

Figure S1

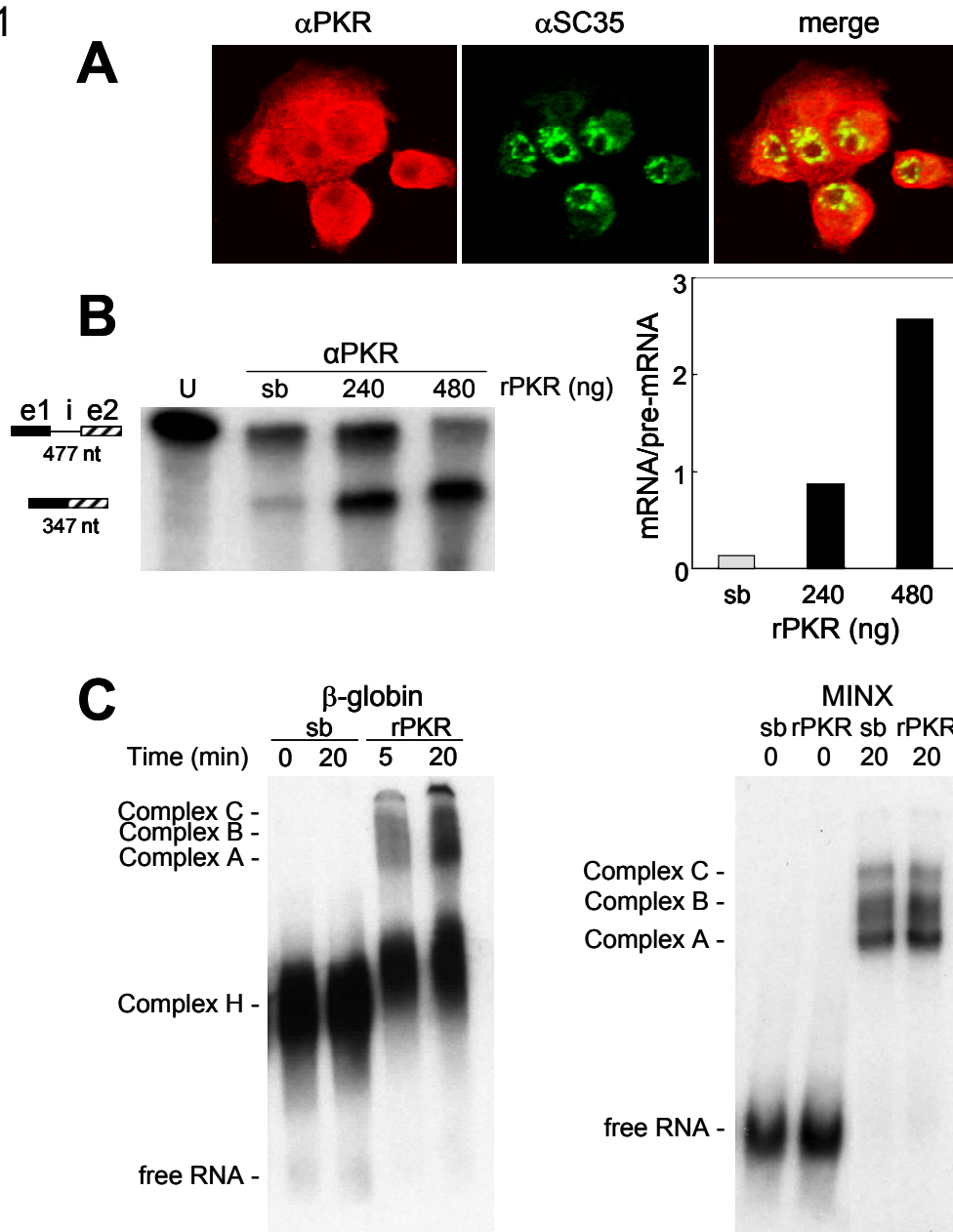


Figure S1. Colocalization of PKR with SC35 and reconstitution of β -globin pre-mRNA template splicing and spliceosome formation by recombinant PKR. (A) Colocalization of PKR with SC35. Monkey Vero cells were incubated for 1 h with α PKR (red) or α SC35 (green). Confocal microscopy is shown. Merge (yellow) shows PKR and SC35 in nuclear speckles. (B) Reconstitution of β -globin pre-mRNA template splicing by recombinant PKR. Nuclear extract was made 500 mM in NaCl and after incubation for 18 h at 4°C with α PKR Ab, the immunoprecipitate was removed using protein A/G Sepharose beads. The depleted extract was dialyzed before in vitro splicing was performed for 2 h as for Figure 3C, with rPKR or storage buffer (sb) added as shown; mRNA/pre-mRNA ratio is plotted on the right. U, input unspliced RNA. (C) Reconstitution of spliceosome formation on β -globin pre-mRNA template by recombinant PKR. Formation of spliceosomes was assayed as in Figure 3F, using extract depleted and dialyzed as in B, adding 260 ng rPKR where indicated. Panel on right shows MINX RNA, assayed in parallel.