-eIF2 $\alpha$ -dependent splicing of *A* $\gamma$ -globin mRNA.** (A) The *s1b* mutation abrogates PKR activation and eIF2 $\alpha$  phosphorylation. Activation of PKR and phosphorylation of eIF2 $\alpha$  in rabbit reticulocyte ribosomal fraction was assayed in the presence of 1.2 and 2 nM 477-nt *wt* or *s1b*  $\beta$ -globin pre-mRNA template. (B) The *s1b* mutation leaves affinity for PKR intact. Uniformly  $^{32}$ P-labeled 477-nt *wt*  $\beta$ -globin pre-mRNA template ( $10^4$  cpm) was incubated for 20 min on ice with 250 nM rPKR in splicing buffer and 3 mM MgCl<sub>2</sub>, in the absence (-) or presence of the indicated amounts of unlabeled *wt* or *s1b* mutant  $\beta$ -globin pre-mRNA template as competitor. Reaction mixtures (25  $\mu$ l) were made 10% glycerol and separated on 5% native polyacrylamide gels. Left lane shows free labeled RNA in water. (C) PKR $\Delta$ 6 blocks splicing of *A* $\gamma$ -globin pre-mRNA template. In vitro splicing was performed as for Figure 2E, using *A* $\gamma$ -globin pre-mRNA template. Where indicated, rPKR $\Delta$ 6 or storage buffer (sb) was added. (D) Activation of PKR by  $\beta$ -globin and *A* $\gamma$ -globin pre-mRNA templates. Activation of PKR (68 kDa) in rabbit reticulocyte ribosomal fraction was assayed in the presence of the indicated RNA concentrations. (E) *A* $\gamma$ -globin exon 1 RNA activates PKR and induces eIF2 $\alpha$  phosphorylation. Activation by 145-nt *A* $\gamma$ -

*globin* exon 1 RNA of PKR and phosphorylation of eIF2 $\alpha$  (38 kDa) was assayed in rabbit reticulocyte ribosomal fraction. **(F)**  $\alpha$ PeIF2 $\alpha$  mAb inhibits splicing of  $^A\gamma$ -*globin* pre-mRNA template. In vitro splicing was performed as for Figure 5H, using  $^A\gamma$ -*globin* and  $\beta$ -*globin* pre-mRNA templates, in the absence (-) or presence (+) of anti-phospho-eIF2 $\alpha$  mAb ( $\alpha$ PeIF2 $\alpha$ ; 1  $\mu$ g). Bar graph shows normalized splicing efficiencies.